MONITORING DIOXIN-LIKE AND ESTROGENIC ACTIVITY USING THE ERE- AND DRE-CALUX BIOASSAY: CASE-STUDY IN THE ZENNE RIVER CROSSING THE BRUSSELS REGION (BELGIUM)

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Introduction

Emerging pollutants (EPs) are a group of chemical substances that have been recently detected in fresh waters, as a result of human activities[1]. According to the NORMAN website these substances are "pollutants currently not included in routine monitoring programmes (at least at the EU level) but which could pose a significant risk requiring regulation, depending upon their potential (eco)toxicological and health effects and on their levels in the (aquatic) environment".

Among the various substances that can be categorized as EPs, endocrine disrupting chemicals (EDCs) are of special concern because these substances interfere with hormone biosynthesis, metabolism, or their action results in a deviation from normal homeostatic control or reproduction[2]. The group of molecules identified as EDCs is highly heterogeneous and includes synthetic chemicals used as industrial solvents/lubricants and their by-products, plastic/resin precursors, plasticizers, pesticides, pharmaceutical and personal care products and flame retardants. The peculiarity of EDCs is that these substances are extremely diverse and do not apparently share any structural similarity other than usually being of small molecular mass (<1000 Daltons), and often composed of fused aromatic rings that may contain halogen group substitutions by chlorine and bromine. This structural diversity makes that EDCs can interact with a large number of nuclear receptors as analogs or antagonists[2].

Many EDCs are still unregulated or are in process of being regulated. Therefore, water bodies currently contain thousands of chemicals about which there is little information regarding levels and occurrence of such chemicals[3]. They may or may not pose risks to the aquatic environment and human health, but in the absence of regulation they are not routinely monitored. Recently in January 2013, the European Commission (IP/12/88) proposed to add 15 chemicals to the list of 33 pollutants already regulated by the Environmental Quality Standards Directive (2008/105/EC). Pharmaceuticals are proposed for the first time and include amongst others the 17 α-ethinylestradiol (EE2) and the 17 β-estradiol (E2). Also found in this amendment are the dioxin-like compounds. Scientific questions that arise towards their impact on ecosystems and human health are justified by (i) their diffuse and continuous release into the aquatic environment, which despite generally low concentrations (ng/L to µg/L) confers to EDCs a pseudo-persistent character[4,5], and (ii) the fact that EDCs are chemicals with the potential to elicit negative effects on the endocrine systems of humans and wildlife at low dose[2,6].

In order to address these important environmental issues, the main objective of this study is to monitor the occurrence of EDCs in the Zenne, a previously polluted river crossing the Brussels region (Belgium). Although instrumental analysis can be used to identify and quantify known EDCs, hazard evaluation based on chemical monitoring is complicated. Since EDCs are structurally highly distinct compounds, mixture interactions have to be taken into account and many compounds responsible for estrogenic activity in sediment samples remain to be elucidated[7]. Dioxin responsive elements (DRE-) and estrogen responsive elements (ERE-) CALUX-assays (Chemical Activated Luciferase Gene Expression) were developed as mechanism-based, rapid and extremely sensitive in vitro reporter gene bioassays to assess dioxin-like and estrogenic activity[8,9]. They provide useful information about the total dioxin-like and estrogenic potential of complex mixtures of chemicals in environmental samples and are thus able to account for both unknown active compounds and mixture interactions in a sample. In this study, we have used both bioassays and focused on two categories of endocrine disrupters: (i) those interacting with the aryl hydrocarbon receptor (AhR) such as the halogenated aromatic hydrocarbons involving polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs), and (ii) those interacting with or activating the estrogen receptor (ER) called estrogenic EDCs (EEDCs) such as natural hormones, pharmaceutical estrogens, phytoestrogens, surfactants, as well as other industrial compounds like bisphenol A.

Materials and methods

Water samples were collected at the following sites: 3 within upstream Brussels, 1 site inside Brussels (at 39km from Lembeek) and 3 sites in the downstream region. Sediments were collected on 5 sites using a bottom sediment grab sampler. The samples were well mixed and aliquots were taken for physical, chemical and microbiological analyses.

For water samples, only the estrogenic activity was monitored as the ultimate fate of dioxin-like compounds in aquatic environments is their adsorption to sediment and suspended matter because of their high hydrophobicity. The sediments were dried, lyophilized, and a 2g aliquot was extracted using the Accelerated Solvent Extraction (ASE) with hexane/acetone, 1:1 (v/v). Water samples were extracted using the Oasis HLB cartridge for estrogen-like compounds.

Dioxins and dioxin-like PCBs were analyzed separately with the DRE-CALUX bioassay[10] and the overall estrogen activity is analyzed using the ERE-CALUX[11]. Briefly, extracts are diluted and dosed in 10 different concentrations yielding a full dose-response curve fitted with the "non-linear 4 parameter Hill function" to calculate an Effective Concentration on 50% of the curve (EC_{50}). The Bioanalytical Equivalent concentration (BEQ), in pg standard-equivalence/g or l sample, is calculated as the ratio of the EC_{50} of the standard and the sample curve.

