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Formation potential of *N*-nitrosamines during the disinfection of treated wastewaters with sodium hypochlorite

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ABSTRACT

This study investigated the formation of eight *N*-nitrosamines (*N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodi-n-propylamine (NDPA), *N*-nitrosomorpholine (NMOR), 1-nitrosopyrrolidine (NPYR), 1-nitrosopiperidine (NPIP), and *N*-nitrosodibutylamine (NDBA)) after the chlorination (with NaOCl) of wastewater effluents from two wastewater treatment plants (WWTPs) located in North East Spain. Two different WWTPs, one with nitrification-denitrification process (CB1) and the other without (CB2) have been chosen. The concentrations of individual N-nitrosamines in non-chlorinated tertiary treated wastewater effluents ranged from <LOD to $75\,\mathrm{ng\,L^{-1}}$. That range increases to 114 and $1092\,\mathrm{ng\,L^{-1}}$ after chlorinating tertiary-treated wastewater samples at 5 and $15\,\mathrm{mg\,L^{-1}}$ of free chlorine, respectively. NDEA and NMEA are the most abundant compounds in both non-chlorinated and chlorinated secondary and tertiary-treated wastewater effluents. The disinfection of tertiary treated wastewater at $5\,\mathrm{mg\,L^{-1}}$ of free chlorine gave a total *N*-nitrosamines concentration of about $300\,\mathrm{ng\,L^{-1}}$ in samples from the two studied WWTPs. Total *N*-nitrosamine concentrations in chlorinated secondary treated wastewater samples were significantly higher than those obtained from the chlorination of tertiary treated wastewaters ($p < 0.05$). This may be due to the capacity of the tertiary treatment processes for removing *N*-nitrosamines precursors. The experiments have revealed that in addition to NDMA, relatively high concentrations of other *N*-nitrosamines are produced when treated wastewater effluents are disinfected with chlorine for agricultural irrigation purposes.

Keywords: Chlorination; Disinfection by-products; *N*-nitrosamines; Reclaimed water

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1. Introduction

Climate change and the increasing of population are driving the shift to a new paradigm in water resource management. With many world communities approaching the limits of available water supplies, water reclamation, and water reuse have become an attractive option [1]. Reclaimed water is obtained from treated wastewater (WW) after the application of different treatments that normally include the use of chlorine as a disinfection agent [2]. Chlorine is intended to kill bacteria and inactivate viruses, but it can also react with the constituents present in the treated effluents from WWs and increase the

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formation of disinfection by-products [2]. The detection of *N*-nitrosamines in rivers, WWs, and drinking waters is of growing concern because over the years many *N*-nitrosamines have been identified as probable human carcinogens [3]. These compounds can be released directly into the environment from industrial sources or can be formed from different precursors, and under a wide variety of nitrosation or oxidation reactions taking place in wastewater treatment plants (WWTPs) [4,5]. Specifically, the presence of chlorine catalyses the reaction between monochloramine and either dimethylamine or tertiary amines present in treated WW to form hydrazine intermediates which are later oxidized to form *N*-nitrosamines [6].

N-nitrosamines are polar compounds, usually soluble in water [3]. Due to their large hydrophilicity and recalcitrant nature, they contribute to the ground and surface waters contamination risk [7]. Several *N*-nitrosamines have been found in secondary effluents in different WWTPs in the range from <limit of quantification (LOQ) to 188 ng L^{-1} [8]. *N-N*-nitrosodimethylamine (NDMA) is the most commonly detected *N*-nitrosamine in drinking water and WWs after chloramination or chlorination [9–11]. *N*-nitrosamines are considered as carcinogenic compounds to multiple organs in at least 40 animal species including higher primates. Epidemiologic evidence has also correlated dietary intake of *N*-nitrosamines with the risk of stomach, esophageal, and nasopharyngeal cancers [12]. The Environmental Protection Agency (US EPA) has classified *N*-nitrosamines in B2 group, indicating their probable carcinogenic effect on humans. Maximum permitted concentration in drinking water was established for three of them at very low concentration level such as 7 ng $L^{-1}$for NDMA, 20 ng L^{-1} for *N*-nitrosomethylethylamine (NMEA) and 2 ng L^{-1} for *N*-nitrosodiethylamine (NDEA) with risk estimation of 10^{-5} [13]. Whereas notification levels in drinking water were established at 10 ng L^{-1} for NDMA, NDEA, and *N*-nitrosodi-n-propylamine (NDPA).

