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Review

Evaluation of Ecotoxicity of Wastewater from the Full-Scale Treatment Plants

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Abstract: In this work, the influence of wastewater from full-scale wastewater treatment plants (WWTPs) on aquatic and soil biota was reviewed and presented. Moreover, the methods and model organisms used in testing the ecotoxicity of wastewater were shown. It was found that wastewater usually affected the biochemical activity and growth of organisms such as bacteria, algae and protozoa. They contributed to the immobilization and death of *inter alia* crustaceans and fishes. The values of degree of inhibition or lethality widely varied dependent on the type of wastewater, the sampling point (influent or effluent) and the model organisms applied in the biotests. Thus, a battery of ecotoxicity tests using model organisms of different sensitivities should be employed. So far, bacteria (e.g., *Vibrio fischeri*), green microalgae (e.g., *Raphidocelis subcapitata*) and crustaceans (*Daphnia magna*) have been frequently used organisms in the biological assessment of wastewater. They were applied in almost half (bacteria) or more than half (microalgae, crustaceans) of papers analyzed in this study. In almost all studies, the reduction of wastewater toxicity after treatment processes was found. It was proven that the conventional activated sludge systems were efficient in the removal of wastewater toxicity from both municipal and industrial wastewater, while the tertiary stage of treatment, in particular chlorination or ozonation, contributed to the increase in wastewater toxicity.

Keywords: aquatic environment; biota; ecotoxicity; full-scale wastewater treatment plant; soil; wastewater

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1. Introduction

Wastewaters contain a variety of chemicals of diverse, often unknown, origins. These are not only nutrients but also pharmaceuticals, surfactants, disinfectants and other compounds that occur in the influent of wastewater treatment plants (WWTPs) in very low concentrations, ranging usually between 0.1 and 10 $\mu g \times L^{-1}$ [1–3]. Due to their low concentrations in wastewater, these compounds have been defined as micropollutants [2]. Simultaneously, it should be regarded that wastewaters are the heterogeneous mixture of compounds of different particle sizes: dissolved substances, colloids and suspended solids [4]. It is obviously impossible to determine all chemicals present in wastewater using even advanced chromatographic techniques. Thus, it is worth considering the application of the bioassays that are able to detect the cumulative effects of known and undetected chemicals of wastewater by treating the wastewater sample as a whole [5,6].

The composition of wastewater influences the treatment processes, in particular the biochemical transformations of the pollutants and the efficiency of their removal [4,7]. Furthermore, it affects the quality of the effluent of WWTPs, which is introduced to the surface waters or to the terrestrial compartment. Taking the fact into account that, according to European Environment Agency, in 2015 only 40% of Europe's surface water bodies achieved good ecological status (https://www.eea.europa.eu/themes/water; accessed on 26 May 2022), it is extremely important to increase the efficiency of wastewater treatment processes and, as a result, to protect the environment and secure the drinking water supply for the population.

Apart from the technological and scientific advances on water and wastewater treatment processes, much political and legal effort has also gone into improving the status of water over the past decades. In the European Union (EU), the Water Framework Directive (WFD) 2000/60/EC was implemented [8]. It requires the EU member states to achieve a good status for all bodies of surface water and groundwater. This directive delivers the framework and describes the steps to reach the common goal instead of adopting the more traditional limit-value approach. With regard to surface water, two elements, i.e., “good ecological status” and “good chemical status”, were introduced to evaluate the status of water bodies (https://ec.europa.eu/environment/water/water-framework/info/intro_en.htm, accessed on 26 May 2022). Ecological status is influenced by water quality (e.g., pollution) and habitat degradation and is used as a proxy for the overall status of water bodies (https://www.eea.europa.eu/ims/ecological-status-of-surface-waters, accessed on 26 May 2022). The WFD [8] was supplemented inter alia by the Directive 2013/39/EU [9], including the list of the priority substances and environmental quality standards (EQS) for the substances in surface waters. These two directives indirectly encourage the ecotoxicological evaluation of wastewater, because chemical pollution of surface water caused inter alia by discharged streams of wastewater contributes to the toxicity towards aquatic organisms and poses a threat to the environment [9]. The application of ecotoxicity tests is beneficial at the beginning of wastewater treatment processes, i.e., before the biological part of the WWTP [6,10–13], as well as at the end of treatment for the effluent that is directed to the aquatic or terrestrial ecosystems. In the first of these cases, it allows for the prediction of potential disturbances in biological wastewater treatment processes, while in the second one, it enables the assessment of the ecological quality and safety of the effluent.

In order to evaluate the ecotoxicity of wastewater, a variety of bioassays using different model organisms (e.g., bacteria, algae, crustaceans, fishes) can be employed [10,12,14–16]. The results of these tests (e.g., the effect concentration EC50 or the lethal concentration LC50) are often recalculated to toxic units (TU) in order to classify the potential toxicity of wastewater. In 2003, Persoone et al. [17] introduced the classification of the water and wastewater toxicity that became the most commonly used system in the last two decades [12,17–21]. This classification is the result of the international cooperation between the Flemish community in Belgium and the countries of Central and Eastern Europe [17]. The water/wastewater samples are classified into one of five categories based upon the highest value of TU found in one of the ecotoxicity tests made for them. For wastewater discharged into the aquatic environment, the following categories were distinguished: class I–no acute toxicity, $TU < 0.4$; class II–slight acute toxicity, $0.4 < TU < 1$; class III–acute toxicity, $1 < TU < 10$; class IV–high acute toxicity, $10 < TU < 100$; class V–very high acute toxicity $TU > 100$ [17].

The assessment of wastewater toxicity is still drawing the attention of scientists, who are continually publishing papers on this subject in the latest years of 2019–2021 [6,10,14,18,19], and practitioners, who need knowledge of the latest results and advances in this area. In order to check the last achievements of the potential impact of wastewater on biota regarding the species level, the papers published between 2011 and 2021 were analyzed and compared in this work. It was divided into the three following parts: types of wastewater and sampling points, organisms and tests used for evaluation of wastewater toxicity and effects of wastewater on biota. The main aim of this review is to present the influence of wastewater from full-scale wastewater treatment plants on aquatic organisms (species level) and to indicate the methods and model organisms used in the evaluation of wastewater biotoxicity.

2. Methods Used in the Review of Literature

In order to review the literature concerning the evaluation of wastewater toxicity, two databases, i.e., EBSCOhost Web and Web of Science Core Collection, available via the Library of Lodz, University of Technology, (Poland) were searched. The review was performed between 3 January and 2 March 2022. The keywords “wastewater”, “sewage”, “toxic”, “ecotoxic”, “wastewater treatment plant” and “WWTP” were selected to search for the relevant data using the mode “Advanced search” in each database. Three of these keywords were joined with the help of Boolean “AND” and used in each query in each database in the field "Abstract". The following combinations of the keywords were used for the purpose of searching: (1) "wastewater" AND "toxic" AND "Wastewater treatment plant", (2) "wastewater" AND "ecotoxic" AND "Wastewater treatment plant", (3) "wastewater" AND "toxic" AND "WWTP", (4) "wastewater" AND "ecotoxic" AND "WWTP", (5) "sewage" AND "toxic" AND "Wastewater treatment plant", (6) "sewage" AND "ecotoxic" AND "Wastewater treatment plant", (7) "sewage" AND "toxic" AND "WWTP", (8) "sewage" AND "ecotoxic" AND "WWTP". Moreover, all types of documents, written in English only, were sought covering the time span from 2011 and 2021. It was performed for about the last 10 years in order to show the most up-to-date review of the results of ecotoxicity tests performed in full-scale WWTPs. The total number of documents found varied from 2 to 188, dependent on the combination of keywords and the database used for searching. In order to refine the results obtained in each query, the abstracts of the documents were initially reviewed to check whether their content was suitable to the subject of this study. The scientific papers presenting the results of the ecotoxicity of real wastewater (liquid phase) from the full-scale WWTPs towards aquatic or, if needed, soil living organisms were selected for further studies. Finally, about 25 papers published from 2011 to 2021 were subjected to the thorough analyses and then cited in this work.

