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# Environmental risk assessment of Polish wastewater treatment plant activity

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## HIGHLIGHTS

- Bioassays prove to be efficient tool in EIA.
- Water treatment greatly affects water bodies receiving WWTP effluents.
- Treatment of wastewaters transforms their matrix interactions.

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## ABSTRACT

Wastewater treatment plants (WWTPs) play an extremely important role in shaping modern society's environmental well-being and awareness, however only well operated and supervised systems can be considered as environmentally sustainable. For this reason, an attempt was undertaken to assess the environmental burden posed by WWTPs in major Polish cities by collecting water samples prior to and just after wastewater release points. Both classical and biological methods (Microtox®, Ostracodtoxkit F™ and comet assay) were utilized to assess environmental impact of given WWTP. Interestingly, in some cases, water quality improvement indicated as a toxicity decrement toward one of the bio-indicating organisms makes water worse for others in the systems. This fact is particularly noticeable in case of Silesian cities where heavy industry and high population density is present. It proves that WWTP should undergo individual evaluation of pollutant removal efficiency and tuned to selectively remove pollutants of highest risk to surrounding regional ecosystems. Biotests again proved to be an extremely important tool to fully assess the impact of environmental stressors on water bodies receiving effluents from WWTPs.



## 1. Introduction

Biotests are conducted to prove the presence of environmental stressors' mixture, show their combined effect and holistic impact on the environmental compartments (Kapanen et al., 2013; Kudłak et al., 2014, 2015; Manusadzianas et al., 2003; Pessala et al., 2004; Tigini et al., 2011; Tsakovski et al., 2009). For this reason, bioassays can be conducted on unicellular and microcosm systems where organisms from different trophic levels are sensitive to different toxins (Szczepańska et al., 2016; Dubiella-Jackowska et al., 2010).

Another advantage of biotests is the possibility of detecting what is of great importance in view of the possible carcinogenic properties of environmental stressors: the endocrine and mutagenic potential of tested samples. Current knowledge in this field proves that toxicity may be the result of:

- the interactions of toxins with receptors,
- the breaking down of the molecular membrane,
- chemical reactions with cell elements,
- the inhibition of enzymatic activity (Cohen and Van Heyningen, 1982; Kudłak et al., 2015).

Bioassays constitute an important branch of analytics and gain more and more interest next to classical instrumental methods in conducting environmental impact assessments (EIA) (Kokkali and

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Van Delft, 2014). Due to increasing consumption of pharmaceuticals and also industrial/agricultural chemicals the impact on water bodies increases. Simple instrumental determination of stressor concentration levels will never give a full answer on the real threat posed by a vast number of substances reaching WWTPs (Frenzilli et al., 2009; Ohe et al., 2004; Gana et al., 2008). In the present state, one common approach is to link chemical concentrations to toxicity data. This is a typical univariate strategy which relies on traditional Quality Guidelines.

For these reasons, an effort has been undertaken to determine the possibility of utilizing biotests to assess efficiency of pollutant removal in the industrial and municipal waste water treatment plants of Poland and to determine the burden placed upon water bodies receiving theoretically treated waste waters. The selected battery of tests has been to respond to both acute and chronic toxicity at cellular and higher levels of biota organization.

## 2. Methodology

### 2.1. Instruments, chemicals and reagents

Chemicals used for Microtox® and Ostracodtoxkit F™ were purchased from ModernWater Ltd. and MicroBiotests, Inc., respectively. These included 2% NaCl solution, lyophilized *Vibrio fischeri*, Microtox Diulent, Microtox Acute Reagent, Osmotic Adjusting Solution, Reconstitution Solution, vials with algal food for chronic toxicity tests and matrix dissolving medium, spiruline, 6-well test plates, and certified dormant eggs of *Heterocypris incongruens*. Epithelial colon cancer cells HT-29 were obtained from American Type Culture Collection (Manassas, USA). McCoy's 5a (Modified) Medium, supplemented with 10% foetal bovine serum and antibiotic (1% penicillin-streptomycin), DMSO (CAS no. 67-68-5), $H_2O_2$ (CAS no. 21-67-63), $N_2$EDTA (CAS no. 6381-92-6), trypsin-EDTA solution, NaCl (CAS no. 7647-14-5), NaOH (CAS no. 1310-73-2), Trizma®-base (CAS no. 77-86-1), Trizma® hydrochloride (CAS no.1185-53-1), phosphate buffered saline (PBS), Triton™ X-100 (CAS no. 92046-34-9), low and normal melting points agarose, trypsin-EDTA and SYBR® GREEN I nucleic acid gel stain were purchased from Sigma-Aldrich (Germany). Sterile serological pipette (25, 10 and 5 ml), cell cultures bottles, UltraFine™ tips, coverslips, microscope slides, 10-ml syringes, sterile centrifuge tubes and filters were purchased from VWR (Poland). All reagents were of analytical grade purity or better, in the case of reagents for microbiological purposes. The instruments and equipment used during the study were: Microtox® 500 of Modern Water Ltd., electronic pipettes (Rainin, Eppendorf), analytical balance from Radwag (Poland), CP411 Metron pH-meter (Poland), and a binocular microscope from Ceti NV (Belgium).

