Occurrence and fate of benzothiazoles and benzotriazoles in constructed wetlands
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ABSTRACT
A suite of benzothiazoles and benzotriazoles was analysed by comprehensive multi-dimensional gas chromatography coupled to time-of-flight mass spectrometry in four different wastewater treatment systems. The selected wastewater treatment systems include one conventional wastewater treatment plant (WWTP) and for the first time, two constructed wetlands. Although benzothiazole (BT), 2-(methylthio)benzothiazole (MTBT), 2-hydroxybenzothiazole (OHBT) and benzotriazole (BTri) occurred in all the raw wastewater samples, 4- and 5-methylbenzotriazoles (4- and 5-TTri) were only detected in the industrially impacted wastewater. Concentrations of benzothiazoles and benzotriazoles in raw wastewater ranged from 0.2 to 2.2 $\text{mg} \cdot \text{L}^{-1}$ and from 0.06 to 36.2 $\text{mg} \cdot \text{L}^{-1}$, respectively. The benzothiazoles removal efficiencies ranged from zero to up to 80% in the conventional WWTP and from 83 to 90% in constructed wetlands. For BTri, removal efficiencies ranged from 65 to 70% and from 89 to 93% in conventional WWTP and constructed wetlands, respectively. The higher performance of CWs in the removal of these compounds might be attributed to the contribution of biodegradation, photodegradation and plant uptake.

Key words | benzothiazoles, benzotriazoles, constructed wetlands, removal, wastewater, WWTP

INTRODUCTION
Benzotriazoles and benzothiazoles are high production volume chemicals that are used in industrial and household applications. Benzotriazoles, namely 1H-benzotriazole (BTri) and 4- or 5-methyl-1H-benzotriazole (4- or 5-TTri), are used as corrosion inhibitors, in de-icing fluids for aircrafts, automotive antifreeze formulations, brake and metal-cutting fluids, industrial cooling systems and silver protection in household dishwashing agents (Cancilla et al. 2003; Weiss & Reemtsma 2005). Benzothiazoles such as benzothiazole (BT), 2-hydroxybenzothiazole (OHBT) and 2-(methylthio)benzothiazole (MTBT) are degradation products from vulcanization accelerators used in rubber production, as biocides in paper and leather manufacturing and also as corrosion inhibitors (Jungclaus et al. 1976; Kloepfer et al. 2005).

Benzotriazoles have been classified as being toxic to aquatic organisms, causing long-term adverse effects (Pillard et al. 2001). Although Reemtsma et al. (1995) reported acute aquatic toxicity for benzothiazoles in various bioassays at concentration of part per million (i.e. specific respiratory inhibition tests and Microtox bioassay), there is a lack of information at the environmental levels (parts per trillion or billion).

Owing to widespread application, several benzothiazoles and benzotriazoles have been detected in surface waters at concentrations ranging from 0.1 to 6 $\mu \text{g} \cdot \text{L}^{-1}$ (Cancilla et al. 1998; Breedveld et al. 2003; Kloepfer et al. 2005; Giger et al. 2006; Reemtsma et al. 2006; Voutsa et al. 2006; Ni et al. 2008; Richter et al. 2008). Water from industrial wastewater systems and surface runoff from urban roads and airports contain a significant amount of these compounds, which gives rise to elevated concentrations of benzothiazoles and benzotriazoles in wastewater treatment plant (WWTP) influents (Giger et al. 2006; Weiss et al. 2006; Ni et al. 2008).
Nevertheless, a low removal efficiency for some of these compounds in conventional domestic activated sludge WWTPs, ranging from 0 to 74% for benzotriazoles and from 5 to 28% for benzothiazoles has been reported (Kloepfer et al. 2005; Voutsa et al. 2006). In addition, there is a lack of similar studies conducted in alternative wastewater treatment systems such as constructed wetlands working as either secondary or tertiary treatment. Constructed wetlands are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent-rooted wetland vegetation (Cole 1998). Until now, only the removal efficiency of pesticides and some emerging compounds such as pharmaceuticals and personal care products have been evaluated. These results revealed that removal efficiencies were similar to and in many times even higher, depending on the contaminant, than conventional WWTPs (Schulz et al. 2003; Matamoros & Bayona 2006). Although Castro et al. (2005) suggested the feasibility of constructed wetlands for the removal of benzotriazoles, there is no experimental information to support it.

