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Occurrence and removal of endocrine disrupters in wastewater treatment plants for small communities

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ABSTRACT

The current study examined the fate of endocrine disrupting compounds in three small communities' wastewater treatment plants (WWTP), including stabilization ponds, trickling filters and activated sludge. The results showed that WWTP of small communities were affected by estrogenic and dioxin-like compound contamination ranging from 1.6 to 50 ng.L^{-1} estradiol equivalents and 5.3 to 73 ng.L^{-1} dioxin equivalents. The stabilization pond system seemed to be the most effective for estrogenicity removal, with 96% efficiency, compared to 51% for a trickling filter alone. Total removal of estrogenic compounds was increased when a stabilization pond system was used as a finishing treatment or when an additional physical treatment was conducted. Activated sludge treatment removed 75% of dioxin-like activity. Additional physical treatment had no impact on dioxin-like compound removal. Although the large contact area with air in maturation ponds represents a risk for air contamination of the water, maturation ponds seemed effective for dioxin-like compound removal. The efficiency of stabilization ponds as a finishing treatment system for the removal of estrogenic and dioxin-like compounds should be taken into account when selecting wastewater treatment systems for small communities.

Keywords: Activated sludge; Endocrine disrupter; Estrogenic; Dioxin-like; Stabilization ponds; Trickling filter

1. Introduction

There is increasing concern over the potential endocrine effects of xenobiotics present in the environment. Endocrine disrupting compounds (EDCs) can disrupt normal functioning of the endocrine system, causing degenerative effects such as reproductive and developmental abnormalities [1]. EDCs include natural and synthetic hormones, alkylphenols, some polycyclic aromatic hydrocarbons (PAH), some polychlorinated biphenyls, some organochlorine pesticides, and many more molecules. Domestic and industrial wastes are the primary sources of EDCs in surface water. These sources converge in wastewater treatment plants (WWTP) where they should be eliminated. Conventional WWTP efficiency parameters such as biological oxygen demand (BOD) and chemical oxygen demand (COD) do not take EDC elimination into account. In fact, EDCs are only partially eliminated during treatment, i.e. hormones, alkylphenols and PAH have been found in the effluents of different types of WWTP [2–6]. As EDCs represent a potential hazard for the fauna and human health, their presence and removal in waste water treatment plants is a major issue. Among EDCs, some compounds are able to bind to es-

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trogen receptors (ER) or dioxin receptor (AhR, aryl hydrocarbon receptor). We used in vitro bioassays to assess the presence and fate of estrogenic and dioxin-like pollutants in WWTP [7]. These bioassays enable the detection of compounds which bind to ER and AhR, giving a signal whose intensity is proportional to the quantity of estrogenic and dioxin-like compounds present in the samples. Even though the fate of EDCs during wastewater treatment has been studied in different treatment processes—basically activated sludge and trickling filters — very little information is available on their distribution or fate in small extensive treatment systems. Many factors such as treatment technologies, hydraulic retention time (HRT) or WWTP performance may influence the relative distribution, fate and treatability of these chemicals in municipal effluents. The current study examined EDCs in three WWTP of small communities, including stabilization ponds, trickling filters and activated sludge. The performance with respect to the removal of these substances from treatment systems was studied according to the WWTP characteristics. Water sampling covered the input, output and intermediary steps of the three systems. Total estrogenic and dioxin-like activities were determined in each sample via luminescent cell bioassays.

2. Materials and methods

2.1. Wastewater treatment plant features

Three wastewater treatment plants (WWTP) were chosen for this study to represent different treatment procedures. A general outline of the WWTPs is shown in Fig. 1. The three WWTPs are located in the south of France. The main characteristics of the sewage plant are reported in Table 1. Biological and chemical oxygen demand (BOD, COD) and suspended solids (SS) were analysed according to standard methods [8]. WWTP 1 consists of a series of different waste stabilization ponds. The first treatment unit is an anaerobic pond, with a residence time of 3.5 d followed by a step-fed facultative recirculation pond with a residence time of 28 d, and three maturation ponds with a residence time of 47 d. The performances and characteristics of WWTP 1 are given by Picot [9]. WWTP 2 consists of an Imhoff tank followed by a trickling filter and three tertiary ponds in series with a total hydraulic retention time (HRT) of 20 d. WWTP 3 is divided into two different treatment lines. The first consists of an activated sludge reactor (chain 1) whereas the second one consists of a chemical treatment (iron chloride) combined with activated sludge (chain 2).

