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Ecotoxicity of Wastewater in the Czech Republic †

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Abstract: The following article is intended as an introduction to ecotoxicology/wastewater toxicology, as this has currently become a widespread and highly discussed topic with scarce information available to date. With the beginning of the COVID-19 pandemic, when disinfectants began to be used in larger quantities, problems also began at wastewater treatment plants. This situation was the reason for beginning to monitor the ecotoxicity/toxicity of the wastewater flowing into the studied wastewater treatment plant; the wastewater treatment plant entered a state of emergency and was unable to treat the inflowing wastewater. In this critical period, the following parameters were monitored at the inflow of the wastewater treatment plant—BOD5, CHSKCr, NL, N-NH4, N-inorg, N-total, P-total, RAS and pH. The effluent from the wastewater treatment plant was also monitored for BOD5, CHSKCr, NL, N-NH_4, N-NO_3, N-NO_2, N-inorg, N-total, P-total and RAS. Ecotoxicity (fish, barnacles, algae) and toxicity (*Vibrio fischeri*) were monitored at the inflow and outflow.

Keywords: ecotoxicity; water quality indicators; wastewater treatment plant; operation; maintenance; design example

1. Introduction

Ecotoxicity is a key factor regarding environmental protection in the European Union. In this regard, the European Union has strict standards and regulations to minimize the negative effects of chemical substances on the environment and human health.

The European Chemicals Agency (ECHA) [1] and the European Food Safety Authority (EFSA) [2] are responsible for evaluating and regulating chemicals for their ecotoxicity. These organizations carry out scientific evaluations of the effects of substances on aquatic organisms, soil, and other environments. The European Union has also introduced the registration, evaluation, authorization, and restriction of chemicals (REACH) system [3], which aims to improve the protection of human health and the environment from the risks associated with chemical substances. REACH requires the manufacturers and importers of chemicals to demonstrate their safety and carry out tests regarding their ecotoxicity. Another tool used by the European Union for ecotoxicity assessments is the system of classification, labeling, and packaging of chemical substances (CLP) [4]. This system establishes standardized criteria for classifying substances as hazardous to the environment and requires that such substances be appropriately labeled, and the information shared. The parameters of ecotoxicity and toxicity are not monitored, as a standard, in wastewater. However, in my view and in the opinion of some experts, these parameters should be monitored at least at the level of industrial producers, due to toxic wastewater discharges into public sewerage systems. Toxic wastewater can subsequently cause considerable damage to wastewater treatment plants, e.g., the mortality of nitrifying bacteria in activation reactors. These European standards are gradually being implemented by individual EU states.

The issue of toxicity/ecotoxicity is not legislated for operators of wastewater treatment plants in the countries of the European Union. Currently, there are valid European standards

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and domestic national standards of individual member states that indicate the method of determining the effect of ecotoxicity on living microorganisms.

In the Czech Republic, a Methodological Instruction has been issued by the Ministry of the Environment of the Czech Republic—Waste Department [5], which defines ecotoxicity as a hazardous property of waste under code H14. In the Czech Republic, it is not mandatory to monitor the ecotoxicity/toxicity indicators for wastewater treatment plant operators. Some operators set this indicator themselves, due to the increased concentrations of toxic substances in wastewater. The reason for the increased levels of toxic substances was the disease COVID-19, which caused a higher concentration of disinfectants in wastewater and medicines. The increased concentration of toxic substances has a direct effect on the biological processes taking place in the activation tank, i.e., it can partially or completely stop the biological cleaning processes.

In the Slovak Republic, the monitoring and assessment of ecotoxicity is monitored according to Government Regulation No. 269/2010 Coll. of the Regulation of the Government of the Slovak Republic, which establishes specific requirements for achieving good water status [6]. This government regulation defines that the indicator of ecotoxicity on aquatic organisms has an indicative character, which is monitored regarding industrial and municipal wastewater discharged directly into surface waters.

Each member state of the European Union approaches the methods of determining ecotoxicity/toxicity differently in terms of legislation and standards. There is no standard for determining ecotoxicity in the Czech Republic. Ecotoxicity is determined according to the Slovak standard STN 83 8303 for testing the hazardous properties of waste, ecotoxicity, acute toxicity tests on aquatic organisms, and testing the inhibition of the growth of algae and higher cultivated plants using *Sinapis alba*, green algae, pearl oysters, and fish [7].

Wastewater toxicity affects living organisms, aquatic organisms, and soil biota. Wastewater discharged into the recipient waters affects the biochemical activity and growth of organisms (algae, bacteria, and protozoa). In the case of animal organisms, their immobilization or outright death occurs [8,9].