The goals of this study were to evaluate the removal efficiency and aquatic mass discharge rate of benzothiazoles (i.e. BT, OHBT and MTBT) and benzotriazoles (i.e. BTri, 4 and 5-TTri) in one conventional activated sludge WWTPs, and for the first time in two constructed wetlands, one working as a secondary treatment system and another as a tertiary treatment system.

### MATERIAL AND METHODS

#### Chemicals

Gas chromatography (GC) grade (Suprasolv) hexane, methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac (Barcelona, Spain). BT, OHBT, MTBT, BTri, XTri (xyllyltriazole) and 5-TTri analytical grade were purchased from Sigma-Aldrich (Steinheim, Germany). Strata-X polymeric solid-phase extraction (SPE) cartridges (100 mg/6 mL) were obtained from Phenomenex (Torrance, CA, USA) and the 0.45 μm glass fibre filters of ø = 47 mm were purchased from Millipore (Bedford, MA, USA). Table 1 lists the chemical structures, acronyms and octanol-water partition coefficients (log $K_{OW}$) of these compounds.

#### Treatment plant description

Table 2 shows the main features of the treatment plants studied. The Granollers urban WWTP serves 154,000 inhabitants (Granollers, Barcelona, Spain). The WWTP influent comprises approximately 45% industrial wastewater and the remaining 55% of domestic sewage, with an average total flow of 23,700 m$^3$ d$^{-1}$. The WWTP carries out pre-treatment, primary clarification, activated sludge treatment and secondary clarification. The effluent is mainly diverted to the Congost River, which is a tributary of the Besòs River. In addition, the Granollers WWTP...
effluent discharge (0.27 m$^3$ s$^{-1}$) accounts for surges in the flow of the Congost River during drought periods.

The Can Cabanes surface flow constructed wetland (SFCW) is fed with a small portion of treated effluent (secondary effluent) from the Granollers WWTP. The SFCW is made up of a single cell with a surface area of 1 ha. It has shallow stretches of water (0.3–0.4 m deep) planted with Typha latifolia and Phragmites australis, deep stretches of water with no plantations (1.5 m deep) and a small island. This wetland treats approximately 100 m$^3$ d$^{-1}$ (0.4% of the WWTP effluent) with a hydraulic retention time (HRT) of approximately 1 month. In SFCWs, the organic pollutants are mostly degraded through aerobic and photodegradation processes (Matamoros et al. 2008). The Trige vertical flow constructed wetland (VFCW) is located in the Trige municipality (Aarhus, Denmark) and consists of a pilot constructed wetland with an unsaturated bed flow in which aerobic conditions prevail. Hence organic matter is mostly removed by aerobic processes. Since it is a pilot system, it only treats a small portion of wastewater from the Trige municipality. Finally, the León WWTP treats domestic wastewater from León city (Castilla y La Mancha, Spain). The WWTP carries out a pre-treatment, primary clarification, activated sludge treatment and secondary clarification before it discharges the treated effluent into the Bernesga River, which is a tributary of the Duero River.

**Sampling strategy**

Between four to ten influent and effluent grab samples were collected from each wastewater treatment system, with the exception of Granollers WWTP where only effluent samples were collected (Can Cabanes SFCW influent is the 0.4% of Granollers WWTP effluent). In addition, five samples from the León WWTP and four from the SFCW were seasonally collected. A total of 44 water samples, including influent and effluent were taken from February 2006 to December 2007.

Samples were collected in 1 L amber glass bottles and kept refrigerated during transportation to the laboratory, where they were then stored at 4°C until analysis (within two days). The collected samples were analysed for benzothiazoles and benzotriazoles as described below.