3. Types of Wastewater Tested and Sampling Points

Full-scale WWTPs treating different types of wastewaters, i.e., municipal, domestic, storm water and industrial wastewater, have been subjected to the ecotoxicological investigations so far. About half of the studies published in the last decade concerned domestic and/or municipal wastewater (Table 1, Table 2, Table 3, Table 4 and Table S1). With regard to the industrial wastewater, these were wastewaters from textile companies [22], pharmaceutical manufacturers [23], hospitals [11] as well as piggery wastewater [14] or wastewater containing acrylonitrile [21]. These articles show that various types of industrial wastewater were tested, and there was no domination of one type of wastewater that was analyzed in terms of ecotoxicity more frequently than the other ones (Table S1). At the same time, the effect of stormwater on biota was rarely (only one paper) examined in the last years [24].

Wastewater treated in WWTPs located in various countries and continents was tested regarding its ecotoxicity, which was included in this review. This was wastewater from Europe (e.g., Italy, Germany, Poland), Asia (China, Thailand), Africa (South Africa, Tunisia) and Australia. The studies on wastewater from such different locations gives the audience a broad view of the variation of toxicity of wastewater all over the world.

The samples for bioassays were usually withdrawn either from the effluent or from both the influent and effluent of WWTPs, as illustrated in Figure 1. It shows the approaches used in the evaluation of wastewater toxicity in the last decades. In some works, the changes of wastewater toxicity along the wastewater treatment system were determined, and for this purpose several sampling points were selected and subjected to analysis (Figure 1). Apart from the influent and effluent samples, there were additional samples taken from the anaerobic, anoxic and aerobic parts of the activated sludge chamber [12,13]. In other studies, the samples from the post-treatment processes, such as ozonation or UV-radiation—i.e., the secondary and tertiary effluents—were tested [11,16]. It was conducted to check whether the application of the advanced chemical oxidation processes or other post-treatment physicochemical methods did not cause the undesired increase in wastewater toxicity. A particular group of by-products formed, i.e., nitro-products, as a result of advanced oxidation processes (AOP) were more toxic than primary pollutants [25]. A relatively large number of works published in the last decade concerned the direct impact of full-scale WWTPs on the receiver, and thus in these works the whole effluent toxicity (WET) approach was applied. This approach enables the identification of the detrimental effects of the pollutants present in the treated wastewater on various ecosystems, in particular on their biotic parts [16]. It was included in the legislation of many countries [16,26]. For example, EPA recommends using the WET tests in the National Pollutant Discharge Elimination System (NPDES) permits together with the requirements based on chemical-specific water quality criteria (epa.gov/npdes/permit-limits-whole-effluent-toxicity-wet#requirements, accessed on 20 May 2022). The WET requires the application of the battery of bioassays comprising tests on living aquatic organisms (plants, vertebrates, invertebrates) representing different trophic levels (https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods, accessed on 20 May 2022) [16].

Determination of wastewater toxicity was also applied for the comparison of the effectiveness of various technological solutions, in particular biological processes, used in the full-scale WWTPs. The majority of the papers analyzed (about 65%) dealt with this issue (Table S1). For example, Librelato et al. [27] compared the quality of the effluents from the sequencing batch reactor (SBR) and membrane bioreactor (MBR), while Hamijanda et al. [11] evaluated the effectiveness of the conventional activated sludge (CAS) and rotating biological contactor (RBC). The influence of wastewater treatment technology applied in the WWTPs under study on the toxicity of wastewater is addressed in Section 5, which addresses the effect of wastewater on aquatic organisms.

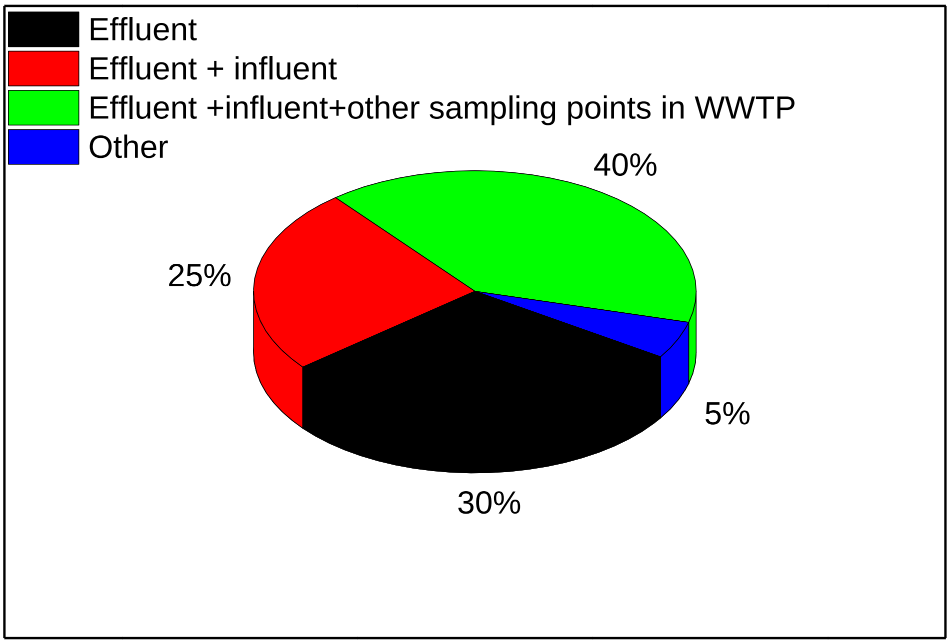

Figure 1. Sampling points of wastewaters analyzed for their impact on aquatic and soil biota.

4. Organisms and Tests Used for Evaluation of Wastewater Toxicity

A wide variety of organisms were used for the evaluation of wastewater toxicity in the full-scale WWTPs (Table S1). Aquatic organisms belonging to bacteria, algae and crustaceans dominated in these tests [10–16,18–24,26–28]. Apart from those, protozoa [10,14,18,19], fish [12,21], snails [13], worms [13] and aquatic plants [22] were also employed in the assessment of wastewater toxicity. Typically, terrestrial species were used as model organisms in about 8% of the studied works [5,22]. Their application is connected with the fact that treated wastewater might be discharged to the soil and reused for irrigation in the countries suffering from problems induced by a lack of water.

In analyzing the selection of the model organisms in terms of the type of wastewater (e.g., municipal, industrial), it was found that the selection of species was independent of the type of wastewater. Regarding bacteria (Table 1) and crustaceans (Table 3), about 45% and 54% of the analyzed papers, respectively, concerned industrial wastewater. At the same time, in the case of algae (Table 2), the papers dealing with industrial wastewater made up about 30% of the studies subjected to analysis in this work. It proves that bacteria and crustaceans were used with approximately the same frequency for the determination of toxicity of industrial and municipal wastewater. Moreover, it reveals that these two groups of organisms were the most often used model organisms in the assessment of the ecotoxicity of industrial wastewater (Table S1).

