ON THE POSSIBILITY OF USING BIOLOGICAL TOXICITY TESTS TO MONITOR THE WORK OF WASTEWATER TREATMENT PLANTS

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Abstract — The aim of this study was to ascertain the possibility of using biological toxicity tests to monitor influent and effluent wastewaters of wastewater treatment plants. The information obtained through these tests is used to prevent toxic pollutants from entering wastewater treatment plants and discharge of toxic pollutants into the recipient. Samples of wastewaters from the wastewater treatment plants of Kragujevac and Gornji Milanovac, as well as from the Lepenica and Despotovica Rivers immediately before and after the influx of wastewaters from the plants, were collected between October 2004 and June 2005. Used as the test organism in these tests was the zebrafish *Brachydanio rerio* Hamilton – Buchanon (Cyprinidae). The acute toxicity test of 96/h duration showed that the tested samples had a slight acutely toxic effect on B. rerio, except for the sample of influent wastewater into the Cvetojevac wastewater treatment plant, which had moderately acute toxicity, indicating that such water should be prevented from entering the system in order to eliminate its detrimental effect on the purification process.

Key words: Monitoring, biological toxicity, wastewater treatment plants

INTRODUCTION

The environment in the 21st century is becoming increasingly polluted, and surface and underground waters are directly or indirectly threatened by every day discharge of wastewaters with various composition.

Wastewaters are waters that after use have altered their physical, chemical, and biological properties and increased content of admixtures, colloids and solutions that can be of organic or mineral origin (S t o i m e n o v, 2001).

Before discharge of wastewaters into the recipient, it is necessary to purify them with adequate treatments in a way and to an extent that does not threaten natural processes and does not reduce the possibilities of their multi-functional use.

The presence of harmful chemicals in aquatic environments is routinely monitored using physico–chemical and biological parameters. However, chemical procedures alone cannot provide sufficient

information on the potential harmful effects of chemicals on aquatic environments. The complex nature of effluents cannot be overcome by specific chemical approaches. The toxic effects of unknown and often undertermined substances in complex mixtures or with possible synergistic effects among compounds on wastewater can be detected only by toxicity testing. Even though the quality of effluent wastewaters conforms to allowed standards, wastewaters can in certain cases still be toxic. Many chemicals that are discharged into aquatic environments are not directly monitored (S p o n z a , 2003).

Toxicity tests are used to establish and explain negative effects of wastewaters on aquatic organisms. Toxicity can be determined experimentally in the laboratory by exposing sensitive organisms to the influence of wastewaters. For selection of test organisms, it is very important that they can be easily maintained in laboratory conditions, reproduce rapidly, and are sensitive to even minimal quantities of toxic substances. Toxicity tests are sooner used for assessment of combined pollution factors than as a

conventional method of monitoring chemical toxicity (SETAC, 2004).

In order to assess the toxicity of a sample, test organisms such as fish, invertebrates, or algae are exposed to diluted or undiluted wastewaters in controlled conditions. When diluted water is used in toxicity testing, the test can reflect natural mixing of the effluent with the receiving water. The diluted water experiment often shows that the situation is improved when the quantity of water in the flow is increased, with a dilution effect, which probably happens during rainy periods.

Combining the results of toxicity tests and chemical analyses with other information can provide a more complete and realistic picture of the influence of wastewaters on aquatic ecosystems (SETAC, 2004). The obtained results can be used to prevent discharge of toxic pollutants into aquatic systems (M a n t i s et al., 2005).

Discharge of industrial wastewaters is allowed only if it does not increase the presence of pollutants above the established standards and on condition that the industry involved ensures purification of its wastewaters to the degree stipulated by the relevant agencies in charge of regulating water utilization and protection (Đ u k i ć, 2005).

Wastewaters treatment at plants in the cities of Kragujevac and Gornji Milanovac consists of preliminary processes, primary and secondary treatment, and treatment of sludge.

In wastewater treatment, removal of crude and fine substances and elimination of sand on the sand separator (preliminary processes) are followed by the primary precipitation stage. In the biological block, aeration is conducted with active sludge, after which precipitation occurs in secondary precipitators. The purified water is discharged from the secondary precipitators into rivers.

Besides the wastewaters of the aforementioned cities, some industrial plants are also connected to the treatment plant (situated on the banks of the Lepenica and Despotovica Rivers respectively, downstream from the city center), and they discharge untreated or partially treated wastewaters into sewage.

The central process in both treatment plants is microbiotic degradation of organic substances in bioaerating tanks. The prerequisite for the functioning of microbiotic degradation is pretreatment and removal of heavy metals and other toxicants from industrial wastewaters.

