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Research Article

Monitoring Wastewater Treatment Plant Toxicity using Two *Vibrio fischeri* Bioluminescene Inhibition Bioassays

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Abstract

Aim and Background: The assay based on the bioluminescence inhibition of *Vibrio fischeri* is probably the most widely applied bacterial test in Whole Effluent Toxicity (WET) assessment. Most commercial systems apply the ISO 11348 standard. As it has been recognized that this protocol might overestimate the toxicity of turbid and/or coloured samples, a kinetic version was developed and standardized. This kinetic protocol has been mostly applied on solid samples such as soil or sediment. As such, the main aim of the study was to compare the performance of the kinetic protocol in comparison to the conventional one in case of whole effluent samples. The study was also targeted to assess the operation of a municipal wastewater treatment plant.

Materials and Methods: Whole effluent samples were collected at the municipal wastewater treatment plant of Szombathely and were tested with the Microtox® 100 and the Ascent Luminometers according to the respective standard protocols (filtered samples for Microtox, ISO 11348-3 protocol; raw samples for Ascent, kinetic ISO 21338:2010 protocol).

Results: In general, the Microtox system detected lower toxicity in comparison to the kinetic protocol. Microtox readings, however, showed better correlation with analytically derived parameters such as COD, indicating the proper sensitivity of the system to organic pollutants.

Conclusions: The Microtox protocol showed better sensitivity based on correlation of ecotoxicity with organic pollutants and the clear dynamic pattern which reflected meteorological conditions. However, this protocol might underestimate the toxicity in case of highly turbid samples.

Keywords: Ascent; Ecotoxicity; Microtox; *Vibrio fischeri*; Wastewater

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* fulfills these requirements and is probably the most widely applied bacterial test in WET assessment (Principles and new developments are reviewed by Ma et al. [1], commercially available test systems are reviewed by Kokkali and van Delft [2]). *V. fischeri* is considered 'The Most Sewage-Sensitive Organism' [3].

For water and wastewater samples, the ISO 11348 standard (Water Quality-Determination of the Inhibitory Effect of Water Samples on The Light Emission of *Vibrio fischeri* /Luminescent bacteria test/) applies. The Microtox system, which follows this

standard, has been the most thoroughly investigated tool for wastewater toxicity evaluation (reviewed by Ren, [4]). Dalzell et al. [5] measured the toxicity of heavy metals, organic pollutions and industrial wastewater using Microtox®. The most sensitive bioassay was *V. fischeri*. analyzed samples of wastewater for ecotoxicological and physicochemical parameters. The Microtox test proved to be the most sensitive and showed good correlation with organic pollutions. (BOD_5/COD). However, it has been recognized that this protocol might overestimate the toxicity of turbid and/or coloured samples. Due to physical effects (The so-Called Tyndall-Effect), these factors might reduce the light output recorded by the luminometer [6]. Though the ISO 11348-3 prescribes that samples should be filtered if turbid, toxicity assessment of filtered samples might give a false estimation if the toxic effect depends mostly on particle-bound compounds [7].

Lappalainen et al. [8] presented a kinetic protocol (Referred to as 'Flash') for testing the toxicity of solid and/or colored samples, also available as an ISO standard (ISO 21338:2010: Water quality - Kinetic determination of the inhibitory effects of sediment, other solids and colored samples on the light emission of *Vibrio fischeri* / kinetic luminescent bacteria test/). As the light output in the sample is assessed independently from the control, disturbing effect of colour and/or suspended particles can be reduced.

The Flash method, though most often applied for assessing toxicity of solid samples such as contaminated soils or sediments (e.g. [9-12], or sewage sludge [13] has also gained recognition in wastewater toxicity assessment [14]. Masner et al. [15] designed a portable luminometer for effluent toxicity assessment using the kinetic protocol. Heinlaan et al. [16] analyzed the toxicity of nanosized bulk ZnO, CuO and TiO_2 using Flash Assay. The test was relatively sensitive compared to other bioassay tests.

However, very few studies have been conducted to compare the two different *V. fischeri* test protocols (conventional and kinetic ones) [17]. As such, the main aim of the study was to make a comparative assessment of the performance of the two protocols in WET testing, based on sensitivity and correlation with other, analytically derived parameters. The study was designated to find out if turbidity and/or color of municipal wastewater really cause virtual toxicity reading in case of the ISO 11348-3 protocol, and also to assess how filtering affects toxicity in case of Microtox measurements.