Results and discussion

The main source of estrogenic active compounds in the aquatic environment is the result of their use by patients and the treatment of domestic and hospital waste in wastewater treatment plants[4,12,13]. This estrogenic activity includes both natural and synthetic estrogens such as xenoestrogens and pseudoestrogens. Figure 1a shows that the estrogenic activities, expressed as pg Bioanalytical Equivalent (BEQ) per L oscillate around 200 pg BEQ/L until km29 (inside Brussels) to increase sharply up to 938 pg BEQ/L at km39 (Vilvoorde, an industrial area). The activity then decreases to 397 pg BEQ/L at km41 (downstream Brussels).

While most of the literature data deals with the occurrence of estrogenic chemicals in water, these substances were also detected in sewage sludge and sediments[5,14,15]. In particular, steroids are relatively lipophilic; these molecules can be adsorbed on suspended solids and sediments where they can contaminate benthic organisms[16]. A peak of estrogenic activity in sediment of the Zenne river is found at km39 (Fig. 1b).

A totally different pattern is observed for the dioxin-like compounds. For both fractions (PCDD/Fs and dl-PCBs), results are in the range of what has been previously reported in the Scheldt mouth[17]. The Beersel station located at km13 shows a markedly higher PCDD/F potency compared to the other sampling stations, while the PCB potency tends to decrease from upstream to downstream Brussels (Fig. 1c).

To highlight relationships between the results of the CALUX tests and some environmental variables, we applied a principal component analysis (PCA). The PCA loading plot for the water samples (Fig. 2a) displays the relationships between 10 variables at the same time, visualised in a two dimensional plane defined by the first two principal components. The first component axis explains 68% of the variation and the second component axis 21%. The coordinates of the original variables in the plane express their correlations with the new principal components. Variables carrying similar information or varying in a comparable way are grouped together, i.e., they are correlated. When negatively correlated, they are positioned on opposite sides of the plot origin, in diagonally opposed quadrants. The further away a variable lies from the origin, the stronger the influence of that variable has on the PCA model. For example, T°C, COND, DOC & POC and to a lesser extent SPM & EEDCs are strongly correlated with PC1, whilst O_2 & pH are anti-correlated. In contrast, %C-SPM is mainly correlated with PC2 and d50-SPM is in an intermediate situation. Orthogonal vectors in the loading plot indicate low correlations between variables, and thus a low predictive ability.

