Journal of Hazardous Materials 172 (2009) 166–171

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Ecotoxicological screening of reclaimed disinfected wastewater by *Vibrio fischeri* bioassay after a chlorination–dechlorination process

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ARTICLE INFO

Article history:
Received 3 February 2009
Received in revised form 25 June 2009
Accepted 26 June 2009
Available online 4 July 2009

Keywords:
Disinfection
Wastewater
Vibrio fischeri
Toxicity
Microtox®
Regression model

ABSTRACT

It is well known that different substances can react with chlorine in a water disinfection process to produce disinfection by-products (DBPs). Some of these substances have proven to be carcinogenic in humans and animals. Because it is not possible to detect all DBPs produced in chlorinated wastewater, toxicity tests have been proposed as a useful tool for screening toxic chemicals in treated wastewater. In this study, the Microtox® bioassay with *Vibrio fischeri* was used to evaluate the formation of toxic by-products in wastewater, after a chlorination–dechlorination disinfection treatment. All the variables were found to be normally distributed, so analysis of variance could be directly applied without transformation of variables. Significant correlations were obtained between toxicity values and total carbon, total inorganic carbon, total nitrogen, chlorine, and pH. In contrast, total organic carbon, chemical oxygen demand, electrical conductivity and turbidity had no effect on toxicity formation. Toxicity increased with the Cl_2:NH_4^+ ratio at a higher chlorine concentration released from combined chlorine. Regression models provided a good fit for effective concentration (EC50) as a function of total carbon and total nitrogen, after 5, 10, and 15 min of exposure. These models had greater multiple determination coefficients than previously reported for similar studies, without autocorrelation in the residuals as indicated by the Durbin–Watson statistic test. The measured and predicted ecotoxicity values were strongly correlated.

1. Introduction

Wastewater treatment plants are complex systems, with different physicochemical and biological phenomena taking place simultaneously. Wastewater collected from municipalities and communities must ultimately be returned to receiving waters or to the land; due to limited water resources, great attention is being paid to the reclamation and reuse of wastewater from municipalities, mainly for irrigation [1]. In all cases, water quality standards are becoming more stringent.

Prior to the industrial development, most municipal wastewater was generated from domestic sources. Nowadays, an increasing amount of industrial wastewater is being discharged to municipal collection systems. In the Region of Murcia, situated in the Southeast of Spain, industrial wastewater now represents 50% of the wastewater discharged into municipal collectors [2].

The use of physicochemical parameters to monitor pollution is a limited procedure, because these parameters may indicate the nature of the pollutants but do not yield any information about the biological effects. In this sense, ecotoxicological tests can be used as a global evaluation of effects that cannot be done by specific chemical analysis [3]. Ecotoxicological screening is included in the new European regulatory framework [4].

These bioassays are interesting, in particular for industrial or mixed wastewaters, where a complex mixture of various organic and inorganic substances may be discharged. In addition to this fact, a great amount of unknown products can be formed during water chlorination, commonly called disinfection by-products (DBPs). Although trihalomethanes (THMs) and haloacetic acids (HAAs) constitute two major classes of DBPs identified in water, in the last 30 years more than 600 different DBPs have been reported in the literature, i.e., halonitromethanes (chloropicrin), halofuranones, haloamides, tribromopyrrole among others [5]. However, only a small number have been assessed either in quantitative or health–effect studies.

The objective of this manuscript was to assess the ecotoxicity of biologically treated water from a municipal wastewater treatment plant (WWTP) after a chlorination–dechlorination disinfection process. Water was monitored by means of physicochemical and ecotoxicological parameters, with the use of the biological response of the bioluminiscent marine bacterium *Vibrio fischeri* [6]. From this evaluation and analysis, quadratic polynomial models were developed to predict toxicity.

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doi:10.1016/j.jhazmat.2009.06.157

Table 1
Quality parameters of raw water at WWTP of Cartagena and that of reclaimed water after chlorination–dechlorination.