### 2.2. . Sampling

Sewage water samples were collected in 2012–2013 from 76 WWTPs receiving effluents from major Polish cities, each time at 2 points for every WWTP: in the hydrologic course prior to inflow of wastes (PO), and from the water course after release of wastes from the WWTP (ZO). Water samples were collected in the largest Polish cities. Data on technological processes taking place in particular WWTPs were collected from annual reports of Voivodship Environmental Protection Inspectorates (Poland). Water samples were collected in glass bottles and stored at 4 °C prior to being transported to a laboratory, filtered with a Cronus 25 mm PES Sterile Syringe Filter (0.2 μm) and frozen.

### 2.3. Instrumental

Major ions ($Na^+$, $K^+$, $NH_4^+$, $Mg^{2+}$, $Ca^{2+}$, $F^-$, $Cl^-$, $Br^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$, $PO_4^{3-}$) were determined with a Dionex 3000i chromatograph (column: Ion Pac®AS22 (2 × 250 mm)); injection volume: 5 μL; suppressor: ASRS-300, 2 mm, mobile phase: 4.5 mM $CO_3^{2-}$, 1.4 mM $HCO_3^-$, flow rate: 0.38 ml/min, detection: conductivity, column: Ion Pac® CS14 (3 × 250 mm); suppressor: CSRS-300, 2 mm, mobile phase: 38 mM metasulfonic acid, flow rate: 0.36 ml/min, detection: conductivity (DIONEX, USA). Total organic carbon was measured with Shimadzu TOC-V CSH analyser. Metals (Na, K, Ca, Mg, Cu, Cd, Ni, Zn, Cr, Co, Fe) were determined with SensAA (GBC, Poland). All instrumental analyses were conducted with standard calibration curve methods.

### 2.4. Biotests

#### 2.4.1. Microtox®

The Microtox® biotest utilizes *Vibrio fischeri* bacteria and their ability to bioluminescence. Acute toxicity was assessed by determining inhibition of the luminescence of the marine Gram-negative bacterium *Vibrio fischeri* (Leusch et al., 2014; Weltens et al., 2014), after a 30-min exposure to different samples. The bacteria were purchased in freeze-dried form and activated by rehydration with a reconstitution solution (specially prepared nontoxic Ultra-Pure Water) to provide a ready to-use suspension of organisms. The light emission of this bacterium in contact with different samples and exposure times was measured using the Microtox 500 analyser and bioluminescence inhibition was calculated and utilized as an endpoint for chemometric studies. The data were processed using the Microtox Omni Software, according to the Basic Test Protocol (81.9%). The design of the procedure is presented in Supplementary Figure A. Chromium sulphate was used as a positive control of the test.

#### 2.4.2. Ostracodtoxkit F™

Ostracodtoxkit F™ is the best known and first biotest for direct contact of crustaceans with freshwater and brackish samples. Unlike bacteria, ostracods have a fully developed gastrointestinal tract, through which toxic substances can enter an organism (easily bioavailable pollutants can also enter via body shells and gills). An Ostracodtoxkit F™ toxkit containing vials with *Heterocypris incongruens* cysts, vials with spiruline and algae, reference sediment and dissolving medium were purchased from MicroBioTests, Inc. (Belgium). An optical microscope was used to assess the number of living organisms and for measurements of the length of the organisms according to the procedure presented in Supplementary Figure B. (Kudłak et al., 2011). Growth inhibition and mortality (according to the producer's and ISO 14371:2012 guidelines) were considered as endpoints for chemometric studies. Control organism growth of 400 μm and mortality of <20% are considered positive indicators for a test.

#### 2.4.3. Comet assay

Epithelial colon cancer cells (HT-29, obtained from American Type Culture Collection, Manassas, USA) were grown in a monolayer culture at 37 °C in a humidified atmosphere of 5% $CO_2$ in McCoy's 5a (Modified) Medium, supplemented with 10% foetal bovine serum and antibiotic (1% penicillin-streptomycin) in a culture flask. The medium was changed twice a week. Single cell suspensions were prepared with a trypsin-EDTA solution (diluted 10 times) and finally re-suspended in McCoy's 5a (Modified) Medium, supplemented with serum and antibiotics.