

## PRESENCE OF COMPOUNDS WITH ENDOCRINE- DISRUPTIVE POTENTIAL IN WASTE WATERS AND THEIR ELIMINATION DURING WASTE WATER TREATMENT

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**Abstract:** *Wastewater treatment plants (WWTPs) receive a large spectrum of molecules from domestic, agricultural, and/or industrial wastes that are not totally eliminated during treatment processes. In addition to that, active metabolites can be formed during treatment processes. In this context, WWTP discharges are considered a major source of estrogenic water pollution. Our study was focused on assessment of composition and specific activities of complex mixtures in waste waters and the efficiency of their removal during the waste water treatment in a modern WWTP located downstream of the second largest city in the Czech Republic (Brno). Two types of passive samplers were exposed in influent and effluent of the WWTP in spring and autumn seasons and composite samples of waste water were collected monthly during whole year. Organic extracts of composite water samples and passive samplers have been tested by battery of in vitro bioassays. Cytotoxic compounds and chemicals able to induce aryl hydrocarbon receptor have been detected in all samples of waste water and two types of passive samplers (SPMD, POCIS) exposed in the WWTP influent and effluent. Estrogenic and androgenic activities have been observed predominantly in influent of the WWTP in POCIS and waste water samples. Compounds with cytotoxic, estrogenic and androgenic potency have been efficiently eliminated during waste water treatment in the WWTP.*

**Keywords:** waste water, anti/estrogenicity, anti/androgenicity, dioxin-like activity, passive sampling

## INTRODUCTION

Wide range of compounds of various origin, chemical structure and biological potency enter wastewater treatment plants (WWTPs). Thus, waste water may contain complex mixtures of anthropogenic contaminants, such pesticides, prescription and nonprescription drugs, personal care and common consumer products, industrial and domestic-use materials and degradation products of these compounds (Alvarez et al. 2005). Chemicals in effluents of WWTPs and receiving waters may affect aquatic organisms by modulating their physiological functions, e.g. disrupt endocrine processes, normal reproduction or developmental processes. Many alterations of the reproductive system observed in the aquatic environment are attributed to the presence of endocrine disruptors (EDs) (Sumpter 1998).

## METHODS

The WWTP Brno Modrice belongs to the most modern WWTPs in the Czech Republic and provides mechanical, biological and chemical treatment of municipal and industrial waste waters from the city of Brno and wide surroundings. Influent and effluent waste water was sampled using two types of passive samplers (SPMD for hydrophobic contaminants and POCIS for polar pesticides and pharmaceuticals) (Grabic et al. 2010) deployed for 21 – 28 days in spring and autumn seasons 2007 – 2008 (4 sampling campaigns). In addition to passive sampling, composite samples of waste water were collected monthly during whole year (May 2007 – April 2008) (Jedlickova et al. 2010).

Four reporter gene bioassays have been used to measure receptor-mediated activities of organic extracts of WW and passive samplers. Total steroid/dioxin-like activity was assessed in stably transfected cell lines containing a steroid/dioxin-like responsive element linked to a luciferase reporter gene. Hormonal effects were examined either singly or in co-exposure with competing endogenous ligand (17 $\beta$ -estradiol, dihydrotestosterone). Dioxin-like activity elicited via aryl hydrocarbon receptor (AhR) was determined with H4IIE-luc (rat hepatoma cell line) bioassay, the estrogen receptor (ER)-mediated activity in MVLN cells (human breast carcinoma) (Novak et al., 2009). Anti/androgenicity and glucocorticoid activity was assessed in a bioassay with MDA-kb2 cells (human breast carcinoma) and in recombinant *Saccharomyces cerevisiae* cells (Novak et al. 2009; Wilson et al. 2002). Another yeast strain served for assessment of cytotoxicity (Leskinen et al. 2005). Relative potency estimates (BIOTEQ, EEQ, AEQ), antiestrogenic, antiandrogenic and cytotoxic effects have been calculated according to Villeneuve et al. (2000) and Novak et al. (2009).

Organic extracts of passive samplers (SPMD, POCIS) were analysed for various organic chemicals including conventional organic pollutants as well as compounds of emerging concern (Grabic et al. 2010).