Reclaimed water usage is emerging as a sustainable water resource in the water sector that may help to overcome water shortages in arid and semiarid regions [14]. In this regard, Pehlivanoglu-Mantas et al. [15] observed that chlorine disinfection of treated-WW effluents used for crop irrigation resulted in excessive NDMA formation up to 2 μg L^{-1}, whereas WW nitrification–denitrification processes reduced the formation of NDMA. However, little attention has been paid to the formation of other *N*-nitrosamines during chlorination of treated effluents. Hence, having into account the toxicity and the previous mentioned carcinogenic effect of these compounds [6,16], the use of reclaimed water for agricultural irrigation is of concern, because nitrosamines could be incorporated into the crops and later be consumed by humans. According to a theoretical study developed by Calderón-Preciado et al. [16], *N*-nitrosamines could be accumulated in vegetables and fruits at 11–13 ng kg^{-1} dry weight.

The aim of this study was to assess the formation potential of eight *N*-nitrosamines (NDMA, NMEA, NDEA, NDPA, *N*-nitrosomorpholine (NMOR), 1-nitrosopyrrolidine (NPYR), 1-nitrosopiperidine (NPIP), and *N*-nitrosodibutylamine (NDBA)) after the chlorination of effluents from two different WWTPs, where one of them includes a nitrification–denitrification process while the other do not. To carry out this study, a series of laboratory scale experiments were conducted adding chlorine to both secondary and tertiary WW effluents. WW samples were not filtered to enable the chlorine to react with particulate and dissolved matter. Furthermore, the analytical methodology based on solid-phase extraction (SPE) followed by gas chromatography with nitrogen phosphorus detector (GC-NPD) was validated for the determination of *N*-nitrosamines in treated WW samples at ng L^{-1} level.

2. Materials and methods

2.1. Chemicals

The EPA 8270 Appendix IX Nitrosamine Mix was purchased from Supelco Analytical (Bellefonte, USA), which consist of five branched nitrosamines: NDBA, NDEA, NDMA, NDPA, NMEA, and three cyclic nitrosamines: NMOR, NPIP, and NPYR with a concentration of 2,000 mg L^{-1} each component in methanol. Chlorothalonil PESTANAL® was used as internal standard and was purchased from Fluka (Buchs, Switzerland). Sodium hypochlorite with a 14.1% of free chlorine was from Sigma-Aldrich (Munich, Germany). All solvents (*n*-hexane, dichloromethane, and ethyl acetate) and anhydrous sodium sulfate were supplied from Merck (Darmstadt, Germany). Supelclean™ coconut charcoal SPE cartridges (bed wt. 2 g, volume 6 mL) were purchased from Supelco Analytical (Bellefonte, USA). Glass microfiber filters (GF/F, 0.7 μm and 47 mm Ø) were from Whatman™ (Carrigtwohill, Ireland). Stock standard solutions containing 100 mg L^{-1} of each *N*-nitrosamine were prepared in methanol and stored in a glass-stopped bottle at −20°C in darkness.

2.2. Description of the treatment plants and sampling

The two WWTPs studied are located in two important tourist resorts in the north-east of the Costa Brava (CB), Spain: CB1 (Blanes WWTP with 109,000 population equivalent) and CB2 (Castell-Platja d'Aro WWTP with 175,000 population equivalent). Both WWTPs are placed in the same area, but whereas CB1 has certain industrial input, CB2 does not. Table 1 shows the secondary and tertiary treatment processes included in each WWTP. The secondary treatment in both WWTPs consisted of a conventional activated sludge reactor, but in CB1 the secondary treatment also included a nitrification–denitrification unit, where nitrogenated compounds were eliminated by a two-stage biological process. The biological oxygen demand in raw WW was around 300 mg L^{-1} in both WWTPs, whereas the final values ranged between 4 and 12 mg L^{-1} in tertiary effluents. In all cases, reclaimed water complied with the Spanish regulations for water reuse (R.D. 1620/2007).