In nature, bacteria are common decomposers involved in the early stages of organic matter decomposition. They play a key role in the degradation of the carbonaceous compounds in wastewater, too. Among the ecotoxicity tests involving bacteria, the most often conducted were those in which a bioluminescent Gram-negative marine bacterium *Vibrio fischeri* was used as a model organism (Tables 1 and 5). The bioluminescence reaction of these bacteria is associated with the electron transport system in their cellular respiration and indicates the metabolic activity of the cells, i.e., the decrease in bioluminescence indicates a decrease in the cellular respiration [28]. The *Vibrio fischeri* bioluminescence inhibition test is usually conducted in agreement with the ISO 11348–3 standard [29], while the determination of the kinetics of the wastewater inhibitory effect is performed according to the ISO 21338 [30]. Apart from *Vibrio fischeri*, two other species of bacteria, *Photobacterium phosphoreum* and *Escherichia coli*, were used in the ecotoxicity tests on wastewater (Tables 1 and 5). At the same time, mixed cultures of bacteria (e.g., activated sludge) that usually participate in the wastewater treatment processes were hardly ever used as test organisms. The response of these bacterial consortia on the influent wastewater containing potentially toxic compounds is very valuable and useful for the operators of full-scale WWTPs. It may help the practitioners make a decision concerning the operation of wastewater treatment systems, in particular biological processes, so that the sufficient level of pollutants' removal is held [6,20,31]. Therefore, the ecotoxicity tests with mixed cultures of bacteria involved in wastewater treatment processes should be more frequently used for the evaluation of the effect of the influent on the effectiveness of full-scale WWTPs.

Table 1. Ecotoxicity data for wastewater, obtained in the tests with bacteria used as model organisms.

Wastewater	Species	Experimental Conditions	Method	Endpoint	Result	Remarks	Reference
Influent and effluent from 12 WWTPs in industrial parks (China).	*Photobacterium phosphoreum* T3 spp.	Bioluminescence of photobacterium T3 was measured by a biological toxicity detector.	Bioluminescence inhibition test.	Inhibition percentage	Inhibition degree of influents from 25.9% to 100%, while for effluents it varied from 18.5% to 91%.	Generally, the decrease in inhibition after treatment was found. Only in sequencing batch reactor (SBR) was the increase in inhibition percentage was observed.	[10]
Two types of wastewater from the company processing meat: (1) the washing wastewater, (2) the condensate wastewater. The wastewater were treated in SBR.	*Vibrio fischeri*	Measurement of the emission of light for 15 min with various dilutions of wastewater and a suspension of luminescent bacteria.	Microtox® test made in agreement with DIN ISO 11348–3, 1998.	EC50	Influents were highly toxic (EC50 < 60%), while the effluents from SBR were low or not toxic (EC50 > 82%).	The correlation between the ammonium nitrogen and the toxicity of wastewater from both the influents and the effluents was found.	[28]
Stormwater samples (51) were taken from the urban area of Sydney, Brisbane and Melbourne (Australia).	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	EN ISO 11348–3, 1998.	Toxicity equivalent concentration (TEQ)	TEQ ranged between 0.20 and 2.75 mg $\times$ L^{-1} for most samples.	The results were similar or slightly higher than those obtained for the secondary effluents of WWTPs. Highest effects similar to primary effluents.	[11]

Table 1. *Cont.*

Wastewater	Species	Experimental Conditions	Method	Endpoint	Result	Remarks	Reference
The antibiotics wastewater treatment plant treating wastewater from a drug manufacturer (Shijiazhuang City, China); 15 samples from five sampling points incl. SBR, biofilm reactor and secondary clarifier.	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	ISO 21338, 2010.	Toxicity unit (TU50) calculated upon EC50–15 min.	TU50 varied from 1.40 to 49.75% depending on the sampling point.	The raw wastewater samples were highly toxic, and the wastewater toxicity decreased during the treatment process. A significant, positive linear correlation between TU50 and indicators of wastewater contaminations (e.g., chemical oxygen demand (COD), biochemical oxygen demand (BOD5), NH_4^+ and others).	[23]
Samples taken seasonally (four times per year) for 4 years from two WWTPs with conventional activated sludge systems. Each time samples were taken from influent, secondary and tertiary effluent.	*Vibrio fischeri* (bacteria NRRL B-11177)	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	Microtox® test	EC50 transformed to toxic units (TUs)	TU values varied from 3.8 to 40.0 for the influents. TUs varied from below 0.1 to 1.8 for the secondary effluents and for the tertiary effluents.	Toxicity of the effluents was usually lower in autumn and winter than in spring and summer. *V. fischeri* was less sensitive than *P. subcapitata* and *D. magna.*	[16]
Samples of 21 sites including three WWTPs (Australia) were taken.	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	EN ISO 11348–3, 1998.	Toxicity equivalent concentration (TEQ)	E.g., in the Oxley Creek, WWTP toxicity decreased from 25.6 mg × L^{-1} to 1.26 mg × L^{-1}. In the Caboolture Enhanced Wastewater Treatment Plant (EWTP), with ozonation and activated carbon treatment toxicity was reduced to 0.56 mg × L^{-1}.	Decrease in toxicity was found after activated sludge treatment, reverse osmosis, advanced oxidation. Chloramination and microfiltration caused increase in toxicity.	[32]
Samples of influent and effluent from the conventional WWTP (Zgierz, Poland). Long-term (13 months) and short-term (two weeks) measurement campaigns were conducted.	*Escherichia coli*; Activated sludge microorganisms	Method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. The presence of toxic substances in the sample decreases the rate of resazurin reduction, which can be measured colorimetrically.	ToxTrak™Method 10017, HACH LANGE Manual.	Degree of inhibition (DI), toxicity unit (TU)	DI for raw wastewater varied from 10.2 to 59.8%, whereas for the treated ones from 3.3 to 35.6%. Out of 25 samples of the effluent, 2 belonged to class III and 23 samples were ranked as class IV.	The toxicity of the effluent was always lower than that of influent. The linear correlation between the toxicity of the influent and effluent was found. Lower toxicity of raw wastewater was observed in summer than in winter.	[20]
Effluent samples from two textile WWTPs (Ksar Hellal, Tunisia).	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	Standard UNI EN ISO 11348–3, 2007.	EC50; LOEC; NOEC; TU	One effluent was toxic: EC50 = 3%, LOEC = 0.9, TU = 33.1, while the second one did not cause any toxicity.	*V. fischeri* was relatively good bioindicator for testing toxicity of the textile wastewaters.	[22]

Table 1. *Cont.*

Wastewater	Species	Experimental Conditions	Method	Endpoint	Result	Remarks	Reference
Samples from five sampling points (incl. influent and effluents) from the pilot plant and the full-scale WWTP in Koblenz (Germany).	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	Standard UNI EN ISO 11348–3, 2007.	EC50	EC50 for influent was 1.49 ± 0.41 REF (Relative factor of enrichment). Reduction of toxicity by 86.1% in the full-scale WWTP.	Baseline toxicity was effectively removed in the activated sludge systems.	[13]
Influent and effluent from three full-scale WWTPs in Tuscany (Italy).	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri.*	Standard UNI EN ISO 11348–3, 2007.	Inhibition percentage	Inhibition percentage varied widely from −40.8% to 95.4%. Reduction of toxicity after treatment processes was usually found.	One of two of the most sensitive bioindicators used in this work; 90% of samples induced a significant bacterial inhibition.	[5]
Samples from six sampling points (incl. influent and effluents) from the full-scale WWTP treating pigment-containing wastewater (China).	*Photobacterium phosphoreum*	Acute toxicity tests of biolumineœent bacteria.	Standard National Environmental Protection Administration, China, NEPA, 1995.	EC50; TU	TU varied from 0 to 5.5.	The highest toxicity was found in the anoxic tank effluent (TU = 5.5). Reduction of toxicity after treatment (to TU = 0) in the final effluent was noticed.	[12]