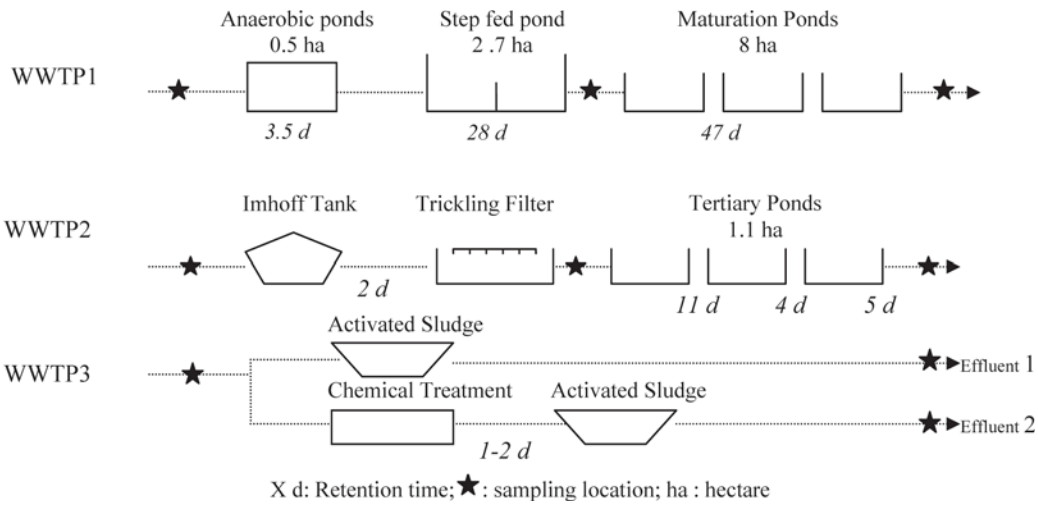

Fig. 1. Description of the three waste water treatment processes.

Table 1
Wastewater treatment plant characteristics

	Population equivalents (PE)	Flow ($m^3 \cdot d^{-1}$)	BOD load ($kg \cdot d^{-1}$)	COD influent ($mg \cdot L^{-1}$)	BOD influent ($mg \cdot L^{-1}$)	SS influent ($mg \cdot L^{-1}$)	HRT (d)
WWTP 1	14,600	2,718	787	690	310	381	78
WWTP 2	2,000	485	107	494	220	218	22
WWTP 3	87,000	15,910	4773	656	300	303	1–2

BOD: biological oxygen demand, COD: chemical oxygen demand, SS: suspended solids, HRT: hydraulic retention time

2.2. Sampling and analysis

24 h composite and grab samples from WWTP influents and effluents were collected on December 2006. Rainwater dilution was avoided since the three water sample collections were performed during dry weather periods. Water samples were stored in glass flasks at 4°C until extraction was performed within next 48 h. Wastewater (250–500 ml) was filtered with a Whatman GF/C filter within 3 h to minimize bacterial degradation. All solvents (ethyl acetate, methanol) used during extraction were either pesticide-grade or HPLC-grade. They were obtained from Carlo Erba Reactifs (Val de Reuil, France).

A C18 reversed-phase cartridge (1 g, 6 mL) provided by Alltech (Carquefou, France) was conditioned with 5 mL of methanol and 5 mL of HPLC-quality water before the sample was applied at a rate of 5 mL·min^{-1}. After solid-phase extraction, cartridges were rinsed with 5 mL of HPLC-quality water, dried under vacuum, wrapped in aluminium foil and stored at –20°C. After thawing, elution was performed with 10 mL of ethyl acetate:methanol (5:1). The ethyl acetate extract was filtered through anhydrous sodium sulfate on a glass microfiber filter and rotary evaporated to dryness at 37°C. Residues were taken up with 1 mL of methanol for bioassay.