Different sampling points were established in the WWTPs. In the case of the CB1 WW, samples were collected from the secondary effluent as well as from the tertiary effluent before the chlorination step. For CB2, samples were only collected from the tertiary effluent before the chlorination step. At CB1 the sampling campaigns were carried out in spring (25 May 2010), summer (15 September 2010), and autumn (25 October 2010), whereas at CB2 only a summer campaign (7 July 2010) was considered. All samples were collected in 1 L clean amber glass bottles stored at 4 °C until being analyzed. The sample holding time was less than 24 h.

2.3. *N*-nitrosamines determination

N-nitrosamine extraction was done following the 521 EPA method [17]. SPE cartridges were first conditioned with 12 mL of dichloromethane, 12 mL of methanol, and 12 mL of Milli Q water. After that, 200 mL of filtered water samples (0.7 µm pore size filters) were percolated through the SPE cartridges (approximately at 10 mL min^{-1}). *N*-nitrosamines were eluted with six portions of 2 mL of dichloromethane. The obtained extracts were treated with Na_2SO_4 to remove remaining traces of water. Samples were preconcentrated using a stream of high purity N_2 (99.999%) and additionally, a change of the organic solvent was made from dichloromethane to ethyl acetate.

N-nitrosamines were separated using a Fisons GC8000 Series gas chromatograph (GC) (Ipswich, United Kingdom). The GC instrument was equipped with a CE Instruments NPD detector (Hindley Green, United Kingdom) and a fused-silica capillary column ZB-1701 (30 m × 0.25 mm i.d. × 0.25 µm, 14% cyanopropylphenyl-86% dimethylpolysiloxane) from Phenomenex (Madrid, Spain). Helium was used as a carrier gas

Table 1
WWTPs description

	CB 1 (Blanes WWTP)	CB 2 (Castell-Platja d'Aro WWTP)
Equivalent population	109,985 hab	175,000 hab
Influent[a]	TSS: 372 mg L^{-1}; NH_4^+: 83 mg L^{-1}	TSS: 266 mg L^{-1}; NH_4^+: 45 mg L^{-1}
Primary treatment	Pre-treatment (screening of large materials) Primary clarification	Pre-treatment (screening of large materials) Primary clarification
Secondary treatment	Activated sludge treatment	Activated sludge treatment
	Nitrification–denitrification	
	Secondary clarification	Secondary clarification
Secondary effluent[a]	TSS: 9 mg L^{-1}; NH_4^+: 11.7 mg L^{-1}	TSS: 8 mg L^{-1}; NH_4^+: 26.7 mg L^{-1}
Tertiary treatment	Coagulation–floculation	Coagulation–floculation Lamellar settlement Filtration (pulsed-bed sand filters)
	Lamellar settlement	UV disinfection
	Filtration (pulsed-bed sand filters)	Chlorination (5 mg L^{-1})
	UV disinfection	
	Chlorination (3 mg L^{-1})	
Tertiary effluent[a]	TSS: 4.4 mg L^{-1}, pH: 7.9	TSS: 7.0 mg L^{-1}, pH: 8.1

[a] Data obtained from Consorci de la Costa Brava (http://www.ccbgi.org, www.ccbgi.org). Average data of year 2010. TSS: Total suspended solids.