Materials and Methods

Research was performed at the regional municipal Wastewater Treatment Plant (WWTP) in Szombathely. The WWTP was designed as 225.000 population equivalent. The wastewater and the rainwater arrives from a combined system drainage from the downtown area, while the recently built parts already use a separated system. The peak plan capacity is 1500 m^3/h with an average daily flow rate of 19600 m^3/day. The wastewater treatment follows the traditional steps (Figure 1): pre-treatment with mechanical removal, primary treatment to remove suspended solids, secondary treatment (biological treatment) by activated sludge process which performs carbon, nitrogen and phosphorus removal. After the secondary clarifiers the effluent flows into the Perint rivulet. The samples were taken (Figure 1) on nine occasions between 06.2012 and 12.2012. from (1.) raw wastewater, (2.) sedimented water from primary clarifiers, (3.) biological treated water from aerobic reactor and (4.) effluent water.

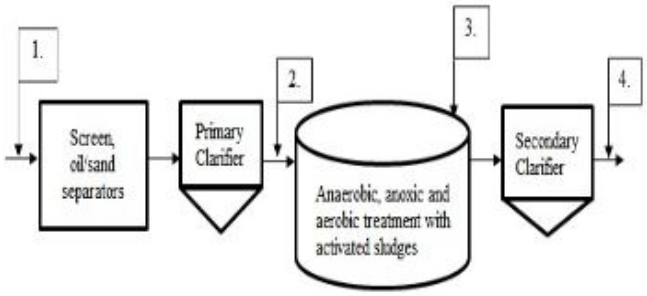

Figure 1: Schematic diagram of the treatment processes at Szombathely WWTP and sampling points.

Samples were used immediately (within 24 hours) for toxicity measurements, whereas they were stored in a freezer at -30°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) were based on the data of the National Meteorological Service (Table 1). Samples were taken at almost the same time for each sampling points, so their direct comparison was not possible, because sewage treatment is a dynamic process. The influent wastewater spends a couple of hours in the primary clarifier, a couple of days in the biological reactor, and then the effluent flows into the Perint rivulet. With samples taken at different meteorological conditions, the wastewater cleaning efficiency can be analyzed.

Date of sampling	Average temperature (°C)	Wet (mm)
25.06.2012.	18	0
10.07.2012.	24,5	3
25.07.2012.	22	24
22.08.2012.	26	0
09.10.2012.	14	2
25.10.2012.	9,5	0
06.11.2012.	6	43
07.12.2012.	-5	0
10.12.2012.	-4	0

Table 1: Temperature and rainfall data.

Toxicity bioassays

For ecotoxicity testing, assays were performed as described by the Microtox® 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). Hereinafter, all results are either referred to as 'Microtox Toxicity' or 'Microtox EC50' or 'Ascent Toxicity' or 'Ascent EC50'.

Chemical measurements

The pH was measured on an Orion™ 3-Star Plus Benchtop pH Meter. Determination of the metal content was performed according to MSZ 1484-3: 2006 standard were based on (Testing of waters. Part 3: Determination of dissolved, suspended and total metals in water by AAS and ICP OES) The preparation of the samples a Mars microwave digestion apparatuswas used. The element analysis was performed on a 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 0,45µm syringe filter. For the measurements ultra pure water (Zeener Power I. Water Purification System) were 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. The correlation of several datas was examined by calculating the Pearson and Spearman correlation coefficients.

Results and Discussion

Toxicity of samples

Toxicity was measured on 36 samples collected at 9 occasions (4 samples each, between 25.06.2012 and 10.12.2012) with the Microtox device filtered (per protocol) and with the Ascent Luminometer unfiltered (Figure 2). It should be noted that high EC50 values mean low toxicity. For the Microtox luminometer the highest measurable effect (Reduced Light Emittance) was below 15% for the 50% diluted samples in the effluent. If the software could not determine EC50 value the next measurement started with 80% dilution in the case of the filtered samples.