2.3. Bioassay

Materials for cell culture were obtained from Life Technologies (Cergy-Pontoise, France). Luciferin (sodium salt) was purchased from Promega (Charbonnières, France). 17-β estradiol (E_2) and dioxin were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). The stably transfected luciferase reporter cell lines (MELN and HAhLP) were obtained as described by Pillon [7]. Basal MELN cell activity was around 15% of maximal activity. Basal HAhLP cell activity was around 20% of maximal activity. For the strain culture, MELN and HAhLP cell lines were grown in phenol red Dulbecco's Modified Eagle's Medium (DMEM F12), supplemented with 5% fetal calf serum (FCS) and 1% antibiotic (penicillin/streptomycin) in a 5% CO_2 humidified atmosphere at 37°C. Considering the phenol red and FCS estrogenic activity, in vitro experiments were conducted in phenol red–free medium supplemented with 5% dextran-coated charcoal (DCC) treated FCS (test culture medium). Cells were seeded at a density of 5 × 10^4 cells/well in 96-well white opaque tissue culture plates (Greiner Cellstar, D. Dutscher, Brumath, France) in 150 μL test culture medium. Water extracts to be tested were prepared 4× concentrated in the same medium and 50 μL was added per well 2 days after seeding. Cells were incubated with the samples for 8 h. At the end of incubation, effector containing medium was removed and replaced by 0.3 mM luciferin containing 5% DCC-FCS. At this concentration, luciferin diffuses into the cell and produces a luminescent signal that is stable for several hours. The 96-well plate was then introduced in a microplate luminometer (Microbeta, Wallac), and intact living cell luminescence was measured for 2 s. The results are expressed as a percentage of maximum luciferase activity. The maximum value, taken as 100%, was obtained in the presence of 10 nM E_2 and dioxin in MELN and HAhLP cell media, respectively. GraphPad Prism statistical software (version 4.0; GraphPad Software, San Diego, CA) was used to evaluate the 50% effective concentration (EC50). The EC50 of estradiol and dioxin are 17 pM and 0.2 nM, respectively. Estrogenic and dioxin-like activities noted at the study sites were expressed in estradiol and dioxin equivalents (E_2 Eq. and dioxin Eq.) per 1 L of water. When maximum activity was lower than 50%, E_2 Eq. and dioxin Eq. were not calculated.

3. Results and discussion

3.1. Estrogenic activity

All samples showed estrogenic activity in different ranges. Fig. 2 shows the dose response curves of samples collected from WWTP 3 which were obtained by testing the samples with the bioassay. Estradiol equivalents (E_2 Eq.) were calculated for all samples in order to determine the estrogenic activity removal efficiency throughout the treatment (Table 2).

Influents from all three WWTPs showed activity in the 50 E_2 Eq. ng.L^{-1} range. These levels were approximately the same as those found in raw sewage from Sweden [10] and France [11], but lower than those noted in Australia [12], which ranged from 108 to 356 ng.L^{-1}. The total estrogenic activity removal ranged from 59% for chain 1 at WWTP 3 (activated sludge only) to 96% for WWTP 1 with stabilization pond systems. In both WWTP 1 and 2, estrogenic removal was remarkably increased in the last treatment step in maturation ponds with a retention time of several weeks. In WWTP 1, only 50% of the estrogenic activity was eliminated by treat-

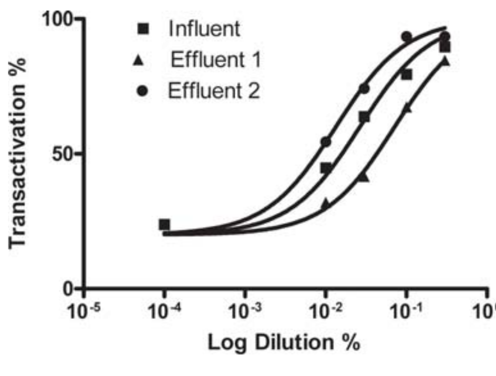

Fig. 2. Estrogenic activity dose-response curve for WWTP 3 (100% transactivation was obtained with 10 nM 17-β estradiol).