at a flow of $1.4\,\mathrm{mL\,min^{-1}}$. The injection port and detector temperatures were kept at 270 and 300°C. respectively. The column oven temperature was held at 65°C for 2 min, then programmed at $10^\circ\mathrm{C\,min^{-1}}$ to 280°C, and held for 10 min, respectively. Compounds were eluted as follows: NDMA (4.7 min); NMEA (5.8 min); NDEA (6.7 min); NDPA (9.3 min); NMOR (9.8 min); NPYR (10 min); NPIP (10.4 min); NDBA (12.2 min); and chlorothalonil (20.7 min). The sample injection was done in the splitless mode, using an injection volume of 2 μL. A validation process was carried out to confirm the suitability of the method for the intended use. The instrumental linearity was determined by performing a calibration curve from 6 to $1{,}000\,\mathrm{\mu g\,L^{-1}}$ for each compound, and the resulting correlation coefficients were always over 0.99. Recoveries were studied spiking secondary treated WW samples at $300\,\mathrm{ng\,L^{-1}}$, by triplicate. The obtained recoveries ranged from 52 (NDMA) to 88% (NPIP). Similarly, low recoveries (57 and 65%) have been reported for NDMA in water samples [18,19], whereas the recoveries for the other studied *N*-nitrosamines were in accordance with those values presented previously by Ripollés et al. [20] and Zhao et al. [21]. The precision was evaluated in terms of repeatability, which is expressed as relative standard deviation (RSD). RSDs ($n = 3$) were analyte dependent and were always lower than 14% for all compounds. The LOD and LOQ of the analytical methodology were determined in ultrapure water ($n = 3$) from the mean background noise plus three or ten times the standard deviation of the background noise, respectively. The LODs and LOQs for the studied compounds were 16 and $23\,\mathrm{ng\,L^{-1}}$ for NDMA, 9 and $14\,\mathrm{ng\,L^{-1}}$ for NMEA, 52 and $80\,\mathrm{ng\,L^{-1}}$ for NDEA, 9 and 15 for NDPA, 17 and $22\,\mathrm{ng\,L^{-1}}$ for NMOR, 79 and $144\,\mathrm{ng\,L^{-1}}$ for NPYR, 19 and $25\,\mathrm{ng\,L^{-1}}$ for NPIP, and 37 and $42\,\mathrm{ng\,L^{-1}}$ for NDBA, respectively. The obtained values are in general agreement with other published methodologies using the same selective detector [22], but higher than those that use mass spectrometry detectors [21,23].

2.4. Experimental design

The water samples collected from each sampling point were split in subsamples of 200 mL, placed into amber glass bottles of 1 L. A group of samples were considered as control (no free chlorine added) and were immediately analyzed for the determination of *N*-nitrosamines. Another group of samples was subjected to disinfection with sodium hypochlorite (NaOCl), in order to study the formation of *N*-nitrosamines. Two levels of chlorination (5 and $15\,\mathrm{mg\,L^{-1}}$ of free chlorine) were studied, adding the corresponding amount of sodium hypochlorite into the bottles containing 200 mL of the sample. The theoretical concentration of free chlorine in ultrapure water was calculated from the total concentration of sodium hypochlorite added to the water sample. The disinfection time was set at 48 h according to a study developed by Pehlivanoglu–Mantas et al. [15], where after the addition of different amounts of chlorine to a WW effluent sample a maximum formation of NDMA was observed at that time. After chlorination step, samples were filtered through a glass microfiber filter of 0.7 μm and processed for the determination of *N*-nitrosamines as described above. All laboratory assays were made in triplicate.

2.5. Statistical analysis

Experimental results were evaluated using the SPSS v.15 package (Chicago, IL, USA). Adjustment of datasets to a normal distribution was always verified to ensure that parametric statistics were applicable. Comparison of means was conducted by the paired samples *t*-test or one-way ANOVA.

3. Results and discussion

3.1. Occurrence of N-nitrosamines in unchlorinated effluents

The individual concentration of *N*-nitrosamines in the nonchlorinated secondary and tertiary-treated effluents of CB1 collected in spring ranged from <10 to $100\,\mathrm{ng\,L^{-1}}$ (Figs. 1 and 2). However, the presence of *N*-nitrosamines was largely dependent on the water composition. In the case of CB1, three out of eight *N*-nitrosamines were detected above the LOD in the secondary effluent (i.e. NDMA, $30\,\mathrm{ng\,L^{-1}}$; NMEA, $67\,\mathrm{ng\,L^{-1}}$; and NMOR, $29\,\mathrm{ng\,L^{-1}}$). These results partially agree with those found by Krauss et al. [8], who found that NDMA ($33\,\mathrm{ng\,L^{-1}}$) and NMOR ($26\,\mathrm{ng\,L^{-1}}$) were the most abundant *N*-nitrosamines in secondary treated WWs from 20 different full-scale WWTPs, whereas NMEA was not detected in any of them. Similarly, in the tertiary effluent from CB1, two out of eight *N*-nitrosamines were found at concentrations above the LOD (i.e. NDEA, $75\,\mathrm{ng\,L^{-1}}$ and NMOR, $41\,\mathrm{ng\,L^{-1}}$). The increase of NDEA and the decline of NDMA and NMEA concentrations after the tertiary treatment process may be explained by the coagulation–flocculation or UV radiation processes occurring in CB1 tertiary treatment (see Table 1). The process of coagulation and flocculation eliminates suspended solids and perhaps stimulates the removal of some