### Analytical methodology

The influent and effluent wastewater samples from every system were filtered and processed as previously reported (Matamoros et al. 2005). Briefly, a sample volume of 500 mL was acidified and then spiked with 1 m$^g$ of XTri as a surrogate standard. The spiked sample was percolated through a previously activated polymeric SPE cartridge. Elution was performed with 10 mL of hexane/ethyl acetate (1:1). The eluate was evaporated until ca. 20 mL under a gentle nitrogen stream and 186 ng of triphenylamine were added as an internal standard. Finally, the vial was reconstituted to 300 mL with ethyl acetate.

The multidimensional gas chromatography coupled to time of flight mass spectrometry (GC × GC–TOF MS) system consisted of an HP 6890N (Agilent Technologies, Palo Alto, CA) gas chromatograph equipped with a split/splittless injector, a secondary oven to fit the second column and a Zoex (Houston, TX) ZX1 two-stage thermal modulator. Liquid nitrogen used for cold pulses was automatically filled from a Dewar using a liquid leveler, which accessed a 60 L liquid nitrogen storage tank. The MS system was a Pegasus 4D TOF system (LECO, St. Joseph, MI) working at 70 eV. The transfer line and ion source was set at 250°C and 200°C, respectively. Scanning was performed from 50 to 500 m/z at 100 Hz with a detector voltage of 1,600 V. As a first dimension, a column of 50 m × 0.25 mm ID coated with 0.25 m film thickness TRB5-MS (5% diphenyl-polydimethylsiloxane) was used. As a second dimension, a 2 m × 0.10 mm ID coated with 0.10 m film thickness TRB-50 HT (high temperature 50% diphenyl-polydimethylsiloxane) was installed. Both columns were
obtained from Teknokroma (Sant Cugat del Vallès, Spain). The oven temperature was ramped from 65°C (1 min) to 290°C at 5°C min⁻¹ with a final holding time of 20 min; the secondary oven was kept at 10°C above the first dimension temperature during the whole experiment. Modulation time was 6 seconds. Data were acquired and processed using the ChromaTOF 3.32 software. Limit of detection (LOD) and limit of quantification (LOQ) ranged from 5 to 56 and from 35 to 99 ng L⁻¹, respectively. Quantification and other quality control parameters have been reported elsewhere (Jover et al. 2009).

Statistical analysis

An SPSS v15 package (Chicago, IL, USA) was used for statistical data analysis. The seasonal influent concentration comparison was analysed using the Mann Whitney test for two independent samples (nonparametric statistics).

RESULTS AND DISCUSSION

Occurrence of benzotriazoles and benzothiazoles in wastewater

Table 3 shows the influent concentration and detection frequency of selected pollutants from three different wastewater systems in Denmark and Spain. The concentration range reported here is in accordance with previously reported data for raw wastewater from different locations (Kloepfer et al. 2005; Voutsa et al. 2006).

Although benzothiazoles occurred in all the wastewaters studied, 4- and 5-TTri were only detected in the SFCW influent. This is in accordance with the high contribution of industrial wastewaters in the Granollers WWTP in which industrial wastewater accounts for ca. 45% of the influent flow.

The concentrations of benzothiazoles and benzotriazoles were higher in the influent of the Trige than in the León WWTP and the SFCW studied. These results can be ascribed to the fact that the León WWTP influent is diluted with groundwater, whereas the SFCW is a tertiary treatment plant from the Granollers WWTP. Isomeric concentrations of 4- and 5-TTri in the Granollers WWTP secondary effluent (SFCW influent, Table 3) differed from the typical values of technical mixtures (5-TTri/4-TTri = 1.2 – 1.3), whereas this isomeric ratio in the Granollers WWTP effluent was 0.28 on average. This may be attributable to a preferential biodegradation of the 5-isomer in the WWTP. Our results are in accordance with a previous study that reported an isomeric ratio of 0.29 in a lab-scale membrane bioreactor (MBR) effluent (Weiss et al. 2006).

In the León WWTP, there were statistically significant differences between the concentrations of BT and BTri influent during winter and summer. The influent concentration of BT was higher in winter than in summer. In the SFCW, varying seasonal influent concentrations (OHBT, BTri and 4-TTri) appeared to be attributable to seasonal applications and/or variations in removal efficiencies in the Granollers WWTP.