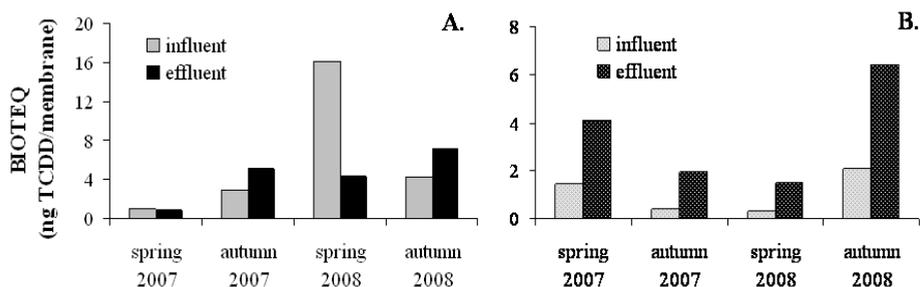
## RESULTS

Samples from both types of passive samplers caused cytotoxic effects in the yeast assay. Treatment processes in the WWTP decreased the cytotoxicity of waste waters in all samples except POCIS samples from autumn 2007. The treatment efficiency for cytotoxic compounds ranged from 73 to 91 % in SPMD and 47 – 78 % in POCIS samples. Some WW influent samples caused 20 % cytotoxicity even at 25times dilution, while effluent samples caused the effect only in real water concentration or were not cytotoxic.

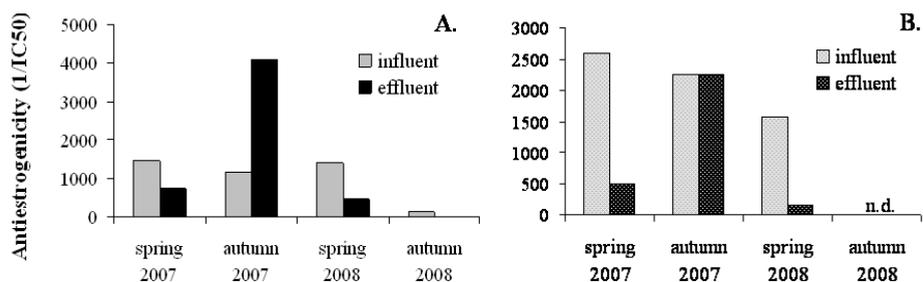
Passive samplers and WW extracts elicited significant AhR-mediated activity. Higher dioxin-like activity was observed in effluents than influents in all POCIS samples and SPMD from autumn seasons (fig. 1). The influent water samples mostly elicited greater dioxin-like activity than the effluent samples. Waste water samples documented 7 to 91 % removal efficiency of the WWTP for AhR-active compounds.

Estrogenic activity was detected only in waste waters and POCIS samples and it was decreased after treatment in the WWTP (80 – 99 %). Passive samplers exposed in the WWTP elicited antiestrogenic activity in most sampling seasons with variable treatment efficiency (fig. 2).

Only waste waters and POCIS samples (except spring 2007) exerted androgenic activity that was efficiently (> 96 %) reduced by treatment processes in the WWTP. All SPMD samples and influent POCIS samples showed antiandrogenic activity. No antiandrogenicity was detected in effluent POCIS samples and influent POCIS sample from spring 2007. SPMD samples elicited higher antiandrogenic activity in influents (except spring 2007).



**Fig. 1:** Dioxin-like activity of (A) SPMD and (B) POCIS samples expressed as TCDD equivalents (BIOTEQ)



**Fig. 2:** Antiestrogenicity of (A) SPMD and (B) POCIS samples (expressed as reciprocal value of IC<sub>50</sub> in membrane/ml)

Wide range of environmental contaminants has been detected in passive samples. Concentrations of some pollutants showed high seasonal variability. There were large differences in elimination efficiency of pollutants during the waste water treatment in the WWTP.

## DISCUSSION

Cytotoxicity, estrogenicity, androgenicity and most of dioxin-like activity in waste water samples have been efficiently decreased during WW treatment. However, extracts of passive samplers did not show decrease of AhR-mediated activity after treatment in most seasons. Higher specific activities in effluent samples than in influent can be caused by lower removal efficiency of contaminants, release of particle-bound pollutants during WW treatment or incomplete sampling of waste water.

Relatively high rate of removal of hormone-like activities, EEQs and AEQs of samples correspond with results reported in other studies. Results from passive samplers point to presence of antiestrogenic and antiandrogenic compounds in the mixtures.

## CONCLUSION

Combination of biological and chemical approach documented the presence of compounds with endocrine-disruptive potential in waste waters and their removal efficiency during the treatment in modern WWTP. The results of in vitro bioassays indicate that both hydrophobic and hydrophilic compounds contributed to the observed biological activities.

## ACKNOWLEDGEMENTS

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