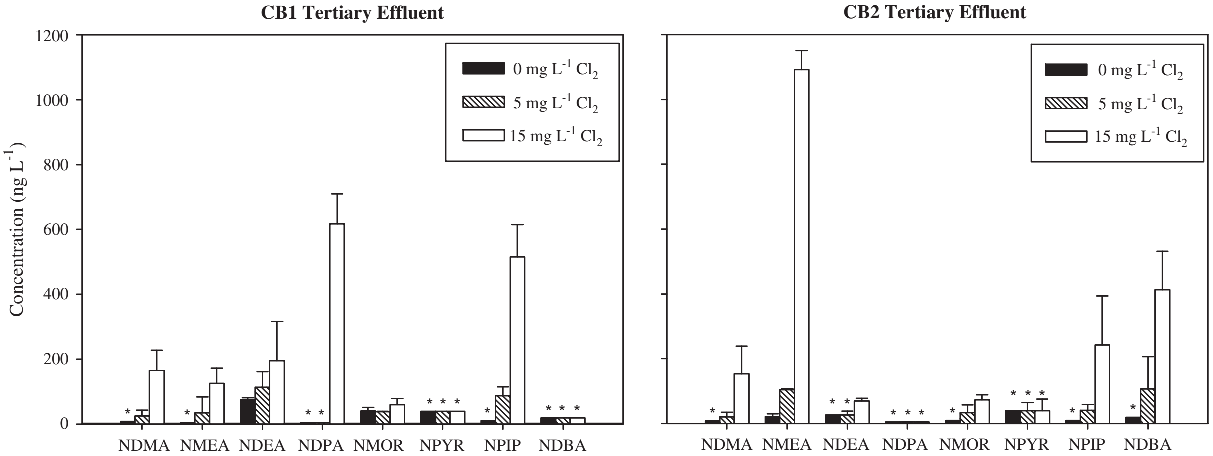

Fig. 1. Average concentration and standard deviation of *N*-nitromamines (n=3) in WW samples after the addition of different amounts of chlorine to tertiary WW effluents from CB1 (spring) and CB2 (summer) WWTPs. *Concentration values under LOD.

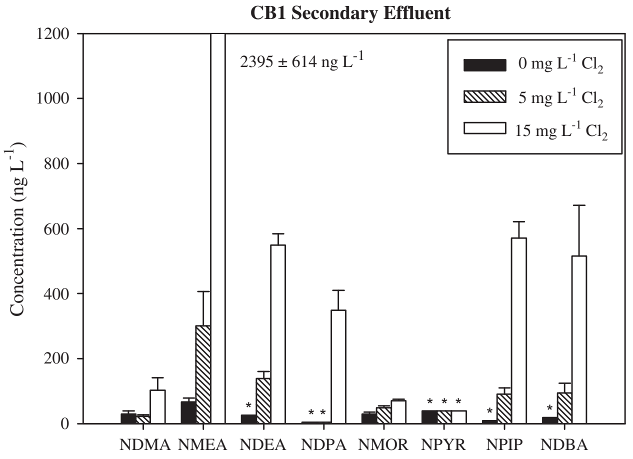

Fig. 2. Average concentration and standard deviation of *N*-nitromamines (n=3) in WW samples after the addition of different amounts of chlorine to secondary WW effluents from CB1 WWTP (spring). *Concentration values under LOD.

N-nitrosamines. Similarly, UV light oxidation can eliminate some compounds, such as NDMA, whilst creating some others (e.g. methylamine, dimethylamine) [24]. Moreover, the *N*-nitrosamine composition may change due to the biological processes occurring in the sand filtration unit. Nevertheless, although our observations and those found by Krauss et al. [8] suggest certain capacity of the tertiary treatments for removing *N*-nitrosamines, a matched-pair *t*-test indicates that there is no significant difference ($p = 0.799$) between the concentration of *N*-nitrosamines found in the secondary effluent (122 ng L^{-1}) and that found in the tertiary effluent (115 ng L^{-1}) from CB1. Finally, only NMEA was detected above the LOD (21 ± 8 ng L^{-1}) in the tertiary effluent from CB2.