Algae are the most commonly used model organisms (about 80% of papers with primary producers applied) representing the trophic level of primary producers that were employed in the evaluation of wastewater toxicity. These are most of all the green microalgae *Raphidocelis subcapitata*, formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata* (Tables 2 and 5). The ecotoxicity tests with these green microalgae adhered to the procedure of ISO norm 8692 [33] and the Organization for Economic Co-operation and Development (OECD) Guideline 201 [34]. Exponentially growing microalgae are exposed to the wastewater in batch cultures over a period of 72 h [29]. Inhibition of the algal growth relative to the control is determined by the measurement of the algal biomass expressed usually by optical density (OD). Based on these data, the growth inhibition rate is calculated [29]. Other species of algae that have been used in the studies for testing of wastewater toxicity are the green algae from the genus *Scendesmus* (e.g., *Scenedesmus obliquus*, *Scenedesmus quadricauda*) or from the genus *Chlorella* (e.g., *Chlorella vulgaris*), and diatoms *Phaeodactylum tricornutum* Bohlin (Table 2). The latter are saltwater phytoplanktonic biological model organisms present in transitional, marine-coastal and marine waters [27]. The toxicity test with *P. tricornutum* is based on the determination of growth inhibition, as it is in the case of *R. subcapitata* [27]. Besides the algae, aquatic and terrestrial plants also representing primary producers were applied as bioindicators to assess the toxicity of wastewater. These tests aimed at the measurement of the seed germination rate and the effects of wastewater on root and/or shoot elongation (Table 4). However, the number of bioassays on plants performed in the last decades is several times lower in comparison to those on algae (Tables 2 and 4). It is most probably due to two reasons. Algae are more typical organisms in the aquatic ecosystems than higher plants. Moreover, algae are more sensitive test organisms than higher plants with regard to testing wastewater toxicity. Palli et al. [5], who used both—i.e., three higher plants (*Sorghum commune*, *Lepidium sativum* and *Cucumis sativus*) and microalgae (*Raphidocelis subcapitata*)— to evaluate the toxicity of wastewater reported that *R. subcapitata* were more sensitive than the terrestrial plants. Furthermore, other authors showed that the green microalgae *R. subcapitata* belonged to the most sensitive aquatic model organisms used in the bioassays testing wastewater toxicity [19, 22]. At the same time, according to Hamjinda et al., [11] higher plants (*Lepidium sativum* and *Cucumis sativus*) turned out to be sensitive towards textile wastewater (Tables 4 and S1).

Table 2. Ecotoxicity data for wastewater obtained, in the tests with algae used as model organisms.

Wastewater	Species	Experimental Condition	Method	Endpoint	Result	Remarks	Reference
Influent and effluent from 12 WWTPs in industrial parks (China).	*Euglena gracilis*	Measurement of absorbance at 610 nm.	Growth inhibition test. Acute toxicity.	Degree of growth inhibition	Degree of inhibition for influents and effluents varied from 42.8% to 77.3%.	For 9 out 12 WWTPs, no significant change in the degree of inhibition between influent and effluent was reported.	[10]
Effluents (93 samples) from SBR and MBR treating domestic, municipal and industrial wastewater. WWTPs were located in Venice (Italy).	*Phaeodactylum tricornutum* Bohlin	*P. tricornutum* was exposed to increasing concentrations of samples for 72 ± 2 h at 20 ± 1 °C and 6000–10,000 lux. Cell density was measured.	Growth inhibition or stimulation was determined according to ISO 10,253 method.	Toxicity unit (TU_{50}); biostimulation unit (BU_{50})	91% of all samples showed a stimulation effect. Among them 7, 30, 31 and 7 samples with low, medium, high and very high effects, respectively, were detected. In general, 90% of samples showed from medium to very high stimulation or toxicity effects.	Toxicity classification system based on inhibition and stimulation of microalgal growth was established.	[27]
Stormwater samples (51) were taken from urban areas of Sydney, Brisbane and Melbourne (Australia).	*Pseudokirchneriella subcapitata*	Inhibition of photosynthesis was assessed after 2 h of exposure using I-PAM (imaging pulse-amplitude-modulated) fluorometry and inhibition of growth rate after 24 h exposure.	The combined algae test integrates the quantification of the inhibition of photosynthesis with specific and non-specific effects on the growth rate.	IPAM: diuron equivalent concentration (DEQ). Algal growth: TEQ	In most samples photosynthesis was more sensitive endpoint than growth inhibition.	Algal toxicity was caused by herbicides in most samples.	[24]
Samples taken seasonally (four times per year) for 4 years from two WWTPs with conventional activated sludge systems. Each time samples were taken from influent, secondary and tertiary effluent.	*Pseudokirchneriella subcapitata*	Algae growth was determined by the measurement of optical density.	Algaltoxkit F^{TM} according to OECD method no. 201, 2011	EC50 transformed to toxic units (TUs)	TUs > 100 for the influents; TUs varied from 1.3 to above 100 for the secondary effluents; TUs varied from 0.4 to above 100 for the tertiary effluents.	Algae were the most sensitive species out of four species tested in this work. Seasonal decrease in the toxicity of effluents in autumn and winter in comparison to spring and summer.	[16]
Samples of 21 sites including 3 WWTPs (Australia) were taken.	*Pseudokirchneriella subcapitata*	Inhibition of photosynthesis was assessed after 2 h of exposure using I-PAM (imaging pulse-amplitude-modulated) fluorometry and inhibition of growth rate after 24 h exposure.	The combined algae test integrates the quantification of the inhibition of photosynthesis with specific and non-specific effects on the growth rate.	IPAM: diuron equivalent concentrations (DEQ). Algal growth: TEQ	E.g., in the Oxley Creek, WWTP DEQ decreased from 2.15 to 0.98 $\mu g \times L^{-1}$. Caboolture Enhanced Wastewater Treatment Plant (EWTP) reduced toxicity from 0.26 to 0.09 $\mu g \times L^{-1}$.	Decrease in toxicity was found after activated sludge treatment, reverse osmosis. Enhanced treatment (e.g., UV radiation, microfiltration, ozonation) did not alter the toxicity towards microalgae.	[32]
Piggery wastewater effluent samples were collected from a farm in Stellenbosch (Western Cape province, South Africa).	*Pseudokirchneriella subcapitata*	72 h growth rate inhibition test	ALGALTOXKIT F, in agreeement with ISO norm 8692 and OECD method no. 201.	Percentage inhibition (%); EC50–48 h	*P. subcapitata* exposed to 1% unfiltered piggery effluent did not show any toxicity. EC50–48 h values for 10 and 20% unfiltered piggery effluent were 49.3% and 13.9%, respectively.	Unfiltered piggery effluent at concentration 10% and 20% can be regarded as toxic.	[14]