Mixed municipal wastewater flows into the sewage treatment plants. The operator should continuously analyze wastewater from important customers (from industrial sites, hospitals, car washes, etc.). Residual concentrations of pollutants in wastewater have a direct impact on the need to optimize wastewater treatment technology [10,11].

On the basis of these wastewater analyses, an evaluation of ecotoxicological risks and the global synergistic effects of ecotoxicity must be conducted, with an emphasis on the residual concentrations of pollutants. An example is hospital wastewater, which has the potential for ecotoxicity and antibiotic resistance in wastewater.

2. Test, Methods, and Evaluation of Ecotoxicological Bioassays

The basic terms employed in the field are ecotoxicology, ecotoxicity, the toxicity of substances, mortality, and immobilization. Their contextual meanings can be described as follows:

Ecotoxicology is a discipline established on the basis of findings from other scientific fields, namely, chemistry, applied ecology, biology, and toxicology. In other words, ecotoxicology can be defined as a discipline that combines knowledge from ecology (a science studying ecosystems) and toxicology (the study of the interaction of chemical substances with living organisms). In 1969, René Truhaut coined the first definition of ecotoxicology: "Study of the toxic effects of chemicals induced by natural and synthetic pollutants to protect natural species and societies" [12].

Ecotoxicity, designated as H14, is a dangerous property of substances with an instant or delayed negative, adverse effect on living organisms or on the environment [12].

Substance toxicity refers to the ability of substances to cause harm to a living organism. Toxicity depends on the physicochemical properties of the given substance, the way the substances enter the organism, their metabolism, and the frequency and dose of the supplied substances [12].

Mortality is a toxic effect that results in the death of the test organism [12].

Immobilization (inhibition of mobility) refers to short-term or long-term immobility, during which the test organism is unable to move in space [12].

Ecotoxicity/toxicity tests are divided into three basic groups—ecotoxicological bioassays, aquatic bioassays, and alternative bioassays.

2.1. Ecotoxicological Bioassays

These tests determine whether a certain substance will have a toxic effect on a given organism in the ecosystem, or whether a biotic bonds in the ecosystem will be disrupted. These tests can be divided according to the exposure time (acute, semi-acute, and chronic), target organism (soil, freshwater, and marine), complexity of the tested sample (natural samples, pure chemical substances, and mixtures of substances), method of sample preparation (direct tests of environmental matrices, the defined concentration of chemical substances, and the testing of leachates or extracts from natural samples), number of test organisms (single species, multi-species, and laboratory mixtures of species), advanced design of the test system (conventional tests with intact organisms, microbiotests, biosensors, biomarkers, and biofunds), degree of complexity of the detection system (enzymes, bioprobes, tissue and cell cultures, intact organisms, population, society), monitored responses (assessment of physiological activity, reproductive activity, and lethal or sub-lethal effects), tested matrix (air, water, soil, waste, sediments, and chemical substances) and trophic levels of test organisms (producers, consumers, and destroyers) [13].

2.2. Aquatic Bioassays

These are the most widespread tests used for determining toxicity. They are conducted with aquatic organisms, most often bacteria, algae, aquatic plants, crustaceans, and fish. They are used to evaluate chemical substances, preparations, and natural water. They are mainly applicable for substances that are soluble in water and for testing aqueous extracts in samples of solid substances. The objective of aquatic tests is to determine the effect of the tested substances on the aquatic ecosystem [13].

2.3. Alternative Bioassays

This type of test is used for several reasons, namely, to save laboratory space and chemicals, to save time, and due to the economic demands of breeding. The breeding of organisms intended for testing takes up a great deal of space in laboratories and is staff-, time-, and chemical consumption-intensive. Alternative bioassays use the resting phases of the organisms [13].

2.4. Determination Methods

Methods to determine ecotoxicity/toxicity in wastewater are described in several international standards, depending on the organism used. These organisms can be divided according to how they receive energy for their life processes (primary producers, primary consumers, secondary consumers, tertiary consumers, and destroyers).