3.2. Chlorination study

3.2.1. Effect of the WWTP

As described in the experimental design Section (2.4), two levels of chlorination were tested (5 and 15 mg L^{-1} of free chlorine). Treated WWs were dosed with 5 mg L^{-1} of free chlorine to emulate real disinfection conditions in WWTPs (between 3 and 6 mg L^{-1} of free chlorine), while 15 mg L^{-1} was applied to study the effect of an excess of this disinfectant agent on the formation of *N*-nitrosamines. Fig. 1 shows the formation of *N*-nitrosamines after treating tertiary effluents from CB1, sampled in spring, and CB2, sampled in summer, at these different concentrations of hypochlorite.

Different behavior was observed after the addition of 5 and 15 mg L^{-1} of free chlorine to the WWs (48 h). The chlorination of WWs from CB1 at 5 mg L^{-1} increased the concentration of NDMA, NMEA, NDEA, NMOR, and NPIP, whereas the addition of chlorine to CB2 increased the concentration of NDMA, NMEA, NDEA, NMOR, NPIP, and NDBA.

The increase of the concentration of free chlorine added (15 mg L^{-1}) gave a greater formation of *N*-nitrosamines due to the oxidation capacity of chlorine. NDPA and NMEA were the most abundant *N*-nitrosamines in CB1 and CB2, respectively. NPIP concentration significantly increased in both WWTPs, as well as NDBA in CB2. As it can be seen in Fig. 1, the concentration of NDMA was approximately the same in CB1 ($165 \pm 62\,\mathrm{ng\,L^{-1}}$) and in CB2 ($154 \pm 84\,\mathrm{ng\,L^{-1}}$), which suggest that a NDMA precursor might exist at similar range in these two water samples. The concentrations of NPYR and NDBA remained under the LOD in CB1, and NDPA and NPYR in CB2.

In summary, although total *N*-nitrosamine concentrations in tertiary treated WW samples after chlorination at $5\,\mathrm{mg\,L^{-1}}$ ($297\,\mathrm{ng\,L^{-1}}$ in CB1 vs. $302\,\mathrm{ng\,L^{-1}}$ in CB2) or $15\,\mathrm{mg\,L^{-1}}$ of free chlorine ($1{,}676\,\mathrm{ng\,L^{-1}}$ in CB1 vs. $2042\,\mathrm{ng\,L^{-1}}$ in CB2) were similar in both WWTPs ($p > 0.05$), the abundance of each individual *N*-nitrosamine was different. This fact can be due to the nitrification–denitrification processes occurring in CB1, which may play an important role in both the removal and generation processes of *N*-nitrosamine precursors [7,10]. Due to the nitrification–denitrification processes, dissolved organic nitrogen (DON) is the main form of nitrogen in the WW effluents, which may act as a precursor of some *N*-nitrosamines [10]. Hence, depending on the remaining WW-derived DON, some *N*-nitrosamines could be formed whereas others do not. It has also to be taken into account that differences between raw WWs can also explain this fact, since CB1 has a relevant industrial input.

The chlorination effect was also studied for secondary-treated WW effluents from CB1 in spring (Fig. 2). As explained in Section 3.1, only NDMA, NMEA, and NMOR were detected slightly above the LOD when no disinfection treatment was applied (no chlorine added). These concentrations increased after adding to the WW samples a normal dosage of $5\,\mathrm{mg\,L^{-1}}$ of free chlorine, NMEA being the most abundant compound after this chlorination treatment (c.a. $301\,\mathrm{ng\,L^{-1}}$). However, there are some *N*-nitrosamines which did not show any effect after this disinfection process, and still remained below the LOD such as NDPA and NPYR. The increase of the disinfectant agent up to $15\,\mathrm{mg\,L^{-1}}$ increased the concentration of *N*-nitrosamines as it did in the tertiary treated WW samples. In this case, NMEA was the most abundant compound ($2395 \pm 614\,\mathrm{ng\,L^{-1}}$), but high concentrations of NDEA ($549 \pm 35\,\mathrm{ng\,L^{-1}}$), NDPA ($348 \pm 62\,\mathrm{ng\,L^{-1}}$), NPIP ($571 \pm 50\,\mathrm{ng\,L^{-1}}$), and NDBA ($515 \pm 157\,\mathrm{ng\,L^{-1}}$) were also found. Although these results partially disagree with those observed in the literature in which NDMA and NDEA are normally the most abundant *N*-nitrosamines after the chlorination of secondary-treated WW effluents [11,25], this may be explained by the different composition of the waters.