Table 2. *Cont.*

Wastewater	**Species**	**Experimental Condition**	**Method**	**Endpoint**	**Result**	**Remarks**	**Reference**
Samples of the effluent from the WWTP in the region of the Western Cape (South Africa).	*Pseudokirchneriella subcapitata*	72 h growth rate inhibition test	ALGALTOXKIT F, in agreeement with ISO norm 8692 and OECD method no. 201.	Percentage inhibition (%); LC; TU	TU varied from 0.234 to 1.000, indicating low acute toxicity.	The WWTP effluent can be toxic to microalgae. Lower toxicity was observed in summer than in winter and autumn.	[18]
Influent and effluent samples from three WWTPs (Bangkok, Thailand) treating hospital wastewater were taken. Conventional activated sludge systems were applied in all WWTPs. In two of them, the effluents were chlorinated.	*Chlorella vulgaris* and *Scenedesmus quadricauda*	72 h growth rate inhibition test	OECD method no. 201.	EC50; TU	The values of EC50 determined for *Ch. vulgaris* ranged from 13.83 to 17.16% (v/v) for influents and from 41.33 to 51.60% (v/v) for effluents. In the case of *S. quadricauda*, these were from 9.81 to 13.63% (v/v) for influents and from 45.8 to 87.1% (v/v) for effluents.	All hospital wastewaters showed similar toxic levels to the test algae. Toxicity decreased after treatment. TU of the effluents was from 1.15 to 2.42. *S. quadricauda* was more sensitive than *C. vulgaris* to hospital wastewater.	[11]
Effluent samples from two textile WWTPs (Ksar Hellal, Tunisia).	*Raphidocelis subcapitata*	72 h growth rate inhibition test	Standard UNI EN ISO 8692:2005.	Inhibition percentage	Inhibition percentage varied from 0 to 69.3%.	*R. subcapitata* is a good bioindicator for testing toxicity of the textile wastewaters.	[22]
Samples from five sampling points (incl. influent and effluents) from the pilot plant and the full-scale WWTP in Koblenz (Germany).	*Desmodesmus subspicatus*	72 h growth inhibition test	Standard ISO 8692, 2012.	Algae cell number	Increase in algal growth in all treatments was found.	The use of the classic growth inhibition test to determine phytotoxic effects of wastewater should be considered.	[13]
Influent and effluent from three full-scale WWTPs in Tuscany (Italy).	*Raphidocelis subcapitata*	72 h growth inhibition test	Standard UNI EN ISO 692:2012, 2012.	Inhibition percentage	Inhibition percentage varied from 10.1 to 98.2%. Reduction of toxicity after treatment.	One of two the most sensitive bioindicators used in this work; 90% of samples induced a significantly algal inhibition.	[5]
Samples from each stage of treatment (incl. influent and effluents) from three full-scale WWTPs of different treatment systems (SBR, conventional activated sludge and Linpur) were tested.	*Scenedesmus obliquus*	72 h growth inhibition test	OECD method no. 201, 2006.	Inhibition percentage (refers to cell density, chlorophyll-A synthesis, super-oxidedismutase (SOD) activity). Percentage of cell viability (refers to cell membrane integrity).	The increase in cell growth was observed in all WWTPs studied. Only the effluent from NaClO disinfection units inhibited the cell growth by 131.8%. Analogous results were found for activity of SOD and chlorophyll-A synthesis. Percentage of cell viability decreased from 0.33% to 17.5%.	The acute toxicity of municipal wastewater on chlorophyll-A synthesis in *S. obliquus* was significantly correlated to phosphorus and organic carbon concentration. SOD activity and chlorophyll-A synthesis were found to be sensitive endpoints for the municipal wastewater studied.	[15]
Effluents from 17 municipal WWTPs of different sizes located in Poland.	*Pseudokirchneriella subcapitata*	72 h chronic growth inhibition biotest	Algaltoxkit, procedure, 1996.	Inhibition percentage	The mean growth inhibition percentage varied from 11 to 100%.	High acute hazard was noted for four WWTPs tested. *P. subcapitata* was sensitive bioindicator for treated wastewater.	[19]

With regard to the consumers, the planktonic crustaceans have been the most frequently applied in the ecotoxicity tests of wastewater so far (Table S1). The absolutely dominant species of crustacean used as a bioindicator was *Daphnia magna* (Tables 3 and 5), while the dominant type of test was the acute immobilization test according to the OECD Guideline 202 [35]. Young daphnids, aged less than 24 h at the start of the test, were exposed to the wastewater for a period of 48 h. Immobilization was recorded at 24 h and 48 h and compared with control values. The results are elaborated in order to calculate the EC50 at 48 h or optionally 24 h [35]. Analogous tests were in some studies performed with the use of *Artemia franciscana* as a model organism [16,22] or *Thamnocephalus platyurus* [19]. The latter organisms are incorporated in the kits in a "dormant" or "immobilized" form, from which they can be hatched or activated prior to the performance of the toxicity tests. Apart from crustaceans, the fish *Danio rerio* (Table 4) representing the consumers was also used in the toxicity tests for the evaluation of the effect of wastewater on aquatic biota [12,21]. *D. rerio* acute toxicity tests were carried out following the OECD Guideline 203 [36] or ISO 7346–3 [37]. The endpoint of these tests was the determination of the mortality of *D. rerio* exposed to wastewater samples.

Table 3. Ecotoxicity data for wastewater, obtained in the tests with crustaceans used as model organisms.

Wastewater	Species	Experimental Condition	Method	Endpoint	Result	Remarks	Reference
Influent and effluent from 12 WWTPs in industrial parks (China).	*Daphnia magna straus*	Determination of the number of immobilized individuals after 24 h and 48 h of exposure.	Immobilization inhibition test. Acute toxicity.	Degree of immobilization inhibition (%)	Degree of inhibition reached 100% after 48 h exposure in 4 out of 12 influents tested.	A large variation of results of tests. Degree of inhibition for influents and effluents varied from several percent to 100%.	[10]
Samples taken seasonally (four times per year) for 4 years from two WWTPs with conventional activated sludge systems. Each time samples were taken from influent, secondary and tertiary effluent.	*Daphnia magna*	Neonates were incubated at the appropriate conditions, and after 24 h and 48 h, the number of dead/immobilized neonates was calculated.	Daphtoxkit F^{TM} according to OECD method no. 202, 2004.	EC50 transformed to toxic units (TUs)	TUs > 100 for the influents; TUs varied from 0.4 to above 100 for the secondary effluents; TUs varied widely from below 0.05 (non-toxic) to above 100 (very toxic) for the tertiary effluents	The toxicity to *D. magna* (48 h) was at the same level as toxicity to *P. subcapitata* determined in this work.	[16]
	Artemia salina	Method based on determination of immobilization of *Artemia nauplii* after 24 and 48 h.	Methodology from US EPA, 2002.	EC50 transformed to toxic units (TUs)	TU values varied from 2.6 to 5.8 for the influents; *A. salina* was not affected by secondary and tertiary effluents of either WWTP (TUs < 0.1)	*A. salina* was the least sensitive indicator out of organisms used in the toxicity tests in this study.	[16]
Piggery wastewater effluent samples were collected from a farm in Stellenbosch (Western Cape province, South Africa).	*Daphnia magna*	48 h mortality/immobilization effect test	DAPHTOXKIT F, in agreement with ISO norm 6341 and OECD method no. 202.	Percent immobile (%); LC50	At concentration higher than 1%, piggery effluent caused significant percentage immobility of *D. magna* after 24 h exposure.	The different percentage concentration of piggery effluent and a high-level dose of mixtures of veterinary pharmaceutical can also cause acute toxicity to *D. magna.*	[14]
Samples of the effluent from the WWTP in the region of the Western Cape (South Africa).	*Daphnia magna*	48 h mortality/immobilization effect test	DAPHTOXKIT F, in agreement with ISO norm 6341 and OECD method no. 202.	Percentage mortality (%); LC; TU	Percentage of mortality after 48 h varied from 5% to 45%. TU varied from 0.944 to 1.	*D. magna* was the least sensitive organism in this study.	[18]