	TOC (mg/L)	TC (mg/L)	NH_4^+ (mg/L)	TN (mg/L)	pH	Turbidity (NTU)	COD (mgO_2/L)
Raw water							
Minimum	8.61	72.74	5.62	36.99	7.57	1.92	23.00
Maximum	11.89	103.20	41.24	51.56	7.86	3.92	84.00
Mean	9.74	87.38	15.96	42.72	7.71	2.83	48.17
SD	1.23	10.94	13.15	4.72	0.09	0.72	22.83
5 mg/L							
Minimum	10.21	27.79	9.02	34.03	7.09	3.60	58.00
Maximum	12.02	62.31	32.44	44.67	7.47	11.61	144.00
Mean	11.31	50.33	20.54	39.80	7.31	6.25	87.80
SD	0.88	13.44	10.22	4.05	0.15	3.28	38.77
10 mg/L							
Minimum	10.35	43.99	5.29	32.75	7.13	2.77	57.00
Maximum	11.78	63.75	78.00	41.32	7.52	10.30	102.00
Mean	11.00	53.46	28.38	37.11	7.34	5.43	75.15
SD	0.69	8.96	2.89	3.55	0.16	3.34	35.68
25 mg/L							
Minimum	9.94	21.06	4.26	29.15	7.12	2.35	48.00
Maximum	15.96	65.86	93.39	43.46	7.46	13.25	110.00
Mean	12.12	54.98	29.93	37.40	7.36	6.73	65.17
SD	2.09	15.74	3.03	4.87	0.12	3.44	22.83
50 mg/L							
Minimum	9.65	25.99	10.37	28.59	7.16	3.15	22.00
Maximum	14.15	73.22	39.28	37.49	7.53	16.20	116.00
Mean	11.42	57.22	20.25	31.96	7.37	8.32	54.17
SD	1.53	15.49	10.94	3.14	0.13	4.86	34.64

$n = 30$.

2. Materials and methods

2.1. Sample collection and chlorination–dechlorination procedure

A total of 30 wastewater samples were processed through different chlorination–dechlorination experiments. They were collected during 2008 from the effluent of the WWTP of Cartagena (Spain). This is a conventional system with a primary treatment and two activated sludge reactors, serving about 140,000 inhabitants and fed by about 35,000 m^3 per day. The samples were collected after biological digestion and clarification, but prior to disinfection. The wastewater samples, kept at 4 °C, were delivered to the laboratory and filtered using a 1.2 μm glass fibre filter (Millipore, Bedford, MA, USA) to eliminate suspended solids. The samples prior to chlorination–dechlorination process will be referred to as "raw water".

Chlorine disinfection was carried out with 100 mL of filtered water. Four different concentrations of available chlorine, i.e., 5, 10, 25, and 50 mg/L, were added to each sample from a 5% (w/v) sodium hypochlorite solution (Panreac, Barcelona, Spain). The experiments were performed in sealed glass bottles. They were incubated for 30 min into a dark isotherm chamber at 20 °C, with slow agitation (20 rpm) provided by a magnetic stirrer. After residual chlorine was measured according to the Standard Methods for the Examination of Water and Wastewater [7], the dechlorination experiments were performed with 0.5% (w/v) sodium sulfite (Panreac, Barcelona, Spain) prepared with ultra-pure water from an Elix 3 Milli-Q system (Millipore, Bedford, MA, USA). Based on the residual chlorine concentration of each experiment, a one-to-one ratio of Na_2SO_3 was used. All chemicals were analytical grade.

2.2. Physicochemical analyses

Total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) were analyzed in a TOC analyzer (Shimadzu TOC-V CSH). Total inorganic carbon (TIC) was calculated based on the difference between TC and TOC. Ammonium (NH_4^+) was measured by HPLC (Metrohm 861 AG, Herisau, Switzerland), using a Metrosep C-250 7 μm (i.d. 250 mm × 4 mm) column. The eluent was a 4 mM tartaric acid (2,3-dihydroxybutanedioic acid) and 0.75 mM dipicolinic acid (pyridine-2,6-dicarboxylic acid) solution, with a flow rate of 1 mL/min. Sample solutions (20 μL) were injected into the

Table 2
Pearson's correlation coefficients of physicochemical and toxicity parameters in wastewater samples.

	EC50-5 min	EC50-10 min	EC50-15 min
TIC	0.771*	0.750*	0.735*
TC	0.763*	0.743*	0.727*
TN	0.629*	0.679*	0.670*
Chlorine	−0.546*	−0.486*	−0.514*
pH	0.725*	0.713*	0.694*
NH_4^+	0.220	0.365	0.363
TOC	−0.273	−0.107	−0.163
COD	0.151	0.248	0.084
EC	−0.118	−0.093	−0.121
Turbidity	−0.253	−0.274	−0.311

* $p < 0.01$.