The aim of this study was to ascertain the toxicity of incoming and outgoing wastewaters at the Cvetojevac (Kragujevac) and Mlakovac (Gornji Milanovac) plants, as well as that of the Lepenica and Despotovica Rivers before and after wastewater inflow with the aid of a toxicity test, i.e., to establish the possibility of monitoring the work of wastewater treatment plants by means of biological toxicity testing.

MATERIAL AND METHODS

The experimental part of this work was perfomed in the Hydrobiological Laboratory of the Institute of Biology and Ecology (Faculty of Science, University of Kragujevac, Serbia) between October 2004 and June 2005.

The toxicity test organism was the fish *Brachydanio rerio* Hamilton–Buchanan (Cyprinidae).

Spawning and breeding of fish for the experiment took place in the spawning facility of Kragujevac Aquarium.

Wastewater sampling sites were selected in keeping with the defined goals. Samples were collected from two plants: the Cvetojevac wastewater treatment plant (outside the city of Kragujevac) and the Mlakovac wastewater treatment plant (near Gornji Milanovac).

The following samples were collected at both plants: incoming wastewater (from the primary precipitator); wastewater from the bioaerating tank; and outgoing wastewater (from the secondary precipitator). Simultaneously, samples were collected from the tested rivers at two points: upstream from the inflow of wastewater from the treatment plant and

downstream from it.

Sampling of wastewaters from the plants and rivers was conducted using plastic bottles of an adequate volume cleaned with water from the tested basins of treatment plants and rivers before and after the inflow of wastewaters. The collected samples were transported with as little change in quality as possible to the laboratory for further testing.

Measurements of the following parameters were conducted in the laboratory: temperature, dissolved oxygen, pH value, and electrical conductivity. Temperature, pH, and electrical conductivity were measured with a combined digital instrument (combo HANNA HI 98129), oxygen concentration with an oxymeter (HANNA HI 9143).

After parameter measurements, the samples were transferred to experimental aquaria of adequate volume, after which the test organisms were introduced. In each toxicity test, 10 B. rerio were used per test vessel containing 5-liter samples.During the experiment (96 h), the test organisms were not fed. After 24, 48, and 96 h, dead organisms were counted in each aquarium, death being the effect used to express acute toxicity. Each experiment comprised two series, including controls.

After the experiment, the results were subjected to statistical processing, where mean values of test organism survival were determined, as well as minima, maxima, and the standard deviation.

Based on the number of surviving test organisms in different samples, acute toxicity of wastewater was determined (S p o n z a , 2003) (Table 1).

RESULTS

Table 2 presents the acute toxicity of wastewater, mean values of survival of the test organisms, and the standard deviation. The acute toxicity results were obtained after two–month sampling of wastewater from treatment plants and from the rivers immediately before and after wastewater influx.

The obtained data indicate significantly lower survival of B. rerio in samples of incoming and outgoing wastewater from the Cvetojevac treatment plant in comparison with survival in samples taken from Mlakovac (7.22 vs. 56.11% for incoming and 51.25 vs. 91.8% for outgoing wastewater). Also, the mean survival value of B. rerio was lower in samples from the bioaeration tank at the Cvetojevac plant in comparison with the tank at the Mlakovac plant (63.89 vs. 71.81%). In samples from the Lepenica before and after wastewater influx, we noticed a lower survival rate of the test organisms in comparison with samples from the Despotovica before and after wastewater influx (87.92 vs. 92.5% before and 55.69 vs. 91.25% after).

It can be seen from Table 2 that the lowest mean value of B. rerio survival was in samples of incoming wastewater at the Cvetojevac plant, the highest in samples from the Despotovica River before the influx of wastewater from the Mlakovac plant.

The obtained data indicate better efficiency of the wastewater treatment system or lower charging of the system with toxic materials at the Mlakovac plant than at the Cvetojevac plant.

DISCUSSION AND CONCLUSION

As the chemical industry grows rapidly, the number of potential toxicants in water is growing at the same place.

Industrial wastewaters cannot be allowed freely into a town's sewage. Before being discharged into sewage, they must undergo preliminary treatment at the industrial site to reduce significantly their

Table 1. Acute toxicity of wastewaters (S p o n z a , 2003).

Percentage of surviving test organisms	Assessment of wastewaters' acute toxicity
< 1 %	Acute toxicity
1 – 10 %	Moderate acute toxicity
10 – 99 %	Low acute toxicity
100%	No acute toxicity

Table 2. *Brachydanio rerio* toxicity test results..