Table 3. *Cont.*

Wastewater	Species	Experimental Condition	Method	Endpoint	Result	Remarks	Reference
Influent and effluent samples from three WWTPs (Bangkok, Thailand) treating hospital wastewater were taken. Conventional activated sludge systems were applied in all WWTPs. In two of them, the effluents were chlorinated.	Microcrustacean: *Moina macrocopa*	48 h mortality/immobilization effect test	OECD method no. 202.	LC50; TU	The values of LC50 were from 32.37 to 38.16% (*v/v*) for influents, while in the case of effluents it was from 45.91 to 59.25% (*v/v*).	Treatment reduced the toxic effect on the tested organism. Chlorination did not give a negative effect on this organism.	[11]
Effluent samples from two textile WWTPs (Ksar Hellal, Tunisia).	*Daphnia magna*	24 h mortality/immobilization effect test	Standard UNI EN ISO 6341:2012	Mortality; TU	One effluent exhibited 100% mortality, while the second one did not cause any mortality.	Good bioindicator for testing toxicity of the textile wastewaters.	[22]
	Artemia franciscana	24 h mortality effect test	ARTOXKIT M in agreement with Ecotoxicological method 8060 of APAT-IRSA, 2003.	Immobilization percentage	Immobilization percentage varied from 0% to 40%.	*A. franciscana* is not recommended for testing toxicity of textile wastewater.	[22]
Samples from five sampling points (incl. influent and effluents) from the pilot plant and the full-scale WWTP in Koblenz (Germany).	*Daphnia magna*	48 h acute immobilization test	Standard ISO 6341, 2012.	Percentage of immobilization	No adverse effect of wastewater on *D. magna*.	Tests with *D. magna* occurred to be of limited relevance in evaluation of toxicity of wastewater.	[13]
Influent and effluent from three full-scale WWTPs in Tuscany (Italy).	*Daphnia magna*	48 h acute immobilization test	Standard UNI EN ISO 6341:2013	Inhibition percentage	Inhibition percentage was 0% except one sample, when it was 100%.	*D. magna* almost never responsed to the samples tested.	[5]
Samples from six sampling points (incl. influent and effluents) from the full-scale WWTP treating pigment containimg wastewater (China).	*Daphnia magna*	48 h acute immobilization test	OECD method no. 202.	EC50; TU	TU varied from 1.1 to 13.6.	The acute toxicity to *D. magna* was reduced by 91.8%, to which the anaerobic and aerobic biological treatment units contributed 65.3% and 12.5%, respectively.	[12]
Samples from eight sampling points (incl. influent and effluents) from the full-scale WWTP treated acrylonitrile containing wastewater (China).	*Daphnia magna*	48 h acute immobilization test	OECD method no. 202, 2004.	LC50; TU	TU varied from below 0.4 to 125.	Systems anaerobic oxic (A/O) and anaerobic oxic-aerobic biological fluidized tank (A/O-ABFT) used in the WWTP were efficient in removal of toxicity to *D. magna*. Effluent was not toxic to *D. magna*.	[21]

5. Effect of Wastewater on Aquatic Organisms

5.1. Effect on Bacteria

Acute toxicity tests using bacteria as model organisms were very often employed to assess wastewater toxicity in the last decades (Table 1). They were primarily used to compare the influent and effluent toxicity [5,20,23] and, in some cases, to follow the changes in toxicity of wastewater along full-scale WWTPs [12,13]. Raw wastewater or wastewater after the primary stage of treatment strongly affected the biochemical activity of bacteria. The degree of metabolic activity inhibition of *V. fischeri* reached even 100% [10] or up to 95.4% for the influents [5]. High inhibition percentages (above 50%) were reported in several studies, in which bacteria other than *Vibrio fischeri* were used for the evaluation of wastewater toxicity. For example, Yu et al. [10] used *P. phosphoreum* and found that the degrees of inhibition for the influents varied from 25.9% to 100%. At the same time, Liwarska-Bizukojc et al. [20] reported that the degree of inhibition of raw wastewater determined towards *E. coli* ranged from 10.2% to 59.8%.

Treatment processes in the full-scale WWTPs contributed to the reduction of toxicity of wastewater [5,12,13,20,23,28]. It was found for various types of wastewater, i.e., municipal and some industrial wastewater, and for different technologies of mechanical–biological treatment processes. The decrease in wastewater toxicity was noticed for various species of bacteria including both pure cultures (e.g., *V. fischeri*, *P. phosphoreum*, *E. coli*) and mixed cultures of bacteria (activated sludge). Volker et al. [13] reported that the reduction of toxicity of municipal wastewater treated in the activated sludge systems reached 86%. It was determined using Microtox® tests with *V. fischeri*. At the same time, similar values of the reduction of municipal wastewater toxicity up to 76.6% were found Liwarska-Bizukojc et al. [20] with the help of Toxtrak™ tests, in which *E. coli* or activated sludge bacteria were used as model organisms. Yu et al. [10] proved that the type of activated sludge system applied in the full-scale WWTP affected the efficiency of toxicity removal from wastewater. They found that most of the anaerobic–anoxic–oxic (A/A/O)-based processes removed wastewater toxicity more efficiently than the sequencing batch reactors (SBR) [10]. The application of the tertiary stage in full-scale WWTPs might contribute to the increase in wastewater toxicity in comparison to the toxicity of the secondary effluents. Macova et al. [32] reported that advanced oxidation processes (AOP) did not increase the toxicity of the tertiary effluents, while the microfiltration and chloramination did (Table 1).

Several studies showed that out of bacterial species used as bioindicators in the evaluation of wastewater toxicity, the bioluminescent bacteria *V. fischeri* turned out to be a good and sensitive model organism [5,22,23]. However, Vasquez and Fatta-Kassinos et al. [16] showed that *V. fischeri* were less sensitive than algae (*Pseudokirchneriella subcapitata*) and crustaceans (*Daphnia magna*) in the ecotoxicological assessment of municipal wastewater. Nevertheless, this simple and fast test with *V. fischeri* should not be excluded from the procedures used for the characterization of wastewater quality; in particular, it concerns the final effluents of WWTPs directed to the water bodies. At the same time, due to the fact that *V. fischeri* are marine bacteria, their usefulness in the determination of the toxicity of influents aiming at the protection of biological parts of WWTPs against toxic compounds seems to be limited.

5.2. Effect on Algae

The results of ecotoxicity tests towards algae showed that the growth of these organisms was affected by wastewater in different ways (Table 2). Both inhibition and simulation of algal growth were noticed. The inhibition of growth of the green microalgae exposed to wastewater was more frequently observed than stimulation effect was. Szklarek et al. [19] even noticed the total cessation (the percentage of inhibition equal to 100%) of *P. subcapitata* growth exposed to the effluents from the full-scale WWTPs treating municipal sewage. Yu et al. [10] reported that the percentage of inhibition of *E. gracilis* growth was from 42% to 77.3%, while Bedoui et al. [22] observed that the growth of *R. subcapitata* was inhibited by wastewater in the range from 0 to 69.3%. Both studies [10,22] concerned the toxicity of industrial wastewater (Table 2). Simultaneously, it should be noticed that the opposite results regarding the effect of wastewater on algal growth were also presented. Volker et al. [13] observed the increase in algal (*P. subcapitata*) growth for all samples of municipal wastewater including the influents and effluents. Zhang et al. [15] observed the increase in *Scenedesmus obliquus* growth exposed to municipal wastewater taken from various sampling points of the full-scale WWTPs. In the latter study, the only exception was the effluent from the disinfection stage that contributed to the inhibition of algal growth [15]. Hamjinda et al. [11] found the increase in wastewater toxicity after chlorination as well. It was caused by the reaction of the disinfectant reagent (NaOCl) with the residual organic matter and formation of organochlorine compounds, which were toxic for the aquatic organisms [11,15].

Therefore, wastewater subjected previously to chemical treatment processes inhibited the growth of green algae [11,15].

Wastewater treatment processes, in particular biological treatment, contributed to the reduction of wastewater toxicity towards algae [5,11]. It was the case of the hospital wastewater treated in the conventional activated sludge systems (CAS) or rotating biological contractors (RBC) [11] and in the case of urban wastewater containing hospital wastewater in the conventional activated sludge system [5]. Moreover, Zhang et al. [15] showed that CAS and SBR removed the toxicity of municipal wastewater towards *S. obliquus* to a higher extent than the Linpor system did.

In some studies, the effect of the seasonal variation of toxicity towards algae was analyzed. Unfortunately, the results of these studies did not allow for the formulation of unequivocal statements. Palli et al. [5] did not observe any evident correlation between the season and wastewater toxicity in the Italian full-scale WWTPs. Vasquez and Fatta-Kassinos et al. [16] reported the increase in toxicity of wastewater towards *P. subcapitata* in summer compared to winter, while Pereao et al. [18] found lower toxicity of wastewater towards *P. subcapitata* in summer than in winter. It shows that the results of ecotoxicity tests of wastewater to algae were strongly dependent on the WWTP under study, and it is difficult to generalize the effect of weather conditions on wastewater toxicity.