Each of the standards describes the testing procedure using one of the organisms. To illustrate this point, we provide a brief overview:

- ČSN EN ISO 6341 Water quality—*Daphnia magna* Straus motility inhibition test (Cladocera, Crustacea)—Acute toxicity test [14];
- ČSN EN ISO 7346-1 Water quality—Determination of acute lethal toxicity of substances for the freshwater fish *Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae) Part 1: Static method [15];
- ČSN EN ISO 7346-2 Water quality—Determination of acute lethal toxicity of substances for the freshwater fish *Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae) Part 2: Semi-static method [16];

- ČSN EN ISO 7346-3 Water quality—Determination of acute lethal toxicity of substances for the freshwater fish *Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae) Part 3: Flow-through method [17];
- ČSN EN ISO 11348-1 Water quality—Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Test on luminescent bacteria)—Part 1: Method using freshly prepared bacteria [18];
- ČSN EN ISO 11348-2 Water quality—Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Test on luminescent bacteria)—Part 2: Method using liquid-dried bacteria [19];
- ČSN EN ISO 11348-3 Water quality—Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Test on luminescent bacteria)—Part 3: method using freeze-dried bacteria [20];
- ČSN ISO 20665 Water quality—Determination of chronic toxicity for *Ceriodaphnia Dubia* [21];
- ČSN ISO 20666 Water quality—Determination of chronic toxicity for *Brachionus calyciflorus* within 48 h [22];
- ČSN EN ISO 10712 Water quality—*Pseudomonas putida* growth inhibition test (*Pseudomonas* cell reproduction inhibition test) [23];
- ČSN EN ISO 8692 Water quality—Freshwater green algae growth inhibition test [24];
- ČSN EN ISO 20079 Determination of the toxic effect of water constituents and wastewater on duckweed (*Lemna minor*)—Duckweed growth inhibition test [25].

3. Course and Evaluation of Ecotoxicological Tests

The procedure for conducting ecotoxicological tests is shown in Figure 1. The preliminary test is performed first, followed by the verification test, the orientation test, and finally, the basic test.

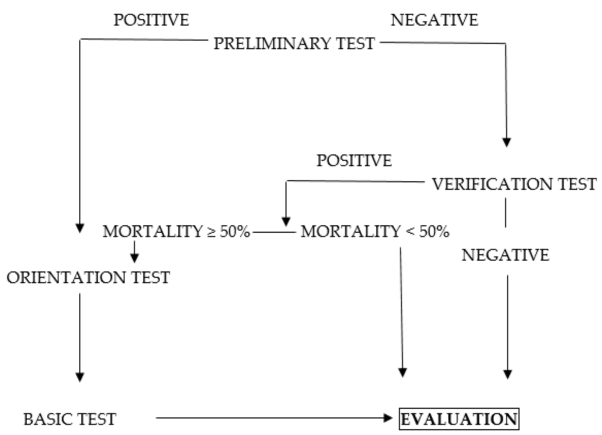

Figure 1. Course of ecotoxicological tests [26].

3.1. Course of Ecotoxicological Tests

Preliminary test: In a preliminary test, a sample of wastewater of unknown toxicity is tested with organisms. Two parallel determinations, including controls, are performed. If the result of this test reveals no mortality of the organism, it is marked as negative, and the verification test is conducted [26].

Verification test: After the verification test is marked as negative, six parallel determinations are performed. If there is no mortality in the wastewater sample at 10% higher than mortality in the control, the test result is marked as negative, and the test is terminated at this point. Otherwise, depending on the immobilization being demonstrated, the orientation test is conducted, namely, when the mortality is higher than 50% [26].

Orientation test: This test is used to determine the range in which a 50% effective concentration can be expected. This concentration is denoted as EC50 (the expressed concentration at which 50% of the tested organisms will die or become immobilized, or there is a change in growth or metabolic activity) [26].

Verification test. For the basic test, the concentration series is selected so that zero and 100% mortality are shown at the extreme concentrations, with the remaining concentrations kept within this range. The EC50 value is determined based on this test [26].

3.2. Evaluation of Ecotoxicological Tests

During these tests, the dependence of the substance concentration on the death of test organisms, and their reproductive capacity, growth, and immobilization are monitored. The so-called "ecotoxicological indices" express the nature of the effect of these substances on organisms (outputs of the toxicity tests). These indices can be used to compare the toxicity of various substances. The ecotoxicological indices describe the level of toxic effects, evaluate the acceptability of substances for the ecosystem, and then divide them into hazard classes. The ecotoxicological indices are divided into two groups—calculated and determined.

Calculated indices: These indices are defined by interpolating experimentally obtained data—EC50, IC50, and LC50. The EC abbreviation stands for effective concentration, LC denotes lethal concentration, IC stands for inhibitory concentration, LD stands for lethal dose, and ED stands for effective dose. The EC50 abbreviation represents the concentration at which 50% of the tested organisms will die or become immobilized, or when there is a change in their growth or metabolic activity.