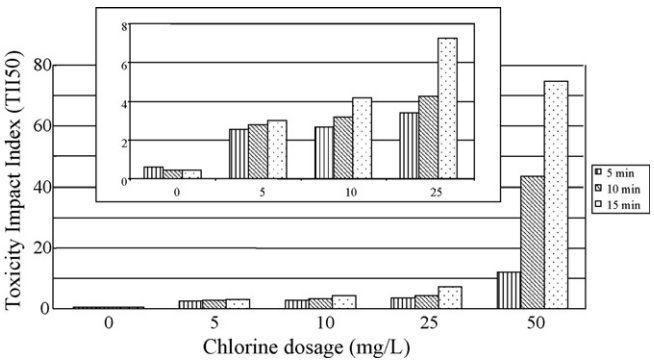

Fig. 1. Toxicity impact indices (TII50) for different chlorine dosages and exposure times ($F = 4.91$, $p < 0.05$; $F = 5.66$, $p < 0.01$; $F = 2.76$, $p < 0.01$, for 5, 10, and 15 min, respectively).

Table 3
Second-order polynomial equations and statistic evaluation of EC50-5 min, EC50-10 min, and EC50-15 min models.

Variable	Coefficient	Coefficient value	Standard error	*t*-Value	*p*-Value
EC50-5 min [$F = 47.292 > F_{0.05\,(4,26)} = 2.743$; $R = 0.963$; $R^2 = 0.927$; $R^2_{adj} = 0.907$; Durbin–Watson statistic = 1.901]					
Constant	β_0	4.823	–	1.429	0.636
TC	β_1	−18.601	4.123	−4.512	0.000
TN	β_2	30.415	7.783	3.908	0.001
TC × TC	β_{11}	0.150	0.029	5.147	0.000
TN × TN	β_{22}	−0.381	0.108	−3.536	0.003
TC × TN	β_{12}	0.184	–	1.231	0.178
EC50-10 min [$F = 50.328 > F_{0.05\,(4,26)} = 2.743$; $R = 0.967$; $R^2 = 0.935$; $R^2_{adj} = 0.916$; Durbin–Watson statistic = 1.887]					
Constant	β_0	9.219	–	2.458	0.651
TC	β_1	−25.000	4.680	−5.342	0.000
TN	β_2	40.285	8.877	4.538	0.000
TC × TC	β_{11}	0.204	0.034	5.957	0.000
TN × TN	β_{22}	−0.512	0.126	−4.059	0.001
TC × TN	β_{12}	0.182	–	1.493	0.428
EC50-15 min [$F = 49.222 > F_{0.05\,(4,26)} = 2.743$; $R = 0.964$; $R^2 = 0.929$; $R^2_{adj} = 0.910$; Durbin–Watson statistic = 2.239]					
Constant	β_0	9.399	–	2.336	0.675
TC	β_1	−26.504	4.643	−5.709	0.000
TN	β_2	43.442	8.812	4.930	0.000
TC × TC	β_{11}	0.214	0.034	6.301	0.000
TN × TN	β_{22}	−0.558	0.125	−4.459	0.000
TC × TN	β_{12}	0.164	–	1.256	0.471

HPLC system in duplicate, and all experiments were carried out at room temperature. Turbidity was determined with the Hach 2100N laboratory turbidimeter (Hach Company, Loveland, USA), and pH with a Crison GLP 22 pH meter (Barcelona, Spain). All were calibrated by means of standard solutions. The chemical oxygen demand (COD) was determined using the Spectroquant NOVA 30 from Merck (Darmstadt, Germany).

2.3. Acute toxicity measurement

Acute toxicity was assessed by determining the luminescence inhibition of the marine Gram-negative bacterium *V. fischeri* (strain NRRL B-11177), formerly referred to as *Photobacterium phosphoreum*, after 5-, 10-, and 15-min exposure to dechlorinated wastewater. This bacterium was purchased in freeze-dried form from Strategic Diagnostics Inc. (SDI, Newark, DE, USA) and activated by rehydration with a reconstitution solution. The light emission of this bacterium in contact with different samples and exposure times was measured using the Microtox® 500 analyzer. Light production is directly proportional to the metabolic activity of the bacterial population. The data were processed using the Microtox Omni Software, according to the Basic Test Protocol. The concentration of the sample (mg/L) which produces a 50% decrease in light after exposure for "*T*" minutes is designated as the effective concentration (EC50). Results are presented in EC50-5 min, EC50-10 min, and EC50-15 min, respectively.

