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Endocrine disrupting activities in sewage effluent and river water determined by chemical analysis and in vitro assay in the context of granular activated carbon upgrade

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ABSTRACT

As part of endocrine disruption in catchments (EDCAT) programme, this work aims to assess the temporal and spatial variations of endocrine disrupting chemicals (EDCs) in River Ray, before and after the commissioning of a full-scale granular activated carbon (GAC) plant at a sewage treatment works (STW). Through spot and passive sampling from effluent and river sites, estrogenic and anti-androgenic activities were determined by chemical analysis and in vitro bio-assay. A correlation was found between chemical analyses of the most potent estrogens (estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2)) and yeast estrogen screen (YES) measurement, both showing clearly a reduction in estrogenic activity after the commissioning of the GAC plant at the STW. During the study period, the annual average concentrations of E1, E2 and EE2 had decreased from 3.5 ng L^{-1}, 3.1 ng L^{-1} and 0.5 ng L^{-1} to below their limit of detection (LOD), respectively, with a concentration reduction of at least 91%, 81% and 60%. Annual mean estrogenic activity measured by YES of spot samples varied from 1.9 ng L^{-1} to 0.4 ng L^{-1} E2 equivalent between 2006 and 2008 representing a 79% reduction. Similarly, anti-androgenic activity measured by yeast anti-androgen screen (anti-YAS) of spot samples was reduced from 148.8 to 22.4 μg flutamide L^{-1}, or by 85%. YES and anti-YAS values were related to each other, suggesting co-existence of both types of activities from chemical mixtures in environmental samples. The findings confirm the effectiveness of a full-scale GAC in removing both estrogenic and anti-androgenic activities from sewage effluent.

1. Introduction

The adverse effects of endocrine disrupting chemicals (EDCs) on aquatic organisms are well documented, and are a focal point of current environmental research worldwide. EDCs have been associated with various hormone-related cancers, e.g. testicular (Toppari et al., 1995) and breast cancer (Wormke et al., 2000), with subsequent implications for human health. Another concern is the potential for EDCs to cause reduced fertility to an organism, a phenomenon observed in fish, which may result in changes to their population structure and size, with implications at other trophic levels (Mills and Chichester, 2005; Sárria et al., 2011).

It is widely accepted that the most prominent source of EDCs into the aquatic environment is via sewage treatment works (STW) effluent (Snyder et al., 2007; Stasinakis et al., 2008; Zhang and Zhou, 2008; Zhou et al., 2009). This is because all humans excrete hormones, EDCs have been found in sewage effluents and adjacent surface waters (Ternes et al., 1999; Baronti et al., 2000; Ying et al., 2002), where fish appear the most affected. Secondly, secondary treatment such as activated sludge in STW has limited effectiveness in degrading EDCs, hence their residues are eventually discharged into rivers (Baronti et al., 2000; Zhang and Zhou, 2008). As a result, many advanced treatment technologies have been tested for their efficiency in further removing EDCs following secondary treatment, these including photodegradation (Zhang et al., 2007), chemical oxidation (Jiang et al., 2005), membrane filtration and activated carbon adsorption (Choi et al., 2005; Snyder et al., 2007). When evaluating a range of adsorbents for EDCs, GAC was found to be the most effective (Zhang and Zhou, 2005). Furthermore, GAC is effective for many other types of organic pollutants e.g. pharmaceuticals (Snyder et al., 2007), hence it is widely promoted as a standard tertiary treatment for EDCs and other emerging pollutants.

The activity of EDCs can be determined by biological techniques such as yeast estrogen screen (YES) and yeast androgen screen

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(YAS) (Matsumoto et al., 2004). In comparison to other in vitro bioassays, yeast-based systems have the advantage over those based on mammalian or fish cell lines in being less susceptible to non-sterile conditions, which can impair cell proliferation, and are therefore more suitable for complex environmental samples e.g. sewage effluent (Streck, 2009). In addition, chemical analysis by sensitive analytical techniques such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) is widely used to quantify EDCs in environmental samples (Céspedes et al., 2004; Liu et al., 2004; Grover et al., 2009).

Water sampling is typically conducted by spot sampling which is rapid and easy to conduct, but provides only an instantaneous measurement of EDC levels. An alternative approach is to use passive sampling devices e.g. polar organic chemical integrative sampler (POCIS) developed by Alvarez et al. (2004), allowing continuous monitoring of aqueous contaminants without using aquatic organisms (Kot et al., 2000; Vrana et al., 2001, 2005; Gorecki and Namiesnik, 2002). In POCIS a sorbent sandwiched between two microporous polyethersulfone (PES) membranes takes a composite sample from water, thereby enabling the chemical concentration to be estimated as follows (Alvarez et al., 2004; Vrana et al., 2006):

$$M_s = C_w R_s t \qquad (1)$$

where M_s is the mass of analytes in the sorbent at time t, C_w represents time-weighted average concentration in water during the deployment period, and R_s is the sampling rate of POCIS.