Generally, green microalgae are believed to be a good bioindicator for the evaluation of wastewater toxicity [16,19,22]. It is worth adding that superoxide dismutase (SOD) activity and chlorophyll-A synthesis were more sensitive endpoints in the evaluation of municipal wastewater toxicity in the full-scale WWTPs in China than traditionally used algal cell density (*S. obliquus*) [15]. In some studies, other organisms occurred to be more sensitive bioindicators than algae [13,18]. In spite of this, the species of microalgae should be used in the assessment of wastewater toxicity, in particular the toxicity of treated wastewater discharged to the environment [16,19,22].

5.3. Effect on Crustaceans and Other Model Organisms

The evaluation of wastewater toxicity towards crustaceans revealed that wastewater might completely immobilize crustaceans or might not affect them at all (Table 3). The results of ecotoxicity tests with *D. magna* varied widely even within the same study. Bedoui et al. [22] reported that daphnids exposed to two types of wastewater (untreated wastewater and biologically treated with *Pseudomonas putida*) exhibited 100% mortality, while in the case of wastewater biologically treated with activated sludge microorganisms, no mortality was observed. All three types of wastewater (untreated and treated using two different biomethods) came from the full-scale WWTP of a textile company [22]. Yu et al. [10] found that four out of twelve influents of the industrial WWTPs immobilized *D. magna* completely (100%), whereas the tests made for eight other influents showed the immobilization of *D. magna* at the level below 40%. In another study, it was proved that *D. magna* almost never reacted to the exposure on pharmaceutical wastewater [5]. Only in two samples taken from one out of three studied WWTPs was the total mortality (100%) found [5]. Moreover, Volker et al. [13] reported the lack of adverse effects of wastewater on *D. magna* mortality. At the same time, Yu et al. [10] found limitations in the application of the test on *D. magna* with undiluted effluents because most of the tested groups of crustaceans were either uninhibited or totally inhibited.

Biological treatment of wastewater usually decreased the toxicity of wastewater on *D. magna*. Vasquez and Fatta-Kassinos et al. [16] observed the reduction of toxicity on *D. magna* from TU > 100 for the influents to TU in the range from 0.4 to about 100 for the effluents. Deng et al. [12] estimated that the toxicity of pigment-contaminated wastewater towards *D. magna* was effectively reduced by 91.8%, to which the anaerobic and aerobic biological treatment units contributed 65.3% and 12.5%, respectively. Na et al. [21] found that toxicity of raw acrylonitrile wastewater to *D. magna* was very high (125 TU), while after biological treatment with the use of the anaerobic oxic-aerobic biological fluidized tank (A/O-ABFT), the final effluent displayed no acute toxicity (below 0.4 TU).

According to some authors, *D. magna* is an organism of limited relevance for the evaluation of wastewater toxicity [5,13]. It is mainly connected with the significant discrepancies in the response of daphnia to the same sample of wastewater. Simultaneously, *D. magna* was classified as more sensitive than the other crustaceans, that is, *A. salina* [16,22]. Thus, *D. magna* as the representative of crustaceans [21,22] might be used for the evaluation of wastewater toxicity, but it should be included in a battery of tests, in which model organisms other than crustaceans are employed.

Table 4. Ecotoxicity data for wastewater, obtained in the tests with other organisms (e.g., plants, protozoan, earthworms, snails).

Wastewater	Species	Experimental Condition	Method	Endpoint	Result	Remarks	Reference
Influent and effluent ww from 12 WWTPs in industrial parks (China).	*Tetrahymena thermophila*	Measurement of absorbance at 492 nm.	Growth inhibition test. Acute toxicity.	Degree of growth inhibition (%).	Degree of inhibition of influents and effluents was between 69.7 and 96.0% and 75.9 and 95.9%, respectively.	For 10 out 12 WWTPs, no change in the inhibition degree between influent and effluent was reported.	[10]
	Vicia faba	The number of micronucleated cells was determined.	Micronucleus test–genotoxicity evaluation.	Pollution index (PI)—the ratio of the mean rate of micronucleus between the treatment and control groups.	The values of PI ranged from 0.38 to 2.00.	A relatively low level of genetic toxicity on *V. faba* for most of the wastewater samples was found.	[10]
Piggery wastewater effluent samples were collected from a farm in Stellenbosch (Western Cape province, South Africa).	*Tetrahymena thermophila*	24 h reproductive inhibition test	PROTOXKIT F, adhered to OECD method no. 202.	Percentage inhibition (%); EC50	EC50 values varied from 4.81 to 52.39% depending on the concentration of piggery effluent.	A relationship between the percentage concentrations of toxicants and percentage growth inhibition of protozoa was found.	[14]
Samples of the effluent from the WWTP in the region of the Western Cape (South Africa).	*Tetrahymena thermophila*	24 h reproductive inhibition test	PROTOXKIT F, adhered to OECD method no. 202.	Percentage inhibition (%); EC50	TU varied from 84 to 89.6.	*T. termophila* was the most sensitive organism in this study. The effluents showed high acute toxicity to protozoa.	[18]
Effluent samples from two textile WWTPs (Ksar Hellal, Tunisia).	*Lemna minor*	7 d growth inhibition rate test	Standard ISO SO/WD 20079, 2001	Inhibition percentage	Inhibition percentage varied from 10.1 ± 4.2% to 52.7 ± 25%	*L. minor* was less sensitive than other bioindicators used in this study.	[22]
	Cucumis sativus and *Lepidium sativum*	72 h seeds germination and early growth tests.	Standard Method ISO 1651:2003, 2003.	Inhibition percentage	Inhibition percentage of germination varied from 20.0 ± 1.6% to 100 ± 0%. Inhibition percentage of root elongation varied from 77.0 ± 1.2% to 100 ± 0%.	Both plants were sensitive organisms to the textile wastewaters.	[22]
Samples from five sampling points (incl. influent and effluents) from the pilot plant and the full-scale WWTP in Koblenz (Germany).	*Potamopyrgus antipodarum*	28 d reproduction test	OECD method no. 242, 2016.	Mortality; number of embryos	Mortality of snails did not exceed 10%. The mean reproductive output was 17.9 ± 5.6 embryos in the control test. Exposure to wastewater effluents increased the reproduction by 10.4–31.1%.	No reproductive toxicity after direct exposure to conventionally treated wastewater.	[13]

Table 4. *Cont.*

Wastewater	Species	Experimental Condition	Method	Endpoint	Result	Remarks	Reference
	Lumbriculus variegatus	28 d reproduction test	OECD method no. 225, 2007.	Number of worms; biomass of worms	No significant effect on reproduction. Biomass exposed to the wastewater decreased compared to the control from 25.5 to 34.2%.	Decrease in biomass of earthworms but no effect on reproduction.	[13]
Influent and effluent from three full-scale WWTPs in Tuscany (Italy).	*Sorghum commune*, *Lepidium sativum*, *Cucumis sativus*	72 h germination and early growth test	Standard UNI 11357:2010.	Inhibition percentage; germination index (GI)	A large variation of results. Both toxic and stimulatory effects were found.	Inhibition phenomenon was observed in 37% of samples.	[5]
Samples from six sampling points (incl. influent and effluents) from the full-scale WWTP treatimg pigment containimg wastewater (China).	*Danio rerio*	96 h static acute toxicity test	Standard ISO 7346–3:1996, 1996.	EC50; TU	TU varied from 2.0 to 3.7.	Only 20% of the acute toxicity was removed.	[12]
Samples from eight sampling points (incl. influent and effluents) from the full-scale WWTP treated acrylonitrile containing wastewater (China).	*Danio rerio*	96 h static acute toxicity test	OECD method no. 203, 1992.	LC50; TU	TU varied from below 0.4 to 29.6.	After going through the A/O and ABFT wastewater treatment systems, the final effluent showed no acute toxicity to *D. rerio*.	[21]
Samples from five sampling points (incl. influent and effluents) from three full-scale WWTPs treated municipal wastewater using A/O system (China).	*Danio rerio*	96 h acute static test	OECD method no. 203, 1992.	Mortality rate (%)	Mortality rate varied from 0% to 50% ± 10%.	Acute toxicity was reduced along with the A/O process of treatment. Acute toxicity on zebrafish decreased in accordance with the COD removal.	[37]
Effluents from 17 municipal WWTPs of different sizes located in Poland.	*Thamnocephalus platyurus*	24 h mortality acute biotest	Thamnotoxkit procedure, 1995.	Mortality rate (%)	The mean mortality rate varied from 3 to 100%.	*T. platyurus* mortality demonstrated a very strong positive correlation with NH_4^+ and a strong with total nitrogen.	[19]
	Tetrahymena thermophila	24 h chronic growth inhibition biotest	Protoxkit procedure, 1998.	Inhibition percentage	The mean growth inhibition percentage did not exceed 40%.	The mean toxicity did not exceed acute hazard for all samples from WWTPs. Stimulation was found.	[19]