Determined indices: This group of indices represents the concentrations of substances detected experimentally, the significance of which was verified by statistical comparison with the control. The most widely used indices are NOEC and LOEC. These values are determined by observation. The NOEC abbreviation denotes the highest tested concentration of a toxic substance at which there is no statistically significant effect on the organisms, compared to a control under the same conditions. LOEC stands for the lowest concentration of a toxic substance at which a statistically significant effect is observed compared to the control. NOAEL designates the toxicant dose at which the effect of the given substance that is observed is not considered undesirable.

After carrying out the above tests and evaluating them, the toxicity class can be determined. The individual toxicity classes are shown in Table 1 [26].

Table 1. Toxicity classes and concentration ranges of toxic substances [26].

Class	Concentration Range ($mg \cdot L^{-1}$)	Evaluation
0	$\geq 10^4$	non-toxic
1	10^3–10^4	very mildly toxic
2	10^2–10^3	mildly toxic
3	10^1–10^2	medium toxic
4	10–10^1	highly toxic
5	10^{-1}–10	very highly toxic
6	$\leq 10^{-1}$	extremely toxic

4. Ecotoxicity Monitoring at a Selected Wastewater Treatment Plant

Operating companies usually do not monitor toxicity/ecotoxicity in wastewater. The main reason for this is the legislation of the Czech Republic, which does not require these indicators to be monitored.

Ecotoxicity monitoring was carried out at the wastewater treatment plant operated by Vodárenská akciová společnost, a.s. The reason for taking a sample for ecotoxicity was an accident at the wastewater treatment plant in 2021, which caused the biological processes of wastewater treatment in the activation tank to stop functioning. In the case of the standard indicators of wastewater analyses, the cause of incapacity was not found; therefore, ecotoxicity and toxicity tests were carried out. Analyses carried out in the laboratory revealed the presence of ecotoxicity in the wastewater at the inflow to the wastewater treatment plant. This wastewater treatment plant is designed for 29,376 PE, with an average daily inflow of 4881.2 $m^3 \cdot day^{-1}$.

Since no substance was detected that would cause this cessation, the wastewater treatment plant mentioned below underwent repeated ecotoxicity monitoring. The monitoring took place at a mechanical–biological wastewater treatment plant with primary sedimentation, nitrification, denitrification, the chemical removal of phosphorus, anaerobic stabilization of sludge by digestion, sludge sanitation, sludge thickening, and dewatering.

4.1. Description of Technology Used at the Wastewater Treatment Plant

Wastewater from the service area flows to the pumping station at the wastewater treatment plant through combined sewerage. Water at flow rates reaching a certain value overflows into the river once the stormwater tank has been filled up. Downstream of the pumping station, the pre-treatment stage commences, consisting of mechanically scraped fine screens, a screening press, and a vortex sand trap. The pumps transport mechanically pre-treated wastewater to the primary sedimentation tank. After primary sedimentation, the wastewater is conveyed to the activation system. The biological treatment stage is designed as two independent lines, with two reactors for each of the lines. The activation process is based on controlled nitrification and denitrification, with the simultaneous chemical precipitation of phosphorus. The reactors are equipped with a fine-bubble aeration system and slow-moving horizontal stirrers. Subsequently, the mixed liquor is discharged over a degassing spillway in a split chamber and moves on to two circular secondary tanks. Following the sedimentation process, the water flows via a measuring channel into the river. Primary sludge is extracted from the primary tank into a primary sludge sump and is then fed into a gravity thickener. After settling, the thickened primary sludge is conveyed to a thickened mixed sludge sump. Surplus biological sludge from the secondary tanks is mechanically thickened using a strainer. The thickened mixed sludge is homogenized via a submersible stirrer and is then transported in a controlled manner to a stirred and heated digester. The digested sludge is pumped into sludge storage tank 1, where it is homogenized, and then passes through a pasteurization system as sanitized sludge into sludge storage tank 2, which stores the sludge before its mechanical dewatering using a decanting centrifuge. Once dewatered, the sludge is transported to the sludge management system storage area, from which it is subsequently removed for further processing [27].

4.2. Monitored Parameters

The monitoring took place in the period from June 2021 to March 2022. In the given period, the following parameters were monitored at the WWTP influent and effluent: BOD, COD, SS, N-NH4, N-inorg, N-total, P-total, RAS, and pH. Ecotoxicity and toxicity were monitored at the influent and effluent. Sampling was carried out on an irregular basis but occurred, on average, about 1–3 times a month.