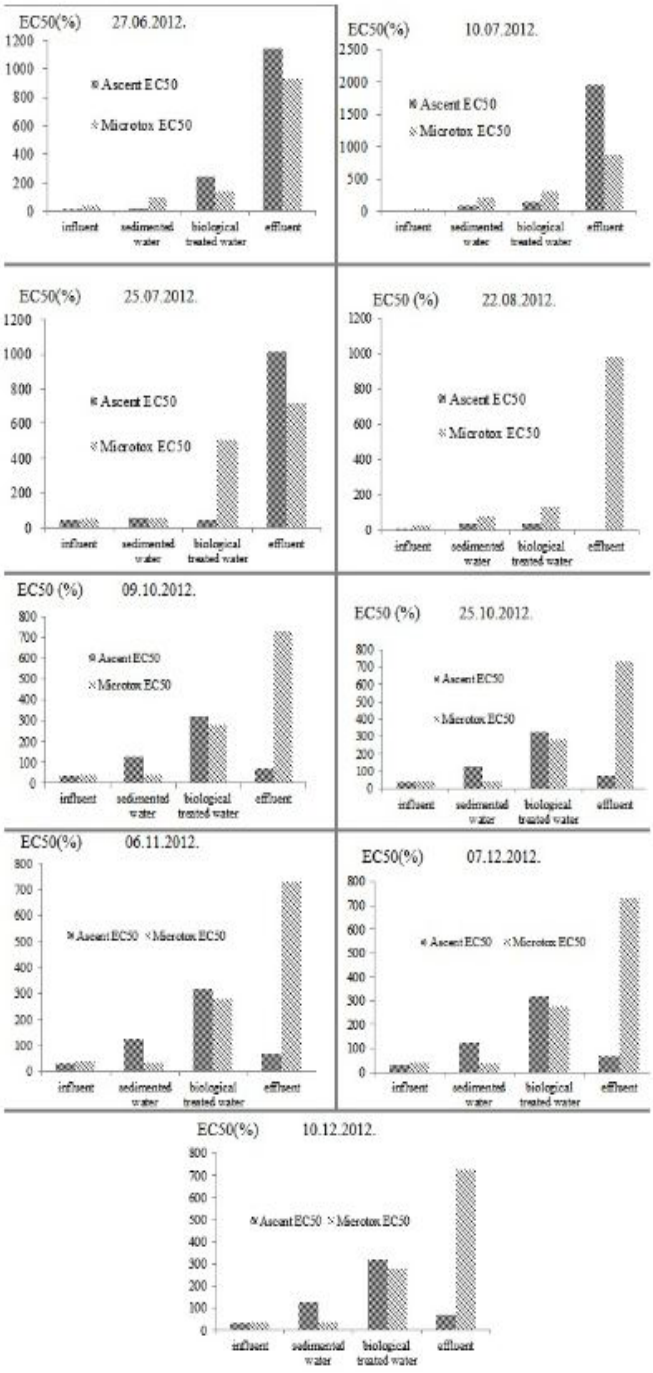

Figure 2: Toxicity of the wastewater samples (06.2012 - 12.2012.) .

During the summer sampling (27.06.2012 - 22.08.2012.) Ascent and Microtox EC50 data showed similar patterns: toxicity continuously decreased from the influent to the effluent. The Ascent

Luminometer showed the influents more toxic than Microtox. The Ascent EC50 values were generally lower (Meaning More Toxic) in the samples from the primary clarifier and biological reactor. None of the devices found the effluent to be toxic.

For autumn and winter samples (09.10.2012-10.12.2012.) the Ascent and the Microtox luminometers differed in estimating purification efficiency of the plant. Measurements performed with the Microtox showed that toxicity decreased again throughout the treatment process in the plant, from the influent to the effluent. The effluent was not toxic. The Ascent Luminometer found the effluent toxic each time. We used analytical measurements to find an answer for this variability of toxicity. Wastewater is a complex chemical and biological system with regard to its compisition. Inorganic (Heavy Metals, etc.) and organic pollutants (Derivatives Of Drugs, Cosmetics, etc.) both play an important role in its toxicity.

Toxic metals in the waste water

Many researchers investigated the sensitivity of *V. fischeri* bacteria to heavy metals. Compared with other test organisms the bacteria were found to be less sensitive [18-20]. Guéguen et al. [21] examined the toxicity of samples taken from a river contaminated with heavy metals using *V. fischeri* and also an algae test (*Pseudokirchneriella subcapitata*). The dissolved, colloidal and total metal content of the samples was also compared to the toxicity data. The algae test was found to be more sensitive to metallic contamination of the samples. While this might be explained by their high metal concentrations, no correlation between metal concentrations and toxicity was observed, suggesting that metal speciation in the truly dissolved fraction may be more important than the absolute metal concentration. The combined presence of metals and organic pollutants in the samples further modifies the toxic effect.

