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PhD Thesis

Ecotoxicological and chemical assay of municipal  
wastewater

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## **INTRODUCTION**

Whole effluent toxicity (WET) testing poses a great challenge in environmental protection, due to the wide variety of possible contaminants and the dynamic nature of wastewaters. Monitoring requires a reliable, sensitive and cost-effective test. The assay based on the bioluminescence inhibition of *Vibrio fischeri* fulfils these requirements and is probably the most widely applied bacterial test in WET assessment.

## **AIMS**

The aim of this study are to

- review the literature on the research topic and collect data in order to process the test results;
- examine the cleaning efficiency of a regional wastewater treatment plant through the ecotoxicological nature of the wastewater;
- to detect the changes in the ecotoxicological properties of the wastewater purifier using chemical and analytical examinations;

- analyze the effects of abiotic ecological factors on toxicity and analytical results;
- compare two *Vibrio fischeri* test devices.

## **MATERIALS AND METHODS**

### **Sampling procedure**

The samples were taken on ten occasions from 06.2012 to 02.2013. from raw wastewater, sedimented water, biological treated water and effluent water. Samples were used immediately (within 24 hours) for toxicity measurements, whereas they stored in a freezer at  $-30^{\circ}\text{C}$  for elemental analysis. Before each sampling we recorded the average temperature and the amount of rainfall during the last 24 hours. The other abiotic parameters (rainfall, temperature) based on the data of the National Meteorological Service. Samples were taken at almost the same time for each sampling points, so their direct comparison is not possible. Sewage treatment is a dynamic process. The influent wastewater spends a couple of hours in the primary clarifier, a couple of days

and in the biological reactor, then the effluent flows into the Perint rivulet. With samples taken at different meteorological conditions, the wastewater cleaning efficiency can be analyzed.

### **Toxicity bioassays**

The samples were tested before (raw) and after filtering. For ecotoxicity testing, assays were performed as described by the Microtox<sup>®</sup> 100 Operating Manual (in compliance with ISO 11348-3 protocol) and kinetic protocol was performed by the Ascent Luminometer Operating Manual (in compliance with ISO 21338:2010).

### **Chemical measurements**

The determination of the metal content of MSZ 1484-3: 2006 standard were based on. The preparation of samples were done by Mars microwave digestion apparatus, the element analysis was measured by the Spectro Genesis ICP-OES instrument. All standards and reagents were of analytical grade (highest purity available), suitable for

elemental analysis. 40 samples were measured for dissolved and total metal content. For the calculation of the total metal content the sample was disrupted without filtration using chemicals from the standard specification. For the calculation of the dissolved metal content the samples were filtered before quantification using a 0,45 $\mu$ m long syringe filter. For the measurements ultra pure water (Zeener Power I. Water Purification System) were used.

For the qualitative analysis of volatile organic pollutants, I have selected a coherent series from three different occasions. For the analysis, the Shimadzu GC-MS QP 2010 gas chromatograph mass spectrometer was used.

Chemical oxygen demand (COD) and 5 days of biological oxygen demand (BOD) were received from the WWTP laboratory.

### **Statistical analysis**

The statistical analysis was implemented using Past 2.17c and Excel 2010 with the Analysis Toolpak extension.

## **RESULTS**

Based on the publications, the effective concentration values are not reached by dissolved metal concentrations in the wastewater samples. Most of the not dissolved metal forms were biologically inactive.

The plant works with good cleaning efficiency with respect to volatile organic pollutants. In the effluent sample, the concentration of volatile organic compounds did not reach the effective concentration of the *Vibrio fischeri* test.

Based on the data from the Microtox Luminometer, the cleaning efficiency of the plant is satisfactory. Microtox values are well correlated with the quantitative data of volatile organic pollutants and the aggregate organic pollutants (BOI<sub>5</sub> and KOI<sub>d</sub>). The conventional protocol (ISO 11348-3 standard) has shown appropriate sensitivity

to organic pollutants. Therefore, based on our research, the conventional protocol (ISO 11348-3: 2010) is more appropriate to evaluate the toxicity of wastewater samples.