2.4. Statistical analysis and model development

Statistical treatment of the physicochemical and toxicological data was carried out using the SPSS (Statistic Package for Social Science) 15.0 statistical software. The Kolmogorov–Smirnov test was used to test the goodness-of-fit to the normal distribution of studied variables. Pearson's correlation coefficient (r) and analysis of variance (ANOVA) were computed between toxicity and physicochemical variables.

The toxicity formation as a function of these parameters was studied by fitting data to different models, and the fitting performance of each model was measured by means of residual values and multiple determination coefficient (R^2). Besides, the adjusted R^2 (*adj*-R^2) was a useful index for comparing the explanatory power of models with a different number of predictors [8]. Residual values were evaluated for independence by means of the Durbin–Watson test, and predicted *versus* measured values allowed us to test the accuracy of each model.

3. Results and discussion

3.1. Water quality characteristics

The main statistical summary for raw and processed wastewater quality parameters is shown in Table 1. Pearson's correlation coefficient for water turbidity according to chlorine levels was 0.569 ($p < 0.01$). Similarly, ANOVA analysis showed a significant increase of this parameter when comparing raw water with chlorinated samples ($F = 7.92$, $p < 0.01$). These results are in agreement with other studies which found a greater turbidity after the chlorination process [9]. Gual et al. [10] reported a decrease of turbidity only when a combined treatment of filtration and chlorination was carried out.

3.2. Toxicity values

Different toxicity values were observed in our study after the chlorination–dechlorination experiment. Toxicity of the wastewater increased with the addition of chlorine (Table 2). Since the dechlorination process removed residual chlorine from disinfected wastewater prior to toxicity analyses, the positive correlation suggested the formation of toxic disinfection by-products (DBPs). This fact has been pointed by different authors, and it is a common concern in the legislation of industrialized countries, especially for drinking water [11,12].

The smallest EC50 value (greater toxicity) corresponded to a 50 mg/L chlorinated sample after 15 min exposure (0.75%), and the greatest value (smaller toxicity) was for a chlorinated sample with 5 mg/L after 5 min exposure (89.14%). In order to observe the toxic impact of each chlorine dosage in an easier way, the Equitox/m^3 or total impact index (TII50) was calculated as 100/EC50 for each incubation time. These indices are depicted in Fig. 1 and, as reported by Farré et al. [13], TII50 are calculated in the same way as toxicity units (TUs), although they represent different concepts. TU is related to an amount of a known substance, whereas TII50 is related to an amount of a mixture of unknown composition. Boluda et al. [14] indicated that samples with toxicity units (15 min) equal to or greater than 10 (i.e., when EC50-15 min < 10%) should be con-