Table 3 | Detection frequency (DF), overall averages and standard deviations of benzothiazoles and benzotriazoles

<table>
<thead>
<tr>
<th>Target compounds</th>
<th>Leon WWTP Cold season</th>
<th>Trige WWTP Cold season</th>
<th>SFCW Cold season</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT</td>
<td>0.39 ± 0.11</td>
<td>0.16 ± 0.01†</td>
<td>1.13 ± 0.09</td>
</tr>
<tr>
<td>MTBT</td>
<td>0.15 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>1.04 ± 0.61</td>
</tr>
<tr>
<td>OHBT</td>
<td>0.38 ± 0.04</td>
<td>0.31 ± 0.14</td>
<td>2.07 ± 0.26</td>
</tr>
<tr>
<td>BTri</td>
<td>3.86 ± 1.08</td>
<td>7.91 ± 1.17†</td>
<td>36.2 ± 4.9</td>
</tr>
<tr>
<td>4-TTri</td>
<td>&lt; 0.06</td>
<td>&lt; 0.06</td>
<td>&lt; 0.06</td>
</tr>
<tr>
<td>5-TTri</td>
<td>&lt; 0.06</td>
<td>&lt; 0.06</td>
<td>&lt; 0.06</td>
</tr>
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</table>

DF = number of samples with concentration greater than LOD/total number of samples.

†Statistically significant differences at a significance level of 0.05.
Removal efficiencies of benzotriazoles and benzothiazoles in wastewater treatment systems

Figure 1 shows the removal efficiency of the selected pollutants in two secondary and one tertiary treatment plants. These results confirm the lower removal efficiency of benzothiazoles and benzotriazoles in conventional WWTPs (Kloepfer et al. 2005; Giger et al. 2006) than in VFCW, both working as secondary treatments. However, the removal efficiency in the SFCW is affected by seasonality. Although there is no seasonal trend seen in the removal efficiency of these pollutants or in the general parameters in conventional WWTPs (i.e. the León WWTP, Table 2), in the SFCW where photodegradation and vegetation development play a significant role in the removal of emerging pollutants (Matamoros et al. 2008), there is a marked variability between warm and cold seasons. In fact, photodegradation is reported as being one of the major removal pathways for benzothiazoles (Brownlee et al. 1992; Andreozzi et al. 1998). In addition, plant uptake and phytotransformation of benzotriazoles appeared to be particularly important in hydroponic studies (Castro et al. 2003). Hence, the higher removal efficiencies observed in the warm season in comparison to those observed in the cold season may be associated with these processes. Nevertheless, biodegradation increase due the temperature rise in summer may play an important role, even higher than photodegradation or plant uptake.

Regarding to individual benzothiazoles behaviour, whereas Brownlee et al. (1992) reported that mercaptobenzothiazole (MBT) is photodegraded to OHBT and BT, De Wever et al. (2001) reported a high biodegradation of OHBT and BT in pilot-scale activated sludge systems. The fact that OHBT and BT concentrations increase throughout the SFCW during winter may be explained by the prevalence of photodegradation processes in winter in comparison to the prevalence of biodegradation processes in summer. In addition, the fact that MBT can undergo biomethylation to lead to the MTBT formation (Brownlee et al. 1992) and MTBT is recalcitrant to biodegradation (De Wever et al. 2001), biotransformation may explain the MTBT increases throughout the sewage sludge treatment of the León WWTP (Figure 1a).

The VFCW showed higher removal efficiency on average (80–90%) than the other treatment systems evaluated. The positive effects of plants on the benzothiazole and benzotriazole elimination may explain such behaviour. Owing to the subsurface flow of the VFCW system, photodegradation is not a relevant factor in this case. Moreover, the high oxygenation rate of these systems, which is relevant for the biodegradation of benzothiazoles, could also help to explain the observed trends (Catallo & Junk 2005). Hence, the VFCW showed higher removal
efficiencies for selected benzothiazoles and benzotriazoles than conventional WWTPs (activated sludge) and emerging technologies such as MBRs (Kloepfer et al. 2005; Weiss et al. 2006). Nevertheless, the high surface area requirement for constructed wetlands limits its applicability to small urban developments (<2,000 inhabitants).