According to Microtox's conventional protocol, the sample had been filtered through a 0.45 μm filter prior to measurement. Therefore, dissolved metal concentrations were compared to the toxicity readings of the Microtox luminometer on the 36 samples. No correlation was found between dissolved metal concentrations and the complete data set or the data sorted per objects. Concentrations of dissolved metals in the influent varied between 9.1 and 218 μg / l. In the effluent the median concentrations of the detected metals decreased slightly, ranging from 0 to 87 μg / l. According to the literature, the measured metal concentrations were way below the effective concentrations of the *V. fischeri* test [18,22-24]. Thus, it can be assumed that the role of dissolved metals in the toxicity of the samples was negligible.

Total metal content, in addition to the dissolved metal forms, includes flakes in the suspended state and the biologically inactive non-free forms. The kinetic procedure enables the measurement of the sample's toxicity without direct filtration. Therefore, total metal concentrations of the 36 samples were compared to the toxicity EC50 values measured on the Ascent Luminometer. No correlation was found between total metal content and toxicity in the complete data set or the data sorted per objects. In the samples from the biological reactor the amount of active sludge was high, and total metal concentration has also increased considerably. The incoming heavy metals are concentrated in the sludge during wastewater treatment, similarly to toxic organic materials. Metals in wastewater are incorporated into biomass through biological processes (Biosorption, Bio-accumulation) [25]. By co-precipitation, in oxidative environments, heavy metals are adsorbed and deposited to precipitating iron hydroxide flakes [26]. There is no accurate information on the proportion of the biologically inactive and suspended parts in the undissolved metal fraction. However, it can be assumed based on metal concentrations and toxicity data that the biologically bound (Inactive) form is in a higher proportion in the samples, because the toxicity of suspended metals should have been significant [16,23]. Total metal concentrations were decreasing from the influent to the effluent. The amount of aluminum, zinc and lead decreased the most, since they had the highest median concentrations in the influent (1183 μg / l, 320 μg / l, 326 μg / l). Total metal concentrations of the effluent water fulfilled the strictest requirements of the corresponding regulations (Zn<1000 μg / l; Cu, Ni<500 μg / l; Cr<200μg / l; Pb<50μg / l). Based on our results, it could be concluded that the role of dissolved and total metal content in toxicity was low in the samples taken at the Szombathely wastewater treatment plant.

Toxic organic pollutions

Biological degradation of some organic contaminants of communal wastewaters is fast, whereas degradation of other compounds is more difficult. Easily degradable substances include carbohydrates, alcohols, organic acids, proteins and fats, which could be found in municipal wastewaters at high concentrations. Slowly degradable organic pollutants are harmful at lower concentrations (μg/l), and they exert their effects mostly through their toxic, carcinogenic, accumulative properties. Organic substances in water can be very diverse and abundant and in many cases the qualitative / quantitative determination of all compounds is not possible. Therefore, organic Matter Content is characterized by so-called sum parameters (COD: Chemical Oxygen Demand, BOD: Biological Oxygen Demand). Nowadays the focus of organic analytical research are: cosmetics [27-29], derivatives of drugs [30-33] and insecticides [34,35]. Yu X. et al. [14] studied drug effluents with *V. fischeri.* Bacterial toxicity correlated well with the COD data. Mendonca et al [36] also found the correlation appropriate between the BOD and COD data and the EC50 value measured by the *V. fischeri* test for municipal effluent and influent wastewater treatment plants.

The relationship between the Microtox, Ascent, the COD and BOD values was analyzed using the influent, sedimented, and effluent wastewater. The COD and BOD values did not correlate

with the Ascent EC50 value (COD: r=-0,21; ρ=-0.48; BOD r=-0.22; ρ=-0.23). Microtox EC50 had a strong negative relationship with BOD and COD (COD: r=-0.75; ρ=-0.81 and BOD: r=-0.80; ρ=-0.78). Negative correlation arises because high EC50 values implicate low toxicity. Organic compounds were rapidly degraded or adsorbed to the sludge during the purification process. In proportion to their decreasing amounts, their EC50 values increased drastically (the toxicity decreased). The influent and the primary clarifiers were the most toxic and analogically, they contained the most organic substances. The effluent contained a small amount of organic pollutants based on the total organic parameters and its toxicity could not be detected in any of the cases. Based on the data series it can be concluded that the toxicity of the wastewater samples can be primarily related to organic pollutants [37-40].