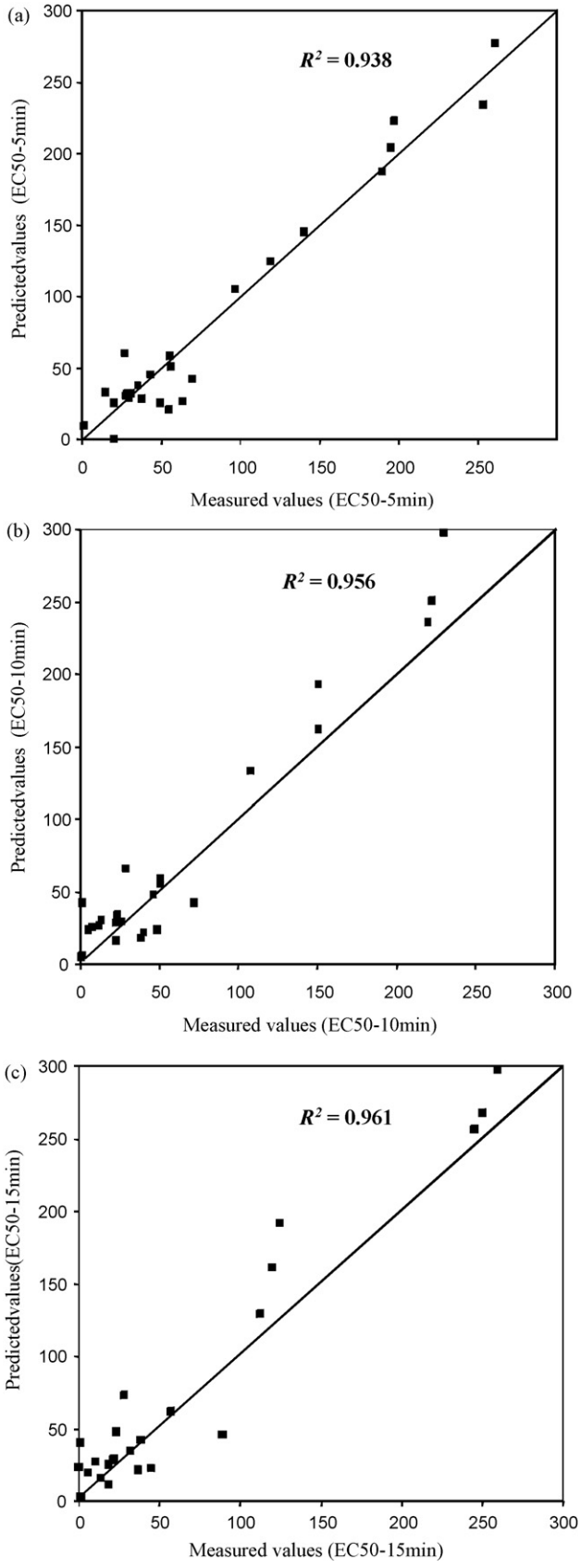

Fig. 2. Measured *versus* predicted values for (a) EC50-5 min, (b) EC50-10 min, and (c) EC50-15 min equations.

sidered as toxic. Following this criterion, only samples disinfected with 50 mg/L of chlorine reached this value. In the same way, and according to the EN ISO 11348-3:2007 standard [6], water samples with more than 20 Equitox/m^3 can be considered toxic. In this case, samples disinfected with 50 mg/L of chlorine after 10- or 15-min exposures were toxic.

3.3. Relationships between chemical characteristics and toxicity

A statistically significant increase of EC50 values could be observed with an increase in TN (Table 2). These values ranged from 28.59 to 51.56 mg/L, corresponding to the treatment with 50 mg/L of chlorine and a raw water sample, respectively. Pearson's correlation coefficient for TN according to chlorine levels was −0.581 ($p < 0.01$). As reported by Deborde and Guten [15] during aqueous chlorination, hypochlorous acid reacts with ammonium to finally generate N_2. This hypochlorous acid would also be an alternative explanation of the small but significant decrease in pH values when samples were chlorinated ($F = 10.70$, $p < 0.001$).

The TC concentration in raw water ranged from 72.74 to 103.20 mg/L, while in chlorinated samples it did not exceed 73.22 mg/L (Table 1). The majority of this carbon was in inorganic form, experimenting an important and statistically significant decrease after the chlorination–dechlorination processes ($F = 8.01$, $p < 0.001$). This fact can be explained by a mineralization, i.e., an important degradation of the carbon compounds to form water and carbon dioxide that will be emitted to the atmosphere. This fact has been previously reported both for water ozonation [16] and in chlorinated swimming pool water [17].

Raw wastewater samples showed a higher and statistically significant TC average concentration, 87.38 ± 10.94 mg/L, than chlorinated ones, 54.39 ± 13.54 mg/L (data not shown), as indicated by one-way ANOVA analysis ($F = 34.43$, $p < 0.001$). When this analysis was developed for all treatments, the same tendency was shown ($F = 8.12$, $p < 0.001$). The differences of the decreasing trend of TC for the first chlorine dosage (5 mg/L) and the increasing tendency for all other dosages may be due to the complex reactions taken into account. Initially, chlorine would act with a high oxidation potential, transforming organic and inorganic carbon into carbon dioxide, as stated before.

But this oxidation does not usually lead only to a complete mineralization, but also to a fragmentation of larger size molecules into smaller sizes after chlorination [18]. Hence, an increase of chlorine levels in treated wastewater would promote halogenation reactions of intermediate products and the formation of DBPs.

Attempts were made to study the evolution of carbon forms according to toxic effects on *V. fischeri*. Using the Pearson's correlation method, a strong and positive correlation was obtained for TC and TIC for 5, 10, and 15 min EC50, as presented in Table 2. However, a weak and negative correlation was obtained for TOC values. In agreement with other reports [19–21], organic carbon can be a broad indicator of the amount of organic matter in the wastewater, but it does not contain specific information about the potential of this organic matter to serve as DBP precursor. According to Marugán et al. [22], total organic carbon measurements must be carefully considered, because of differences in the nature of organic substances present in similar waters.