Sampling site	Mean value of survival, %	Standard deviation	Results (see Sponza, 2003)
Incoming wastewaters – Cvetojevac	7.22	13.94	Moderate acute toxicity
Incoming wastewaters – Mlakovac	56.11	32.26	Low acute toxicity
Bioaerating tank – Cvetojevac	63.89	32.84	Low acute toxicity
Bioaerating tank – Mlakovac	71.81	31.84	Low acute toxicity
Outgoing wastewaters – Cvetojevac	51.28	20.14	Low acute toxicity
Outgoing wastewaters – Mlakovac	91.81	13.79	Low acute toxicity
Lepenica before treatment	87.92	11.11	Low acute toxicity
Despotovica before treatment	92.5	11.65	Low acute toxicity
Lepenica after treatment	55.69	16.19	Low acute toxicity
Despotovica after treatment	91.25	16.04	Low acute toxicity

mechanical and organic charge and the content of toxic substances that aggravate or completely prevent operation of the town's plant. Further purification takes place in wastewater treatment plants, where industrial wastewaters together with utility waters undergo mechanical and microbiological processing treatments (C i b u l i ć, 2000).

The 96-h acute toxicity test is a standard method used for assessing the impact of wastewaters on the environment (L e o n a r d et al., 2005). Fish are good test species for water toxicity assessment (APHA, 1995). Their economic and ecological importance make them relevant test organisms. Generally, salmonid fish species are more sensitive than cyprinid species (N a g e l, 2002).

B. rerio is a highly productive tropical fish frequently used as a model in many studies involving wastewater toxicity assessment.

In our toxicity tests, *B. rerio* was used to assess the toxicity of wastewaters and their impact on behavior of the tested fish, i.e., to establish the possibility of monitoring the work of wastewater treatment plants. Wastewaters of various chemical composition directly influence fish activity. The main symptoms exhibited by fish in tested wastewater samples are red gills and uncontrolled surface swimming.

Based on the number of surviving test organisms, it can be asserted that all samples showed low acute toxicity with small variances in the percentage of survival, except for the sample of incoming wastewater at the Cvetojevac plant, where acute toxicity

was moderate, indicating that such water should not be let into the treatment plant if we want to maintain discharge within defined limits, i.e., reduce the harmful effect of this water on the purification process.

Although acute tests with fish have a significant role, it is sometimes necessary to substitute the embryo test with *Brachydanio rerio* because the acute test covers only situations after chemical spillage. The use of fish embryos in studying toxicity is a promising approach to ecotoxicity testing permitting chemical testing in living and fledgeling organisms (H e r r á e z – B a r a n d a et al., (2005).

G e l l e a r t a n d H e i n r i c h s d o r f f (2001) researched the toxic impact of various concentrations of chemical industry wastewaters on zebra fish (*Brachydanio rerio*) eggs. The research showed age-dependent differences of egg sensitivity related to the ontogenetic phase in which the experiment commenced.

Many authors have investigated acute toxicity of different reagents using aquatic and terrestrial organisms. D o b š í k o v á (2003) researched acute toxicity of carbofuran (a pesticide widely used against insects and nematodes) on various aquatic (the guppy *Poecilia reticulata*, water flea *Daphnia magna*, and green algae *Raphidocelis subcapitata*) and terrestrial (white mustard *Sinapis alba*) organisms. It was established that *D. magna* is the most sensitive of these organisms, whereas *Sinapis alba* is not a convenient (sensitive enough) plant species for carbofuran toxicity assessment.

Neither biological control nor biomonitoring in the narrower sense are proposed in current regulations applied to the control of wastewaters and their impact on recipients, since data on the immediate impact of wastewaters on aquatic organisms are inferred only from scientific papers published in the past few years. They noted a negative impact of urban wastewater on aquatic ecosystem in Novi Sad, Serbia where unpurified utility wastewaters and partly purified wastewaters from industrial facilities flow into the Danube (T e o d o r o v i ć et al., 2004). This research indicated that the recipient water

does not mitigate toxicity of the tested wastewaters. Other tests showed an unfavorable impact of industrial wastewaters on the Nišava River, even in conditions where industry works at reduced capacity and industrial wastewater meets MDK values for discharge into sewage (S t o i m e n o v, 2001). Negative impact of urban wastewaters is also evident in the Veternica, Croatia, where purification of industrial wastewaters is necessary, at the city's central plant even after mandatory preliminary treatment at the industrial site, tests having revealed the presence of hazardous toxic substances in the wastewaters (C i b u l i ć et al., 2000).

Wastewaters of many industries contain a mixture of several complex compounds, and it is often possible only through biological toxicity testing to establish the level of toxicity and determine the method and extent of purification required. In such a way, it is possible to realize significant savings while designing and commissioning purification plants.

REFERENCES

APHA (1995). Standard Methods for the Examination of Water and Wastewater, American Public Health Association, 1015 Fifteenth Street, NW Washington, DC 20005.