Total *N*-nitrosamine concentrations in chlorinated secondary treated WW samples (after chlorination at 5 or $15\,\mathrm{mg\,L^{-1}}$ of free chlorine) were significantly higher than those obtained from the chlorination of tertiary treated WW samples ($p < 0.05$). This may be due to the elimination capacity of the tertiary treatment process in the removal of *N*-nitrosamines precursors, either accounted by the retention in the coagulation/flocculation lamella clarifier unit or photoxidation by the UV light radiation unit (Table 1). From these results, it can be said that the tertiary WW treatment reduces the potential formation of *N*-nitrosamines. Finally, it is worth mentioning that although some studies have claimed NDMA to be the most abundant compound after chlorination of treated tertiary WW effluents [15], other studies have found that NMEA [26] or NDEA are the most abundant N-nitrosamines [25]. According to our results, NDEA and NPIP were the most abundant *N*-nitrosamines in tertiary CB1 effluent after chlorination at $5\,\mathrm{mg\,L^{-1}}$, whereas NMEA and NDBA were the most abundant in tertiary CB2 effluent.

3.2.2. Effect of the seasonality at the CB1 WWTP

Table 2 shows the *N*-nitrosamine concentrations in spring (same data in Fig. 1), summer, and autumn after the chlorination ($5\,\mathrm{mg\,L^{-1}}$ of free chlorine) of the tertiary treated WW effluents from CB1. Laboratory studies reflected that frequency of detection of NDMA (100%), NDEA (66%), and NPYR (66%) were above

Table 2
Average concentration ($\mathrm{ng\,L^{-1}}$) and relative standard deviation (RSD, %) of *N*-nitrosamines in chlorinated tertiary effluents from CB1

Target analytes	Average nitrosamine concentration ($\mathrm{ng\,L^{-1}}$) and RSD (%). Chlorine treatment at $5\,\mathrm{mg\,L^{-1}}$			
	Spring[a]	Summer[a]	Autumn[a]	Significant difference*
NDMA	27 (63)	23 (26)	53 (23)	–
NMEA	34 (147)	<LOD	<LOD	–
NDEA	114 (42)	152 (54)	<LOD	c, d
NDPA	<LOD	<LOD	<LOD	–
NMOR	38 (3)	<LOD	<LOD	b, c
NPYR	<LOD	102 (22)	97 (1)	b, c
NPIP	87 (31)	<LOD	<LOD	b, c
NDBA	<LOD	<LOD	<LOD	–

[a]Samples collected before chlorination step. LOD – Limit of detection. *One-way ANOVA, significant difference at $p = 0.05$. b-spring vs. summer; c-spring vs. autumn; d-summer vs. autumn.

the LOD in most of the seasons, whereas other *N*-nitrosamines (NMEA, NDPA, NMOR, NPIP, and NDBA) were less frequently detected (<33%). NDMA was detected in spring at $27 \pm 63\,\mathrm{ng\,L^{-1}}$, in summer at $23 \pm 26\,\mathrm{ng\,L^{-1}}$, and in autumn at $53 \pm 23\,\mathrm{ng\,L^{-1}}$. NDEA was detected above LOD in spring ($114 \pm 42\,\mathrm{ng\,L^{-1}}$) and summer ($152 \pm 54\,\mathrm{ng\,L^{-1}}$). NPYR was detected in summer at $102 \pm 22\,\mathrm{ng\,L^{-1}}$ and in autumn at $97 \pm 1\,\mathrm{ng\,L^{-1}}$. Hence, NDEA was shown to be the most abundant *N*-nitrosamine after chlorination at $5\,\mathrm{mg\,L^{-1}}$. These results are in agreement with those found by Planas et al. [25] in which NDEA ($161\,\mathrm{ng\,L^{-1}}$), NDMA ($71\,\mathrm{ng\,L^{-1}}$), and NMOR ($41\,\mathrm{ng\,L^{-1}}$) were the most abundant *N*-nitrosamines after the chlorination of WWTP effluents at $6\,\mathrm{mg\,L^{-1}}$.