Total nitrogen average concentrations in wastewater were shown to decrease from not-chlorinated (42.72 ± 4.72 mg/L) to samples disinfected with 50 mg/L of chlorine (31.96 ± 3.14 mg/L) ($F = 6.15$, $p < 0.01$) (Table 1). Moreover, a statistically significant increase on EC50 values could be observed with an increase in TN, as shown in Table 2. Both results were clearly correlated, and can be explained by the same fact: the consumption of chlorine by ammonia nitrogen forms leads to the formation of chloramines,

which produce less DBPs, because of smaller reactivity of combined chlorine than free chlorine. The ammonia reacts rapidly to form inorganic monochloramine.

Sorlini and Collivignarelli [23] and Pavelic et al. [24] reported the same results in artificial lakes and rivers, and in groundwater, respectively. Wang et al. [25] have also reported a decrease in the toxicity of disinfected wastewater when ammonia nitrogen increased from 2 to 30 mg/L. However, Abdullah et al. [26] found no correlation between DBPs and nitrogen in Malaysian drinking water, mainly due to the absence of ammonium in almost all samples.

In order to study the effect of chlorine to ammonium ratios on toxicity formation a one-way ANOVA test was carried out. EC50 for 5, 10, and 15 min exhibited a similar decreasing tendency as Cl_2 to NH_4^+ increased. This means that more DBPs are formed at greater Cl_2:NH_4^+ ratios, as also suggested by Zhang et al. [27] and Diehl et al. [28]. In these conditions, chlorine to chloramines previously formed would hydrolyze to form hypochlorous acid (HOCl) in equilibrium with free chlorine released by this hydrolysis, which would increase DBP formation. Qi et al. [29] found a similar association between Cl_2:NH_4^+ ratio and the formation of haloacetic acids (HAAs), during monochloramination of wastewater.

The formation of toxic compounds in wastewater samples increased significantly with decreasing pH (Table 2). At low pH values, HOCl is the predominant species, responsible for the formation of DBPs because it has a greater oxidative capacity than the OCl^- anion found at greater pH. An increase in pH also enhances the degradation of these DBPs [27,30]. Diehl et al. [28] reported similar results, showing a decrease of DBP formation with an increase in the pH from 6 to 10. Yang et al. [30] found a marked difference in DBP formation in Suwannee river water, in the pH range from 4 to 9; the maximum amounts of DBPs occurred at pH 5–6. In consequence, Sadiq and Rodriguez [31] suggested a control of pH among the operational factors that contribute to a reduction in the formation of DBPs.

In common with previous reports, no significant correlation was obtained between turbidity and COD values and toxicity formation [26].

3.4. Development of regression models

By applying multiple regression analysis on the experimental data, three second-order polynomial equations were fitted between EC50-5 min, EC50-10 min, and EC50-15 min, as dependent variables, and TC and TN values as independent variables, according to the following equations:

$$\text{EC50-5 min} = -18.601\text{TC} + 30.415\text{TN} + 0.150\text{TC}^2 - 0.381\text{TN}^2 \quad (1)$$

$$\text{EC50-10 min} = -25.000\text{TC} + 40.285\text{TN} + 0.204\text{TC}^2 - 0.512\text{TN}^2 \quad (2)$$

$$\text{EC50-15 min} = -26.504\text{TC} + 43.442\text{TN} + 0.214\text{TC}^2 - 0.558\text{TN}^2 \quad (3)$$

All the equations displayed an important and negative influence of TC values, and an important and positive influence of TN values. An opposite trend was obtained for the quadratic effects of both parameters, i.e., TC^2 and TN^2, although with weaker relationships. However, the inclusion of these quadratic terms in the equations was necessary, as the models without them resulted in a lower *adj-*R^2, i.e., 0.579, 0.561, and 0.534, for EC50-5 min, EC50-10 min, and EC50-15 min, respectively, as compared with 0.907, 0.916, and 0.910, respectively, when they were included. These multiple determination coefficients were higher than those reported in previous studies [32]. Golfinopoulos and Arhonditsis [33] also reported the use of second-order equations, including first- and second-degree terms in the independent variables and without a constant term, for the prediction of THM concentrations in drinking water.

The Durbin–Watson statistic test was used to detect the absence of autocorrelation in the residuals from the regression models. When the Durbin–Watson value converges to zero, there is a strong correlation between the regression residuals. On the contrary, a Durbin–Watson value converging to 2.0 indicates a weak correlation or random distribution between successive points [34].