Effects of meteorological factors

The effect of high intensity rainfall was investigated on the toxicity of the samples taken at different objects. Prior to the sampling there were significant amount and high intensity rainfalls on two occasions: on July 25th and November 6th, 2012. During the summer sampling, 24 mm and in autumn sampling, 43 mm rain dropped off the day before the sampling. Due to the falling rain in July, the stormwater tank reached only 65%, so no direct wastewater into the Perint rivulet. The fallen rain had no effect on the toxicity of the influent wastewater because the hydraulic pressure has already left the channel. The dilution effect could not be observed at the primary clarifier, either, due to the short (Some Hours) residence time. The biological reactor is a large object, with multiple days of residence time, the effect of summer rainstorm was detectable in the measured EC50 values (Figure 3). In November, nearly twice the amount of rainfall dropped compared to July. At this occasion the plant was almost washed away by a sudden rain. The stormwater tank became full and a portion of the diluted wastewater flew from the plant to the Perint rivulet. The EC50 values were clearly higher at the effluent (135%), primary clarifier (182%) and biological reactor (570%). During the sampling, the dilution effect reached the primary clarifier which had short (some hours) residence time and the biological reactor, with a residence time of several days.

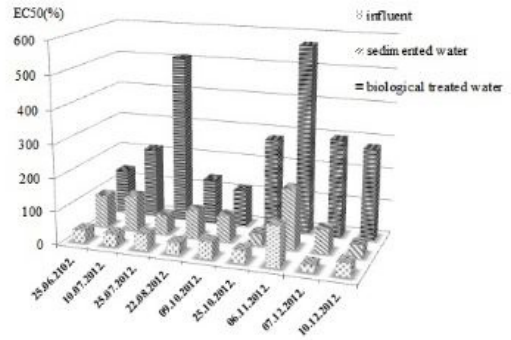

Figure 3: EC50 data of the wastewater samples for three objects.

Toxicity bioassays

When comparing the two practical protocols (Ascent raw and Microtox filtered), it seems obvious that in approximately half of the sampling campaign (2012.06.25.; 2012.07.10.; 2012.08.22.; 2012.11.06.) Ascent raw EC50s were lower (therefore higher ecotoxicity detected) than in the case of Microtox filtered. It can be explained by the fact that filtering wastewater samples for performing the Microtox assay, such particles were removed which could bind toxic compounds, As such, filtering actually decreased toxicity. In case of two other sampling date (2012.07.25., 2012.10.09.) Ascent and Microtox values fell very close to each other, at last for the inflow and the sedimented water. Still, correlation between values measured by the two different protocols was weak for the first three phases of the WWTP, (r=0.50 ;ρ=0.46).

Evaluating the performance of the Wastewater Treatment Plant (WWTP), a constant decrease in toxicity should be anticipated as treatment processes go by. Such pattern can be clearly seen in samples taken on 2012.06.25.; 2012.07.10; 21012.11.06.; 2012.12.07. However, in some cases Ascent and Microtox values show different trends. For samples taken on 2012.07.25.; 2012.08.22., 2012.10.09., 2012.10.25., a low but steady decrease in toxicity is shown by both Ascent and Microtox. between the inflow and sedimented water. However, a further decrease is shown by Microtox, stating that in the biological reactor toxicity cannot be practically detected (EC50 values are above 100% of the concentration of the original sample). On the contrary, Ascent values

show relatively high toxicity in the biological reactor, on all sampling days these values fell very close to the sedimented water or even showing higher toxicity on 2012.07.25. and on 2012.10.25.

When evaluating sensitivity of the two protocols, correlation between toxicity and group of contaminants might give a good indication (if cause and effect relationship can be established). For the raw wastewater samples, however, no correlation could be found between Ascent values and analytically measured parameters. Ecotoxicity of filtered Microtox samples showed significant correlation with COD (-0.916). It might indicate its sensitivity to organic pollutants as well.