In summary, because the low log Kow of benzothiazoles and benzotriazoles (Table 1) and the partial ionization of benzotriazoles (pKa = 7–8, (Hart et al. 2004)) at the wastewater pH of around 8, a moderately low retention into the organic matter should be expected. Hence, although benzotriazoles and benzothiazoles might be removed partially by sorption, the most important removal process observed in wastewater treatment systems might be attributable to other processes. In this regard, whereas in conventional WWTP (León WWTP) the removal of benzothiazoles and benzotriazoles is mostly by biodegradation processes, in constructed wetlands biodegradation, photodegradation and plant uptake coexist. Because of the high efficiency of the constructed wetlands in removing benzothiazoles and benzotriazoles, as it has been previously suggested by Castro et al. (2005), constructed wetlands could be a suitable alternative for treating waste aircraft de-icer fluids and highly impacted runoff and domestic or industrial wastewaters with high concentrations of benzothiazoles or benzotriazoles.

Mass emission rate of selected benzothiazoles and benzotriazoles into the receiving aquatic systems

Figure 2 shows the mass emission rate of the selected pollutants from three treatment systems to the Bernesga River, the Congost River and a small stream in the Trige municipality. The rates are shown in mg day$^{-1}$ population equivalent (PE)$^{-1}$. Mass emission rates ranged from 0.02 to 0.44 and from below detection limit to up to 2.1 mg d$^{-1}$ PE$^{-1}$ for benzothiazoles and benzotriazoles, respectively. In this regard, the Granollers WWTP owns the highest mass discharge rate, attributable to its high industrial contribution. The annual mass emission rates of benzothiazoles and benzotriazoles from the Granollers WWTP were 28 and 127 kg yr$^{-1}$, respectively. The annual mass emission rates of the same compounds from the León WWTP were remarkably lower at 20 and 70 kg yr$^{-1}$, respectively (note that the PE in the León WWTP is twice as high as in the Granollers WWTP).

Figure 2 | Amount of benzothiazoles and benzotriazoles discharged from the three WWTPs studied. 1 Granollers WWTP ($n = 8$); 2. León WWTP ($n = 10$) and Trige VFCW ($n = 4$). Box plot shows 5 and 95 percentile range. Asterisks show that values were below the LOD. PE (population equivalent).
From the three benzotriazoles studied in the Granollers WWTP, 4-TTri showed the highest emission rate followed by BTri, and then 5-TTri. In the other two treatment systems, which corresponded to a high domestic coverage, only BTri was emitted to the aquatic environment. Similarly, the OHBT and BT mass emissions rates were more than ten times higher than those of the MTBT in the WWTP affected by industrial wastewaters (Granollers WWTP). OHBT, BT and MTBT mass emission rates were similar in León WWTP and Trige VFCW, in which domestic wastewater inputs were predominant.

CONCLUSIONS

Benzothiazoles and benzotriazoles were identified and quantified in raw wastewater from three different locations in Spain and Denmark. Although BT, MTBT, OHBT and BTri were found in all raw sewage samples, 4 and 5-TTri were only detected in the industrially polluted raw wastewater system (Granollers WWTP). Constructed wetlands appeared to be the most suitable wastewater treatment system for the removal of these pollutants. Whereas in conventional WWTP the removal of benzothiazoles and benzotriazoles is mostly by biodegradation processes, in constructed wetlands biodegradation, photodegradation and plant uptake coexist. Nevertheless, constructed wetlands exhibited a strong seasonality and require higher surface area than conventional WWTPs.

Finally, mass emission rates of benzotriazoles and benzothiazoles from secondary effluents of three treatment systems were a particularly important source of pollution in the receiving waters, in which they reached values as high as 2.1 mg d\(^{-1}\) PE\(^{-1}\) for 4-TTri. In this regard, as these pollutants are polar and recalcitrant to biodegradation, surveys and monitoring programmes should include them in order to improve the understanding of their fate and effects in the aquatic biota.

REFERENCES