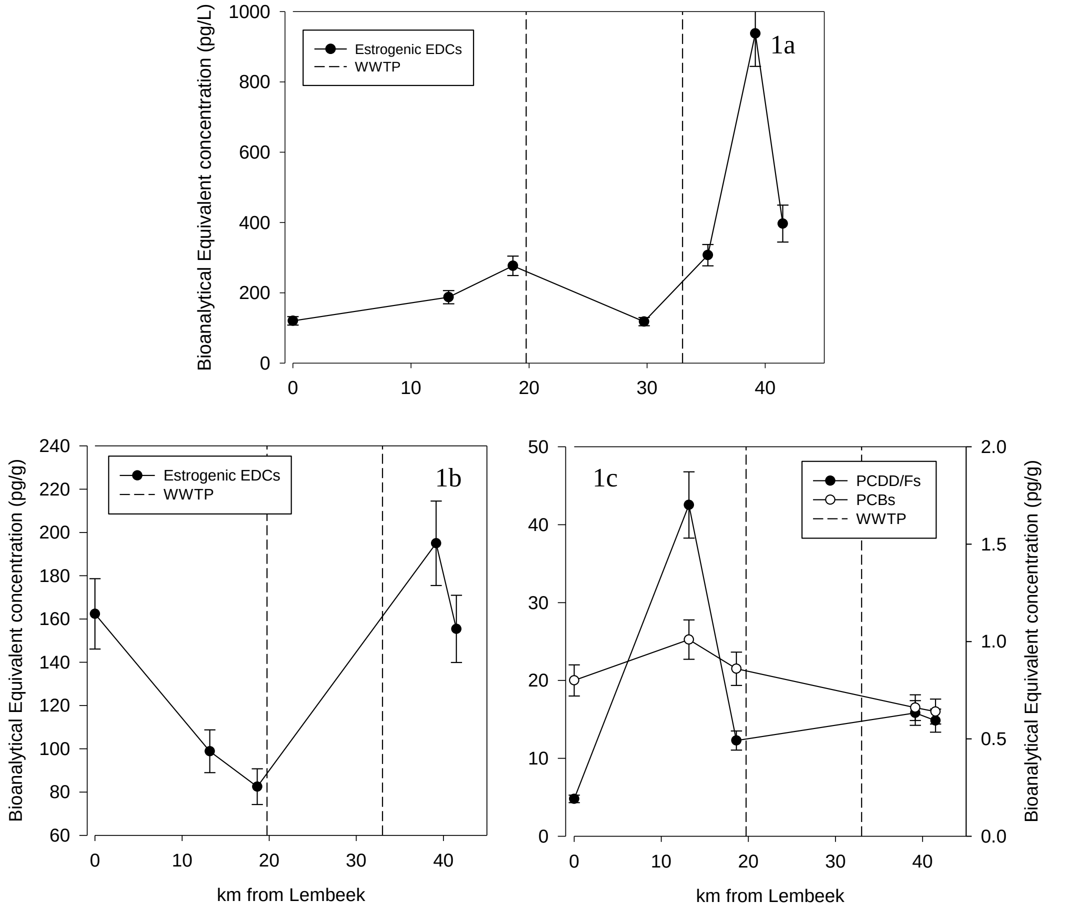

Figure 1: Bioanalytical Equivalent (BEQ) concentration for estrogenic EDCs (EEDCs) and dioxin like compounds in water (1a) and sediment (1b and 1c) of the Zenne (Belgium) in October 2012

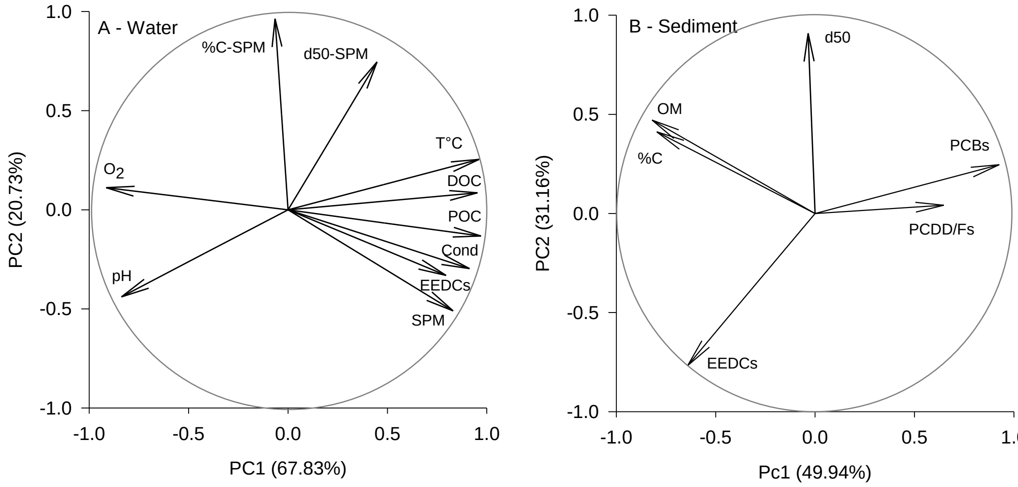

Figure 2: PCA loading plots obtained after Varimax rotation for the whole dataset. The factor loadings represent the correlations between the original variables and the principal components

For instance, it is observed that d50-SPM is not a powerful predictor for EEDCs in water samples; neither is %C-SPM. On the other hand, COND, SPM, POC & DOC are predictor variables of potential interest for EEDCs with Pearson's correlation coefficients ranging from 0.80 to 0.87, $p < 0.05$. It is not surprising that POC and DOC appear as controlling variables for EEDCs given the lipophilic nature of some of the investigated compounds.

For sediment, the PCA loading plot (Fig. 2b) indicates that dioxins like compounds (PCDD/Fs and DL-PCBs) and EEDCs behave differently. The vectors are located in opposite quadrant. None of these compounds exhibits significant correlation with the other environmental variables measured so far (OM, %C and d50).

Data on dioxin and EEDCs activities in sediment are very scarce. Most of the available BEQs are originating from sewage sludge analysis and have been established for the DRE-CALUX. In Belgium, values ranged from 10 to 132 pg BEQ /g dw with a median of 30 pg/g dw (15 pg/g dw in this study). Dl-PCB levels were much lower and ranged from 0.8 to 4 pg BEQ/g dw with a median of 1.8 pg BEQ/g dw (0.8 pg/g dw in this study). One sample reached 14 pg BEQ/g dw but was excluded from the dataset as a probable outlier[10].

An increase in estrogenic activity was noticed in both sediment and water samples at km39 (Vilvoorde, an industrial area), but further investigation is necessary to see if this site could be an active source of EEDCs. Based on these preliminary results we were able to conclude, for water samples, that COND, SPM, POC and DOC are powerful predictors for EEDCs and that PCDD/Fs and EEDCS behave differently in the sediments.

Acknowledgements
The BG1LUC4E2 and H1L7.5c1 cell lines were developed by the University of California-Davis (USA) with funding from a Superfund Research Program grant (ES04699) from the National Institute of Environmental Health Sciences.

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