Table 2
Estradiol and dioxin-like equivalents and removal efficiency

	Sampling sites	E_2 Eq. ($ng \cdot L^{-1}$)	Estrogenicity removal efficiency	Dioxin Eq. ($ng \cdot L^{-1}$)	Dioxin-like removal efficiency
WWTP1	Influent	51.8		52.2	
	Intermediate	40.7	50%	34.3	34%
	Effluent	1.6	96%	5.3	89%
WWTP2	Influent	53.7		72.1	
	Intermediate	26.6	51%	<0.11	>99%
	Effluent	4.0	92%	13.7	81%
WWTP3	Influent	53.7		60.1	
	Effluent 1	21.6	59%	14.5	75%
	Effluent 2	5.2	90%	15.1	75%

ment in the anaerobic and step-fed recirculation ponds, while the maturation pond treatment increased the removal to 96%. In WWTP 2, primary Imhoff tank treatment and trickling filter removed 51% of the total estrogenic activity, which is comparable to previous findings [10,11] and far lower than the efficiency achieved in pond systems. Estrogenic activity removal in WWTP 2 increased from 51% to 92% with the maturation pond. The WWTP1 and 2 removal efficiency results underline the important role of this polishing treatment unit for removal of estrogenic compounds in small communities' wastewater treatment systems. Chemical treatment would also likely improve estrogenic activity removal. In fact, when effluents from chain 1 and 2 from WWTP 3 were compared, they showed that removal was 59 and 90%, respectively. Thus the presence of chemical treatment increases the treatment efficiency. The estrogenic compound removal efficiencies of the activated sludge unit were similar or lower than those reported in the literature [5,10,13].

3.2. Dioxin-like activity

The dioxin-like activity of the influent of the three plants was in the 60 dioxin Eq. ng.L^{-1} range for WWTP 1 and 2 (Table 2). The dioxin-like removal efficiencies were variable for WWTP 1, 2 and 3. WWTP3 showed a removal efficiency of 75% for both effluents, which seems to mean that chemical treatment did not significantly contribute to the removal of dioxin-like activity (Fig. 3).

For WWTP1, we observed a 34% decrease in dioxin-like activity after treatment in anaerobic and step-fed ponds. Maturation ponds increased the removal of dioxin-like compounds to 89%. In WWTP 2, we noted a marked decrease in dioxin-like compounds after the Imhoff tank and trickling filter treatments, i.e. dioxin-like activity was lower than the detection limits, thus highlighting substantial removal of dioxin-like compounds during the first part of the treatment. After the

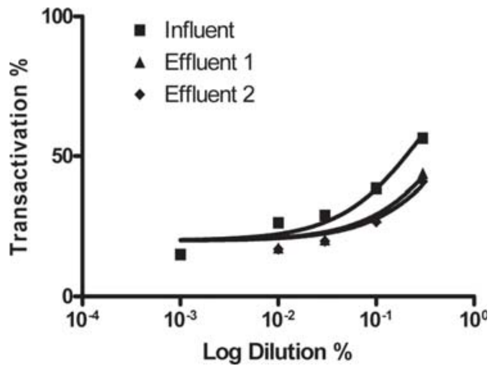

Fig. 3. Dioxin-like activity dose-response curve for WWTP 3 (100% transactivation was obtained with 10 nM dioxin).

maturation ponds, dioxin-like activity increased to 13.7 dioxin Eq. ng.L^{-1}. These results could indicate that dioxin-like components had been introduced in the maturation ponds. All were open air ponds, so there was a large contact area with the atmosphere. Dioxin-like pollutants are molecules such as polycyclic aromatic hydrocarbons (PAH) which can be airborne and could explain the increased dioxin-like activity in the WWTP 2 effluent. Surface water contamination by atmospheric PAH has been observed in France in urban surface water [14,15] and in marine surface microlayers [16]. Morevover, WWTP 2 is near a former oil refinery site and the soil supporting the refinery is currently being remediated and the excavations are suspected to contribute to PAH air pollution in the region. However, when considering only the influent, the total removal of dioxin-like compounds was 81% for WWTP 2.

4. Conclusions

In recent years, several studies have focused on the fate of estrogenic active compounds in the wastewater treatment process, but only a few include dioxin-like compounds as well. To our knowledge, no information about the elimination efficiency for these compounds in wastewater treatment plants for small communities has been reported, especially for pond systems. Stabilization ponds are particularly interesting for small communities as little maintenance is required for treatment, which is effective for the elimination of classical wastewater pollutants and estrogenic compounds. This study underlines the importance of polishing treatment for the elimination of estrogenic and dioxin-like pollutants.

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