As shown in Table 3, the Durbin–Watson statistics for these three models were found to be 1.901, 1.887, and 2.239, respectively, demonstrating that the residuals were independent. Uyak et al. [35] have reported similar results in the development of multiple regression models for predicting the concentrations of different DBPs in drinking water.

Fig. 2 depicts a 1:1 line of measured *versus* predicted ecotoxicity values. All the equations reported very high Pearson's correlation coefficients, i.e., 0.938, 0.956, and 0.961 for EC50-5 min, EC50-10 min, and EC50-15 min, respectively, always with a significance level of 99.9% (p-value < 0.001).

4. Conclusions

The Microtox® test was used for the assessment of the acute toxicity of treated wastewater samples after a chlorination–dechlorination process. Toxicity was strongly associated with chlorine levels, and negatively associated with TIC, TC, TN, and pH. There was only a weak association between toxicity and TOC, COD, EC, and turbidity of the disinfected wastewater samples. When significant factors were included in a regression analysis, only TC and TN remained in the two-order polynomial equations for toxicity prediction (EC50), after an exposure of 5, 10, and 15 min. As indicated by Sadiq and Rodriguez [31], laboratory-scale studies are more reliable than field-scale studies for developing empirical models because of controlled conditions. Our models explained a large proportion of the response variable, with multiple determination coefficients of 0.927, 0.935, and 0.929, respectively. Further surveys could allow us to develop fuzzy rule-based models, in order to improve the predictive capacity.

Acknowledgements

The authors are grateful to the personal of the WWTP of Cartagena for their assistance with wastewater sample collection.

References

[1] P.H. Chen, K.C. Leung, J.T. Wang, Investigation of a ponding irrigation system to recycle agricultural wastewater, Environ. Int. 26 (2000) 63–68.
[2] F. Victoria Jumilla, Guía del Medio Ambiente para Empresas y Profesionales, Murcia, Spain, 1999.
[3] E. Mendonça, A. Picado, L. Silva, A.M. Anselmo, Ecotoxicological evaluation of cork-boiling wastewaters, Ecotoxicol. Environ. Saf. 66 (2007) 384–390.
[4] Council Regulation (EC) No. 440/2008 of 30 May 2008, laying down test methods, pursuant to regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Regulation, Evaluation, Authorisation and Restriction of Chemicals (REACH), Official Journal of the European Union L 142 (2008).
[5] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research, Mutat. Res. 636 (2007) 178–242.
[6] EN ISO 11348-3:2007, Water quality—determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminiscent bacteria test). Part 3. Method using freeze-dried bacteria.
[7] APHA, in: L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, USA, 1998.