Industrial pollution and human excretion are the most relevant reported sources of *N*-nitrosamines in WW influents [7], as a consequence, their concentrations are highly variable. Table 2 shows that concentrations of *N*-nitrosamines resulted to be statistically significantly higher in summer or spring (NDEA, NMOR, and NPIP) than in autumn, which is apparently attributable to the high tourist population as well as the warmer temperature during these seasons which may led to higher *N*-nitrosamine formation rates. This is in accordance with the results observed by Planas et al. [25] who found that the differences in *N*-nitrosamine concentrations depended on the month of sampling, which was attributed to differences in the organic matter content of the WWTP effluent. In addition, it is worth mentioning that in warmer months the chemical reactions are faster.

3.2.3. Agricultural and environmental implications

In addition to the microbiological quality criteria for WW reuse in agricultural irrigation [27], concern about the *N*-nitrosamines present in reclaimed water has also risen due to reported carcinogenic and mutagenic effects of a number of these compounds [25]. Human exposure to these compounds can be by ingestion or inhalation of preformed *N*-nitrosamine compounds, as well as by endogenous nitrosation of naturally occurring precursors [28].

Bearing in mind that reclaimed water is widely used for agricultural irrigation and that we have demonstrated that chlorinated and nonchlorinated reclaimed waters contain *N*-nitrosamines at levels higher than $100\,\mathrm{ng\,L^{-1}}$ (i.e. NDMA from <LOD to $165\,\mathrm{ng\,L^{-1}}$, NDEA from <LOD to $549\,\mathrm{ng\,L^{-1}}$, and NDPA from <LOD to $617\,\mathrm{ng\,L^{-1}}$), we suggest avoiding sprinkler irrigation since it may increase the presence of *N*-nitrosamines in the aerosols, and consequently, cause exposure to pollutants and a health risk for farm workers. Furthermore, human exposure through the daily intake of vegetables and fruits irrigated with this reclaimed water cannot be ignored [15]. For example, it is well established that, among others, dried malt and beer contain trace levels of volatile *N*-nitrosamines [28]. Moreover, the discharge of chlorinated reclaimed water to surface waters may increase the toxicological risk on organisms inhabiting the impacted waters.

In these regards, our results show that the use of tertiary treatment systems decrease the formation of *N*-nitrosamines and consequently may be used to attenuate the presence of these compounds in the reclaimed water.

4. Conclusions

In summary, NDEA was the most abundant nitrosamine in nonchlorinated tertiary treated WW samples from CB1, whereas NMEA was in samples from CB2. Concentrations of *N*-nitrosamines in chlorinated tertiary treated WW effluents ranged from <LOD to $114\,\mathrm{ng\,L^{-1}}$ (chlorination under standard conditions at $5\,\mathrm{mg\,L^{-1}}$ of free chlorine), NDEA being the most abundant *N*-nitrosamine in CB1 and NMEA in CB2. Tertiary treated WW samples from the two WWTPs resulted in similar amount of *N*-nitrosamines after chlorination studies, but with different individual abundance, which may be due to both differences between raw WWs and the role played by the nitrification–denitrification processes occurring in CB1, that may contribute simultaneously to the removal and generation of *N*-nitrosamine precursors. Higher chlorine concentration increases total nitrosamine formation. Furthermore, a higher abundance of *N*-nitrosamines was observed in the spring and summer seasons, probably due to differences in water composition. In this study, we have demonstrated that tertiary treatment allows a significant reduction of *N*-nitrosamine formation under chlorination conditions. Moreover, in addition to NDMA, relatively high concentrations of other *N*-nitrosamines are produced when treated WW effluents are disinfected with chlorine for agricultural irrigation.

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