Đukić, D., and *V. Ristanović* (2005). Hemija i mikrobiologija voda. STYLOS, 153–169

Cibulić, V., Kankaraš, K., and *M. Bogdanović* (2000). Zagađivanje reke Veternice otpadnim vodama grada i industrije Leskovca. 29. konferencija o aktuelnim problemima zaštite voda, Zaštita voda 2000, Mataruška Banja, jun 2000, Knjiga radova, 433–438.

Dobšíkově, R. (2003). Acute toxicity of carbofuran to selected species of aquatic and terrestrial organisms. *Plant Protect. Sci.*, **39**, 103 – 108.

Gellert, G., and *J. Heinrichsdorff* (2001). Effect of age on the susceptibility of zebrafish eggs to industrial wastewater. *Water Res.* **15**, 3754 – 3757

Herráez – Baranda, L. A., Rodríguez, J., Felipe, A., San Segundo, L., and *J. Guinea* (2005). Biological and technological advances for ecotoxicity and human health risk assessment using zebrafish embryos. ALTEX, Abstracts, 228.

Leonard, M., Belanger, S. E., Rawlings, J. M., Vanpoucke, M., Pocher, J. M., and *S. Gimeno* (2005). Evaluation of the fish

ambryo test as a potential replacement for the standard acute fish toxicity test using juveniles. ALTEX, Abstracts, 230.

Mantis, I., Voutsa, D., and *C. Samara* (2005). Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods. Ecotoxicol. Environ. Safety **62**, 397–407.

Nagel, R. (2002). The embryo test with the zebrafish *Danio rerio* – a general model in ecotoxicology and toxicity. ALTEX **19**, 38–48.

SETAC - Society of Environmental Toxicology and Chemistry (2004). Whole Effluent Toxicity Testing. *Environmental Quality Through Science.*

Sponza, D. T. (2003). Application of toxicity tests to discharges

of the pulp – paper indrustry in Turkey. *Ecotoxicol. Environ. Safety* **54**, 74–86.

Stoimenov, S. (2001). Zagađenje reke Nišave otpadnim vodama grada Pirota. 30. konferencija o aktuelnim problemima zaštite voda, Zaštita voda 2001, Aranđelovac, jun 2001, Knjiga radova, 398-403.

Teodorović, I., Đukić, N., Dalmacija, B., Maletin, S., Miljanović, B., and *M. Bokorov* (2001). Procena pouzdanosti indeksa metalozagađenja toksikološkim testovima, 30. konferencija o aktuelnim problemima zaštite voda, Zaštita voda 2001, Aranđelovac, jun 2001, Knjiga radova, 339–344.

Teodorović, I., Đukić, N., Dalmacija, B., Živković, Lj., and *M. Bokorov* (2000). Ocena toksičnosti otpadnih voda grada Novog Sada, 29. konferencija o aktuelnim problemima zaštite voda, Zaštita voda, Mataruška Banja, jun 2000., Knjiga radova, 427 – 432.

МОГУЋНОСТ КОНТРОЛЕ РАДА СИСТЕМА ЗА ПРЕЧИШЋАВАЊЕ ОТПАДНИХ ВОДА БИОЛОШКИМ ТЕСТОВИМА ТОКСИЧНОСТИ

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Отпадне воде представљају комплекс сложених једињења и често је само биолошким тестовима токсичности могуће утврдити токсичност комплексних отпадних вода и према томе одредити начин и обим пречишћавања. На тај начин се остварују велике уштеде код пројектовања и рада система за пречишћавање.

Циљ ове студије је био да се утврди могућност контроле улазних и излазних отпадних вода система за пречишћавање отпадних вода биолошким тестовима токсичности. Информације добијене овим тестовима користе се да би се спречио улазак токсичних загађивача у систем за пречишћавање отпадних вода као и испуст токсичних загађивача у реципијент. Узорци отпадних вода из система за пречишћавање отпадних вода града Крагујевца и Горњег Милановца као и реке Лепенице и Деспотовице непосредно пре и после улива отпадних вода из система сакупљени су у периоду од октобра 2004. до јуна 2005. године. Као модел организам у овим тестовима коришћена је зебрица *Brachydanio rerio* Hamilton–Buchanon (Cyprinidae). Тестови акутне токсичности у трајању од 96 часова показују да испитивани узорци имају мали акутни токсични ефекат на *B. rerio,* осим узорка улазне отпадне воде у систем за пречишћавање отпадних вода Цветојевац који показује умерено акутну токсичност, што указује да такве воде не би требало уводити у систем како би се смањило њихово штетно дејство на процес пречишћавања.