[8] M. Pérez-Moya, M. Graells, P. Buenestado, H.D. Mansilla, A comparative study on the empirical modeling of photo-Fenton treatment process performance, Appl. Catal. B: Environ. 84 (2008) 313–323.
[9] E. Friedler, I. Katz, C.G. Dosoretz, Chlorination and coagulation as pretreatments for greywater desalination, Desalination 222 (2008) 38–49.
[10] M. Gual, A. Moià, J.G. March, Monitoring of an indoor pilot plant for osmosis rejection and greywater reuse to flush toilets in a hotel, Desalination 219 (2008) 81–88.
[11] R.J. Garcia-Villanova, C. Garcia, J.A. Gomez, M.P. Garcia, R. Ardanuy, Formation, evolution and modeling of trihalomethanes in the drinking water of a town. II. In the distribution system, Water Res. 31 (1997) 1405–1413.
[12] V. Uyak, I. Koyuncu, I. Oktem, M. Cakmakci, I. Toroz, Removal of trihalomethanes from drinking water by nanofiltration membranes, J. Hazard. Mater. 152 (2008) 789–794.
[13] M. Farré, M.J. García, L. Tirapu, A. Ginebreda, D. Barceló, Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminiscence inhibition assays, Anal. Chim. Acta 427 (2001) 181–189.
[14] R. Boluda, J.F. Quintanilla, J.A. Bonilla, E. Sáez, M. Gamón, Application of the Microtox® test and pollution indices to the study of water in the Albufera Natural Park (Valencia, Spain), Chemosphere 46 (2002) 355–369.
[15] M. Deborde, U.V. Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment—kinetics and mechanisms: a critical review, Water Res. 42 (2008) 13–51.
[16] B.S. Karnik, S.H. Davies, M.J. Baumann, S.J. Masten, The effects of combined ozonation and filtration on disinfection by-product formation, Water Res. 39 (2005) 2839–2850.
[17] H. Kim, J. Shim, S. Lee, Formation of disinfection by-products in chlorinated swimming pool water, Chemosphere 46 (2002) 123–130.
[18] Q. Ji, H. Liu, C. Hu, J. Qu, D. Wang, J. Li, Removal of disinfection by-products precursors by polyaluminum chloride coagulation coupled with chlorination, Sep. Purif. Technol. 62 (2008) 464–469.
[19] T.F. Marhaba, D. Van, The variation of mass and disinfected by-product formation potential of dissolved organic matter freactions along a conventional surface water treatment plant, J. Hazard. Mater. 74 (2000) 133–147.
[20] D.M. White, D.S. Garland, J. Narr, C.R. Woolard, Natural organic matter and DBP formation potential in Alaskan water supplies, Water Res. 37 (2003) 939–947.
[21] K. Gopal, S.S. Tripathy, J.L. Bersillon, S.P. Dubey, Chlorination byproducts, their toxicodynamics and removal from drinking water, J. Hazard. Mater. 140 (2007) 1–6.
[22] J. Marugán, R.V. Grieken, C. Sordo, C. Cruz, Kinetics of the photocatalytic disinfection of *Escherichia coli* suspensions, Appl. Catal. B: Environ. 82 (2008) 27–36.
[23] S. Sorlini, C. Collivignarelli, Trihalomethane formation during chemical oxidation with chlorine, chlorine dioxide and ozone of ten Italian natural waters, Desalination 176 (2005) 103–111.
[24] P. Pavelic, B.C. Nicholson, P.J. Dillon, K.E. Barry, Fate of disinfection by-products in groundwater during aquifer storage and recovery with reclaimed water, J. Contam. Hydrol. 77 (2005) 351–373.
[25] L.S. Wang, D.B. Wei, J. Wei, H.Y. Hu, Screening and estimating of toxicity formation with photobacterium bioassay during chlorine disinfection of wastewater, J. Hazard. Mater. 141 (2007) 289–294.
[26] M. Abdullah, C.H. Yew, M.S. Ramli, Formation, modeling and validation of trihalomethanes (THM) in Malaysian drinking water: a case study in the districts of Tampin, Negeri Sembilan and Sabak Bernam, Selangor, Malaysia, Water Res. 37 (2003) 4637–4644.
[27] H. Zhang, J. Qu, H. Liu, X. Zhao, Isolation of dissolved organic matter in effluents from sewage treatment plant and evaluation of the influences on its DBPs formation, Sep. Purif. Technol. 64 (2008) 31–37.
[28] A.C. Diehl, G.E. Speitel, J.M. Symons, S.W. Krasner, C.J. Hwang, S.E. Barrett, DBP formation during chloramination, J. Am. Water Works Assoc. 92 (2000) 76–90.
[29] Y. Qi, C. Shang, I.M.C. Lo, Formation of haloacetic acids during monochloramination, Water Res. 38 (2004) 2375–2383.
[30] X. Yang, C. Shang, P. Westerhoff, Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination, Water Res. 41 (2007) 1193–1200.
[31] R. Sadiq, M.J. Rodriguez, Disinfection by-products (DBPs) in drinking water and predictive models for their occurrence: a review, Sci. Tot. Environ. 321 (2004) 21–46.
[32] A.D. Nikolau, S.K. Golfinopoulos, G.B. Arhonditsis, V. Kolovoyiannis, T.D. Lekkas, Modeling the formation of chlorination by-products in river waters with different quality, Chemosphere 55 (2004) 409–420.
[33] S.K. Golfinopoulos, G.B. Arhonditsis, Multiple regression models: a method for evaluating trihalomethane concentrations in drinking water from raw characteristics, Chemosphere 47 (2002) 1007–1018.
[34] D.N. Rutledge, A.S. Barros, Durbin–Watson statistic as a morphological estimator of information content, Anal. Chim. Acta 454 (2002) 277–295.
[35] V. Uyak, K. Ozdemir, I. Toroz, Multiple linear regression modeling of disinfection by-products formation in Istanbul drinking water reservoirs, Sci. Tot. Environ. 378 (2007) 269–280.