Conclusions

Strong correlation of Microtox values with organic pollutants and the clear pattern which reflected meteorological conditions might favour the application of the conventional protocol (ISO 11348-3:2010) for WET assessment. On the contrary, it might seem probable that at some sections of the WWTP (Mainly In The Biological Reactor) filtering removes toxic particles or more precisely, particles which have bound toxic compounds. It should be taken into consideration that Microtox assay might underestimate toxicity in such cases.

References

1. Ma XY, Wang XC, Ngo HH, Guo W, Wu MN, et al. (2014) Bioassay based luminescent bacteria: Interferences, improvements, and applications. Science of the Total Environment 468-469: 1-11.
2. Kokkali V, van Delft W (2014) Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples. Trends in Analytical Chemistry 61: 133-155.
3. Papa M, Ceretti E, Viola GCV, Feretti D, Zerbini I, et al. (2016) The assessment of WWTP performance: Towards a jigsaw puzzle evaluation?. Chemosphere 145: 291-300.
4. Ren S (2004) Assessing wastewater toxicity to activated sludge: recent research and developments. Environment International 30: 1151-1164.
5. Dalzell DJB, Alte S, Aspichueta E, de la Sota A, Etxebarria J, et al. (2002) A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. Chemosphere 47: 535-545.
6. Campisi T, Abbondanzi F, Casado-Martinez C, DelValls TA, Guerra R, et al. (2005) Effect of sediment turbidity and color on light output measurement for Microtox Basic Solid-PhaseTest. Chemosphere 60: 9-15.
7. Harkey GA, Young TM (2000) Effect of soil contaminant extraction method in determining toxicity using the Microtox® assay. Environmental Toxicology and Chemistry 19: 276-282.
8. Lappalainen J, Juvonen R, Vaajasaari K, Karp M (1999) A new flash method for measuring the toxicity of solid and colored samples. Chemosphere 38: 1069-1083.
9. Pollumaa L, Kahru A, Eisentrager A, Reiman R, Maloveryan A, et al. (2000) Toxicological investigations of soils with the solid-phase Flash assay: comparison with other ecotoxicological tests. Alternatives to Laboratory Animals 28: 461-472.
10. Karlsson K, Viklander M, Scholes L, Revitt M (2010) Heavy metal concentrations and toxicity in water and sediment from stormwater ponds and sedimentation tanks. Journal of Hazardous Materials 178: 612-618.
11. Kanarbik L, Blinova I, Sihtmäe M, Künnis-Beres K, Kahru A (2014) Environmental effects of soil contamination by shale fuel oils. Environmental Science and Pollution Research International 21: 11320-11330.
12. Jarque S, Masner P, Klánová J, Prokeš R, Bláha L (2016) Bioluminescent *Vibrio fischeri* Assays in the Assessment of Seasonal and Spatial Patterns in Toxicity of Contaminated River Sediments. Frontiers in Microbiology 7: 1738.
13. Kapanen A, Vikman M, Rajasärkkä J, Virta M, Itävaara M (2013) Biotests for environmental quality assessment of composted sewage sludge. Waste Management 33: 1451-1460.
14. Yu X, Zuo J, Tang X, Li R, Li Z, et al. (2014) Toxicity evaluation of pharmaceutical wastewaters using the alga *Scenedesmus obliquus* and the bacterium *Vibrio fischeri.* Journal of Hazardous Materials 266: 68-74.
15. Masner P, Javůrková B, Bláha L (2017) Rapid in situ toxicity testing with luminescent bacteria *Photorhabdus luminescens* and *Vibrio fischeri* adapted to a small portable luminometer. Environmental Science and Pollution Research 15: 3748-3758.
16. Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71: 1308-1316.
17. Kováts N, Kovács A, Ács A, Ferincz Á, Turóczy B, et al. (2012) Development of a whole-aerosol test protocol. Environmental Toxicology and Pharmacology 33: 284-287.
18. Teodorovic I, Planojevic I, Knezevic P, Radak S, Nemet I (2009) Sensitivity of bacterial vs. acute *Daphnia magna* toxicity tests to metals. Central European Journal of Biology 4: 482-492.
19. Pardos M, Benninghoff M, Guéguena C, Thomasa R, Dobrowolskib J, et al. (1999) Acute toxicity assessment of Polish (waste) water with a microplate-based *Hydra attenuata* assay: a comparison with the Microtox test. The Science of the Total Environment 244: 141-148.
20. Kungolos A, Hadjispyrou S, Petala M, Tsiridis V, Samaras PG, et al. (2004) Toxic Properties of Metals and Organotin Compounds and Their Interactions on *Daphnia magna* and *Vibrio fischeri.* Water, Air, & Soil Pollution: Focus 4: 101-110.
21. Guéguen C, Gilbin R, Pardos M, Dominik J (2004) Water toxicity and metal contamination assessment of a polluted river: The Upper Vistula River (Poland). Applied Geochemistry 19: 153-162.
22. Fulladosa E, Murat JC, Martinez M, Villaescusa I (2005) Patterns of metals and arsenic poisoning in *Vibrio fischeri* bacteria. Chemosphere 60: 43-48.
23. Mortimer M, Kasemets K, Heinlaan M, Kurvet I, Kahru A (2008) High throughput kinetic *Vibrio fischeri* bioluminescence inhibition assay for study of toxic effects of nanoparticles. Toxicology in Vitro 22: 1412-1417.