Apart from bacteria, algae and crustaceans, two other organisms representing protozoa and fishes have been relatively frequently used in the tests aiming at the evaluation of wastewater toxicity (Table 4). These are *Tetrahymena thermophila*, a genus of free-living ciliates, and the zebrafish *Danio rerio* belonging to the minnow family (*Cyprinidae*) of the *Cypriniformes* order. Both species are commonly present in freshwater, lakes and ponds. That is why they are particularly useful to evaluate the ecotoxicity of the final effluent from the full-scale WWTPs discharged to freshwater [14,18,19]. Yu et al. [10] determined the toxicity of the influents and effluents from the full-scale WWTPs of the industrial park in Jiangsu Province in China. The growth inhibition of *T. thermophile* varied from 69.7 to 96.0% and from 75.9 to 95.9% for the influents and effluents, respectively. It occurred that in 10 out of 12 full-scale WWTPs studied, the effects of wastewater on growth of protozoan *T. thermophila* did not change after treatment, while in one WWTP the decrease and in another WWTP the increase in toxicity of the effluent compared to the influent was found [10]. It shows a high variability of the ecotoxicity results obtained in the tests with *T. termophila* and makes the use of this organism for the assessment of wastewater toxicity questionable. Szklarek et al. [19] also noted that both the inhibition and stimulation of growth of *T. thermophila* exposed to the effluents from various full-scale WWTPs treating municipal wastewater were observed. The stimulation of growth of *T. thermophila* was recorded in almost 35% samples of all those analyzed [19]. At the same time, in two other studies *T. thermophila* exhibited toxic effects of effluent from the municipal WWTPs [18] as well as from WWTP treating the piggery wastewater [14].

Pereao et al. [18] indicated that *T. thermophila* was the most sensitive organism in their study, whereas Szklarek et al. [19] demonstrated that this organism had high resilience, even regarding low-quality treated wastewater, and it was less sensitive than the other ones used in their study as bioindicators (e.g., *Pseudokirchneriella subcapitata*, *Thamnocephalus platyurus*).

The results of the ecotoxicity tests with the zebrafish proved that wastewater, in particular the raw wastewater, contributed to the mortality of *D. rerio* [12,21,38]. After treatment, the acute toxicity of wastewater towards the zebrafish was markedly decreased [12,21] or even completely removed [38]. It was found that conventional activated sludge systems were able to remove the toxic effects of wastewater on *D. rerio*. Zhang et al. [38] showed that the A/O process was very efficient in the reduction of acute toxicity of municipal wastewater towards the zebrafish, which was accompanied with the removal of organic pollutants and the decrease in COD below 50 mg L^{-1}. The A/O process was also efficient in the removal of the acute toxicity of acrylonitrile wastewater; however, the residual acute toxicity as well as the organic toxicants in the A/O effluent were further reduced after going through the downstream ABFT process system, and the final effluent displayed no significant acute and embryo toxicity [21]. The decrease in toxicity of wastewater containing pigments after anaerobic–anoxic–oxic (A/A/O) treatment was also observed, but the final effluent still exhibited acute toxicity to *D. rerio* with the toxic units (TU) of 2.0 [12].

Moreover, other than the above-described species of aquatic and soil biota, plants, snails and earthworms, e.g., were used as model organisms in the assessment of wastewater ecotoxicity. The effects of wastewater on these organisms are included in Table 4.

Table 5. Review of organisms most frequently used for the evaluation of wastewater ecotoxicity.

Trophic Level	Group of Organisms	Examples of Species
Primary producers	Algae	*Raphidocelis subcapitata; Chlorella vulgaris; Scenedesmus* sp.
	Aquatic plants	*Lemna minor*
	Terrestrial plants	*Lepidium sativum*
Reducers	Bacteria	*Vibrio fischeri;* *Photobacterium phosphoreum*
	Protozoa (ciliata)	*Tetrahymena thermophila*
Consumers	Crustaceans	*Daphnia magna* *Artemia franciscana*
	Fishes	*Danio rerio*

6. Conclusions and Perspectives

The study of the effect of wastewater on aquatic and soil biota has been underestimated so far. This review shows that it is an important and complementary part of wastewater analysis that should be developed in the future. The presented results of the ecotoxicity tests of wastewater from the full-scale WWTPs prompts the formulation of the following conclusions:

1. Monitoring of physicochemical indicators of wastewater contamination in the full-scale WWTPs should be supplemented with the ecotoxicological assessment. It comprises not only the treated wastewaters (effluents), which are discharged to the environment—i.e., the whole effluent toxicity tests—but also the raw wastewater (influent) entering the WWTPs. Due to testing of the wastewater toxicity in several points of the WWTPs, the treatment processes, in particular the biological treatment, might be carried out more efficiently.
2. The application of the ecotoxicological treatment should concern all types of wastewater (municipal and industrial) and all full-scale WWTPs irrespective of their size, because the small WWTPs are also the source of hazard for the aquatic compartment.
3. Wastewater is a complex matrix of compounds, and therefore, the determination of their toxicity is a difficult task. The results of ecotoxicity tests for wastewater often vary to a high extent not only due to the complex composition of wastewater but also because they are dependent on the organisms, which are used as model organisms in the bioassays. In order to evaluate the toxic hazard of wastewater towards biota, a battery of ecotoxicity tests using model organisms of different sensitivities and representing different trophic levels should be employed. So far, bacteria (e.g., *Vibrio fischeri*), green microalgae (e.g., *Raphidocelis subcapitata*) and crustaceans (e.g., *Daphnia magna*) have been the most commonly used organisms in the biological assessment of wastewater. They were applied in almost half (bacteria) or more than a half (microalgae, crustaceans) of papers analyzed in this study. They are usually regarded as sensitive organisms towards wastewater; however, only the application of different model organisms allows for the identification of the organisms of the appropriate sensitivity for a specific WWTP and/or a specific purpose.
4. The treatment, in particular the biological treatment, of wastewater contributes to the reduction of wastewater ecotoxicity irrespective of the technological solution applied in the WWTPs. The conventional activated sludge systems (e.g., A/O or A/A/O processes) are efficient in the removal of wastewater toxicity. It concerns both municipal and industrial wastewater. At the same time, the tertiary stage of wastewater treatment, in particular chlorination or ozonation, induces the increase in wastewater toxicity.
5. The classification of wastewater toxicity based upon the toxicity units proposed several years ago by Persoone et al. [17] was shown to be very useful and commonly applied. Now, it is necessary to take the next steps in the evaluation of the ecological risks of wastewater and linking this classification with the limit values of wastewater toxicity. These limit values should be primarily established for the final effluent discharged to the aquatic or soil ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14203345/s1. Table S1: Ecotoxicity of wastewater—data selected for review. Reference [39] is cited in the Supplementary Materials.

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