There is ample evidence to confirm the effects of EDCs on the reproductive health of aquatic organisms such as fish in controlled laboratory experiments. However, it is less certain whether wild fish populations are at significant risk (Mills and Chichester, 2005), as there is wide variation in the sensitivity of different fish species to EDCs. As a result, a research programme (EDCAT) was set up to investigate whether some UK fish populations are at risk from environmental estrogens. Specific objective of EDCAT was to investigate whether EDCs in the Rodbourne STW effluent were having effects on fish populations in the receiving River Ray. This work aimed to use chemical measurement and in vitro assay for estrogens and anti-estrogens, to support biological assessments and modelling conducted within the EDCAT programme (Balaam et al., 2010). Specific objectives included assessing the temporal and spatial variations of EDC concentrations in sewage effluent and river water, potential hotspots hence sources of EDC inputs, and complementarities between passive and spot sampling and between chemical analysis and bio-assay results. The benefit of a full-scale GAC in enhancing the removal of EDC residues from sewage effluent was examined.

2. Methods and methods

2.1. Materials

The solvents used including methanol and ethyl acetate were of distiled-in-glass grade from Rathburn Chemicals Ltd., Walkerburn, UK. Standards E1, E2 and EE2 and the internal standard E2-d_2 were purchased from Sigma, Dorset, UK. Separate stock solutions of individual compounds (1000 mg L^{-1}) were prepared in methanol, from which working standards at 10 mg L^{-1} were prepared by dilution. All the standard solutions were stored at −18 °C. Ultrapure water was supplied by a Maxima Unit from USF Elga, Marlow, UK.

The POCIS was based on a version described by Alvarez et al. (2004). Briefly POCIS devices were prepared by using membrane compression discs (54 mm i.d.) sandwiched between PES membrane (0.1 μm pore size) which had previously been conditioned with 20% methanol in water and twice with methanol. Then 300 mg of pre-cleaned strata-X sorbent (Phenomenex, UK) was added to each POCIS with an exposed membrane surface area of 46 cm^2.

2.2. The STW

The River Ray (Wiltshire) is the conduit for Rodbourne STW effluent, which serves 177 000 people and has a daily flow of 49 000 m^3. Influent was subject to preliminary, primary, biological and final treatment. Preliminary treatment involved screening, decoagulation and grit removal. The treated water was pumped to primary settlement tanks where settled sewage overflew to biological treatment aeration channels for nutrient removal. Effluent was then distributed to final settlement tanks for the removal of any remaining suspended solids.

To improve effluent quality and as part of the National EDC Demonstration Programme of the UK, a GAC plant was installed in 2008, to extend the existing conventional treatment at the STW. The GAC plant was fully operational in March 2008.

2.3. Sampling

To monitor temporal changes in EDC concentrations, both spot and passive sampling was conducted four times per year (spring, summer, autumn, winter) throughout the 3-year period. Spot samples in triplicate were taken at four sites on the River Ray and one site on the River Ock (in Oxfordshire) acting as a reference, during 2006–2008 (Fig. 1 and Table 1). Due to site restrictions from the water company concerned on health and safety grounds, influent

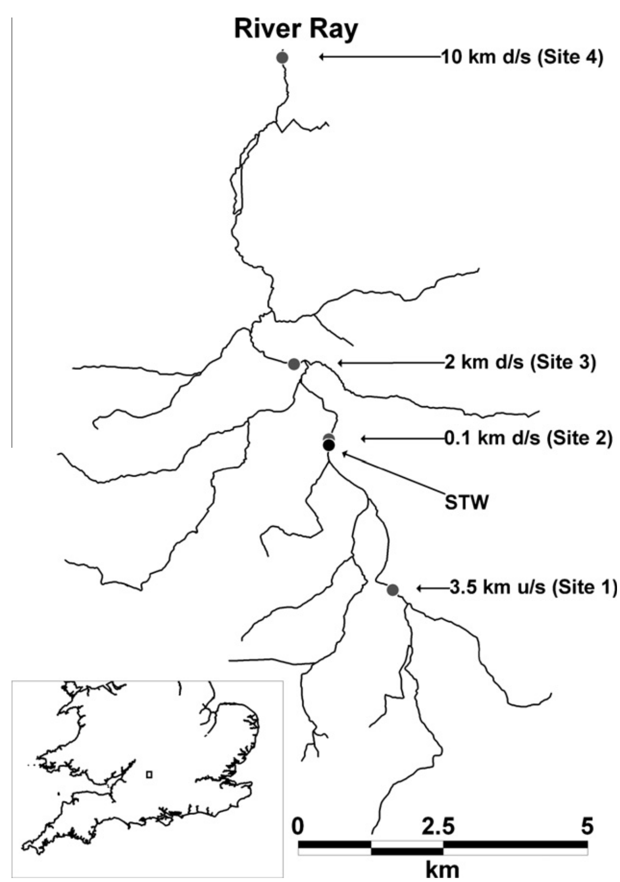

Fig. 1. A site map to show sampling locations on the River Ray indicating distances from the Rodbourne STW effluent (u/s: upstream, d/s: downstream). Site 5 as a reference in the river Ock is not shown.