BOD expresses biochemical oxygen demand, which is defined as the volume of oxygen consumed by microorganisms for the decomposition of organic matter under aerobic conditions. COD stands for chemical oxygen demand, which indicates the total oxygen consumption for organic matter oxidization in a wastewater sample. SS denotes suspended solids, where an exact volume of homogenized wastewater samples is filtered through a dried and weighed glass fiber filter under reduced pressure. The filter is then dried at 105 °C and the SS weight is determined by weighing [28]: N-NH_4—ammonia nitrogen, N-NO_3—nitrate nitrogen, N-NO_2—nitrogen dioxide, N-inorg—inorganic nitrogen, N-total nitrogen, P–total phosphorus, and RAS—dissolved inorganic salts. The test for the presence of toxicity in wastewater was performed on Vibrio Fischer in undiluted samples, 10-times-diluted, and 100-times-diluted samples. Ecotoxicity was tested on fish, pearl oysters, and algae in undiluted, 10-times-diluted, and 100-times-diluted samples.

4.3. Values of the Monitored Indicators

Tables 2 and 3 show the minimum and maximum values of the monitored influent and effluent parameters at the wastewater treatment plant.

Table 2. Values of selected parameters at the inflow to the wastewater treatment plant in mg/L [28].

Indicator	Minimum Value	Maximum Value
BOD	100	480
COD	220	950
SS	80	550
$N\text{-}NH_4$	15	85
N-inorg	15	85
N-total	25	88
P-total	2	10
RAS	720	720
pH	6.8	8.3

Table 3. Values of selected parameters at the effluent from the wastewater treatment plant in mg/L [28].

Indicator	Minimum Value	Maximum Value
BOD	1.5	8
COD	8	73
SS	1	15
$N\text{-}NH_4$	0	10
N-inorg	0.5	16
N-total	0	11
P-total	4	17
RAS	6	18
pH	0.7	3.6

Toxicity at the influent to the wastewater treatment plant, which was tested on Vibrio Fischer, ranged in the given period from 36 to 98% in the undiluted sample, 3–79% in the 10-times-diluted sample, and 0.2–10.9% in the 100-times-diluted sample.

Only one sample was taken at the effluent point and its toxicity was determined at 8% in the undiluted sample. As regards the ecotoxicity test at the influent to the wastewater treatment plant in an undiluted sample, fish mortality was 100%, water flea immobilization totaled 55–100%, and algae inhibition was at 20–100%. For the 10-times-diluted samples, fish mortality was 0–50%, water flea immobilization was 0–63%, and algae inhibition was 0–30%. Only one sample was taken at the effluent point and the values were only determined for the undiluted sample—fish mortality was at 0%, water flea immobilization at 7%, and algae inhibition at 19% [28].

5. Discussion

During the COVID-19 epidemic, the above-mentioned wastewater treatment plant was repeatedly in an emergency situation. The first such "signal" that something was happening at the wastewater treatment plant was the detection of higher nitrite values in the effluent and the impossibility of reducing the concentration of total phosphorus in the wastewater. Over the monitored period, the nitrite values were measured in tens of $mg \cdot L^{-1}$. Such values are already very toxic for living organisms. As regards the problem of reducing the concentration of total phosphorus in wastewater, the doses of the precipitant, ferric sulfate, were increased. However, despite such increased doses, no reduction was achieved. Ferric sulfate dosing lowers the wastewater pH, which should not drop below 6.3. pH values of <6.3 cause the nitrification process to stop. During this period, it was necessary to bring activated sludge from another wastewater treatment plant, which was costly and time-consuming but offered an important solution to the given situation. The water in the secondary tank had a gray color. Another manifestation of the presence of ecotoxicity/toxicity in the wastewater was the formation and accumulation of froth at the effluent point from the wastewater treatment plant. The grass growing around the outlet was yellow-brown (considered to have been "burned" by the wastewater). The centrifuged sludge sample was more or less disinfected. To illustrate this conclusion, the concentrations of thermophilic coliform bacteria in a sample taken before the monitored event were at 57,000 KTJ. In the monitored period, this concentration dropped to a value of 1200 KTJ.

6. Conclusions

The topic that this article deals with has not yet been sufficiently explored in the context of the Czech Republic. In the Czech Republic, there is no valid legislation setting limiting values/boundaries for ecotoxicity/toxicity in discharged wastewater.

Legislation for the assessment of ecotoxicity/toxicity should be introduced in the Czech Republic. This legislation should define the methods of determining ecotoxicity, set limits for the discharge of wastewater into public sewers for significant customers, and set limits for discharges from wastewater treatment plants.

The operators of wastewater treatment plants and basin managers should be more concerned with the issue of ecotoxicity/wastewater toxicity due to potential accidents at wastewater treatment plants, which may result in the pollution of the recipient area. Each operator should continuously evaluate the ecotoxicological risks and global synergistic effects of ecotoxicity on water life.

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