24. Abbondanzi F, Cachada A, Campisi T, Guerra R, Raccagni M, et al. (2003) Optimisation of a microbial bioassay for contaminated soil monitoring: bacterial inoculum standardisation and comparison with Microtox assay. Chemosphere 53: 889-897.

25. Kaur I, Bhatnagar AK (2002) Algae-dependent bioremediation of hazardous wastes. Biotransformations: Bioremediation Technology for Health and Environmental Protection. 36: 461-516.

26. Koppe P, Stozek A, Neitzel V (2008) Biotechnology V. 11a., Edited by: Rehm, H. J. and Reed G. Municipal Wastewater and Sewage Sludge. p. 159-189.

27. Szymanski A, Wyrwas B, Swit Z, Jaroszynski T, Lukaszewski Z (2000) Biodegradation of fatty alcohol ethoxylates in the continuous flow activated sludge test. Water Research 34: 4101-4109.

28. Traczyk L, Szymanski A, Wyrwas B, Jaroszynski T, Lukaszewski Z (2005) Efficiency of Non-Ionic Surfactant Removal in Biological Sewage Treatment Plants. Polish Journal of Environmental Studies 15: 493-499.

29. Eriksson E, Auffarth K, Eilersen A-M, Henze M, Ledin A (2003) Household chemicals and personal care products as sources for xenobiotic organic compounds in grey wastewater. Water SA 29: 135-146.

30. Farré M, Ferrer I, Ginebreda A, Figueras M, Olivella L, et al. (2001) Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with *Vibrio fischeri.* Journal of Chromatography A 938: 187-197.

31. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Research. 42: 3498-3518.

32. Schultz MM, Furlong ET (2008) Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. Analytical Chemistry 80: 1756-1762.

33. Sim WJ, Lee JW, Lee ES, Shin SK, Hwang SR, et al. (2011) Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. Chemosphere 82: 179-186.

34. Bester K (2005) Fate of triclosan and triclosan-methyl in sewage treatment plants and surface waters. Archives of Environmental Contamination and Toxicology 49: 9-17.

35. Ahmed S, Rasul MG, Brown R, Hashib MA (2011) Influence of parameters on the heterogeneous photocatalytic degradation of pesticides and phenolic contaminants in wastewater: a short review. Journal of Environmental Management 92: 311-330.

36. Mendonça E, Picado A, Paixão SM, Silva L (2009) Ecotoxicity tests in the environmental analysis of wastewater treatment plants: Case study in Portugal. Journal of Hazardous Materials 163: 665-670.

37. Kováts N, Refaey M, Varanka B, Reich K, Ferincz Á, et al. (2012) Comparison of conventional and *Vibrio fischeri* bioassays for the assessment of municipal wastewater toxicity. Environmental Engineering and Management Journal 11: 2073-2076.

38. Kudłak B, Wieczerzak M, Yotova G, Tsakovski S, Simeonov V, et al. (2016) Environmental risk assessment of Polish wastewater treatment plant activity. Chemosphere 160: 181-188.

39. Pessala P, Schultz E, Nakari T, Joutti A, Herve S (2004) Evaluation of wastewater effluents by small-scale biotests and a fractionation procedure. Ecotoxicology and Environmental Safety 59: 263-272.

40. Yuan N, Wang C, Pei Y (2016) Bacterial toxicity assessment of drinking water treatment residue (DWTR) and lake sediment amended with DWTR. Journal of Environmental Management 182: 21-28.