## **THESES**

- In the examined wastewater treatment system, with the usage of ecotoxicological methods, no direct correlation was found between the total metal concentration and the toxicity. Most of the not dissolved metal forms were biologically inactive. The amount of dissolved metals was below the effective concentration of the test organism.
- Significant correlation was found between the volatile organic impurities of wastewater samples and the amount of oxidisable organic pollutants and Microtox EC50 values. In the wastewater samples containing complex pollutants, the test organism was sensitive to organic compounds.



- The wastewater treatment plant had good cleaning efficiency with respect to the degradation of volatile organic pollutants. Alcohol ethoxylates and its metabolites also had 100% removal efficiency, even under extreme weather conditions.
- At the Szombathely regional wastewater plant, the dilution effect of the rainfall can be demonstrated well on the data measured on Microtox, concentrations of all metal content, and volatile organic pollutants. Rainfall did not reduce the proper cleaning efficiency of the plant.
- The cleaning efficiency of the wastewater treatment plant is sufficient, based on the data measured by the Microtox Luminometer. From the influent to the effluent, the level of toxicity is decreasing, even under extreme weather conditions.
- The toxicity measured on the Ascent luminometer with the kinetic method was compared with the conventional Microtox method for samples taken at the Szombathely regional wastewater plant. The conventional protocol showed better performance based on a strong correlation of ecotoxicity with

organic pollutants and a dynamic pattern of meteorological conditions. For these reasons, conventional protocol (ISO 11348-3: 2010) should be should be applied.

## **PRACTICAL APPLICABILITY OF THE RESULTS**

In the study, the cleaning efficiency of a regional wastewater treatment plant was analyzed through changes in ecotoxicological properties with parallel chemistry and analytical tests. The monitoring period included both the summer, autumn and winter periods. Based on this, it can be stated that the plant operates with good cleaning efficiency even in extreme weather conditions. Total heavy metal content is well below the emission limit value in the effluent samples. Volatile organic pollutants are almost completely decomposed during the purification process. Alcohol ethoxylates were completely decomposed in all three investigated periods. In specialized journals, the analyzed compound was removed with 96-99% efficiency. From the measurements, it can be concluded that the

ecotoxicological examination using the Microtox luminometer with the traditional method has a strong correlation with the amount of organic compounds measured and it also represents the meteorological conditions well. Therefore, it is clearly recommended to evaluate and monitor the toxicity of the wastewater samples. It would be advisable to further widen the research towards the full detection of organic pollutants, as there was a clear connection with toxicity.

## **PUBLICATIONS**

### **Publications in reviews and conference proceedings:**

**Farsang Á.**, Kováts N., Molnár P., Horváth B., Rétfalvi T., Béres Cs. (2017): Comparison of two *Vibrio fischeri* bioluminescence inhibition bioassays for monitoring toxicity at a wastewater treatment plant. *Applied Ecology and Environmental Research* (proofreading is in progress).

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**Farsang Á.**, Béres Cs., Ács A., Kováts N., Rétfalvi T. (2013): Abiotikus tényezők hatása egy kommunális szennyvíztisztító ökotoxikológiai paramétereire. In: Mesterházy Beáta (szerk.): XII. Természet-, Műszaki- és Gazdaságtudományok alkalmazása Nemzetközi Konferencia (2013.05.11.) Konferencia kötet.

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**Farsang Á.,** Béres Cs., Ács A., Kováts N., Rétfalvi T. (2013): Nehézfémek ökotoxikológiai hatása a kommunális szennyvízben. In: Mesterházy Beáta (szerk.): XII. Természet-, Műszaki- és Gazdaságtudományok alkalmazása Nemzetközi Konferencia (2013.05.11.) Konferencia kötet. Szombathely: Nyugat-magyarországi Egyetem. pp 120-126.

### **Presentations:**

**Farsang Á.,** Béres Cs., Ács A., Kováts N., Rétfalvi T. (2013): Egy kommunális szennyvíztelep tisztítási hatásfokának elemzése *Vibrio fischeri* tesztorganizmussal. Fiatal kutatók konferenciája Sopron 2013.08.30. pp.117-123.

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**Farsang Á.,** Béres, Ács, Kováts N., Rétfalvi (2013): Impact of abiotic factors on the ecotoxicological parameters of a communal wastewater treatment plant. In: Hatvani Zsolt (szerk.): 10th János Szentágotthai

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