sustainability

MDPI

Article

A Battery of Simple Bioassays for Domestic and Industrial Wastewater Treatment Plants in Konya, Turkey

Süheyla Tongur * and Hande Atmaca

Department of Environmental Engineering, Faculty of Engineering and Natural Sciences, Konya Technical University, 42250 Konya, Turkey; e198228001016@ktun.edu.tr

* Correspondence: stongur@ktun.edu.tr

Abstract: Wastewater ingredients present risks to the environment and can cause health problems. The aim of this study was to identify the toxicological effects of influent and effluent wastewater from Konya Urban Wastewater Treatment Plant (KU WWTP) and Konya Organized Industrial Zone Wastewater Treatment Plant (KOI WWTP). Three different trophic level toxicity tests were conducted to determine the possible harmful effects of wastewater on the environment. The base toxic unit values of the Lepidium sativum toxicity test for the inlet and outlet samples of KU WWTP were found to be 1.43 and 1.10, respectively. Both values classified the analyzed wastewater into the "toxic" category. Wastewater entering the KU wastewater treatment facility was classified as "toxic" for the presence of toxic substances according to the Vibrio fischeri toxicity testing. Influent samples from the KOI wastewater treatment plant were classified as "toxic" with the Vibrio fischeri toxicity test. In addition, based on the fish bioassay value (TDF), wastewater from the KOI treatment facility was also classified as "toxic". It was concluded that increased chemical oxygen demand and concentrations of total nitrogen and phosphorus and of certain heavy metals above the limits played a decisive role in classifying the samples as "toxic". The results of this study suggest that all three tests have the potential to assess wastewater toxicity and that changes in wastewater properties may result in differences in test sensitivity.

Keywords: wastewater treatment plant; industrial wastewater; domestic wastewater; acute toxicity; fish bioassay (TDF); *Lepidium sativum*; *Vibrio fischeri*

Citation: Tongur, S.; Atmaca, H. A Battery of Simple Bioassays for Domestic and Industrial Wastewater Treatment Plants in Konya, Turkey. *Sustainability* **2024**, *16*, 316. https://doi.org/10.3390/su16010316

Academic Editor: Mirna Habuda-Stanić

Received: 25 November 2023
Revised: 15 December 2023
Accepted: 27 December 2023
Published: 29 December 2023

1. Introduction

Toxicology is a science branch related to poisons. Poison is a term describing any material that harmfully affects living organisms. Any substance unconsumed in the appropriate dosage and manner results in a toxic effect. Numerous chemicals that are nutrients for living organisms are released in excrement and urine into sewers without being altered. However, in addition to untreated and treated effluent wastewater from treatment plants, high concentrations of such chemicals are potentially observable in drinking water and in surface and underground waters [1]. One of the primary sources of water pollution is uncontrolled wastewater released from various industries into water masses and other environments. Wastewater is primarily produced by the industrial sector, which is advancing quickly and is essential for the growth and expansion of national economies [2]. Despite the rapid improvements in analytical chemistry, constraints such as cost and time prevent analyzing and identifying all pollutants in wastewater [3]. Pollutant removal efficiency, based on chemical oxygen demand (COD) and biochemical oxygen demand (BOD), is evaluated to assess the efficacy of wastewater treatment plants (WWTP) and optimize wastewater treatment processes [4]. However, the systematic COD and BOD monitoring is relatively expensive and time-consuming [5]. Only toxicity testing can reveal the unknown and often otherwise undetectable toxic effects of components in complex mixtures or the possible synergistic effects of compounds in wastewater [6]. In some cases, even if chemical concentrations in wastewater do not exceed the discharge limits, wastewater may be toxic [7]. Testing is used to identify and predict the harmful effects of chemicals on populations, communities, and ecosystems [8]. It is now clear that toxicity testing is essential for risk assessment of waste, wastewater and industrial chemicals [9]. Toxicity tests are used to identify waste toxicity level, the impact of wastewater treatment technologies on tested species, the effect of environmental factors on the toxicity of waste for aquatic life, the degree of treatment required, and the permissible wastewater discharge rates in water pollution control experiments [10].

This study aimed to evaluate the influent and effluent wastewater from KU WWTP and KOI WWTP and analyze its toxicological effects by employing three different methods, namely, the Lepidium sativum, fish bioassay (TDF), and Vibrio fischeri toxicity tests, which designate diverse trophic levels and were conducted to define the potentially harmful effects of wastewater toxic matter on the environment. Two distinct characterizations of the wastewater toxicity levels indicated the conditions of the receiving environment. The relevance of the toxicity test methods used in this study is great in terms of their utilization in studies on wastewater toxicity.

2. Materials and Methods

2.1. Wastewater Samples

In this study, the toxic effects of wastewater samples were defined with two different methods. Inlet and outlet wastewater samples were collected from Konya Organized Industrial Zone Wastewater Treatment Plant and Konya Urban Wastewater Treatment Plant. Konya Urban Wastewater Treatment Plant uses the 4-stage biological Bardenpho treatment process. Konya Organized Industrial Zone Wastewater Treatment Facility uses biological and chemical procedures. Wastewater from both facilities can be very harmful to the environment. The treatment plants where the samples were taken are shown in Figures 1 and 2.

Figure 1. *Cont.*

Figure 1. Konya Urban Wastewater Treatment Plant (KU WWTP).

Figure 2. Konya Organized Industrial Zone Wastewater Treatment Plant (KOI WWTP).

2.2. Physical and Chemical Properties of the Wastewater Samples

The characterization of the wastewater samples was performed, including the determination of pH, electrical conductivity (EC), chemical oxygen demand (COD), suspended solids (SS), total nitrogen (TN), total phosphorus (TP) and toxic effects in different bioassays. COD and SS were measured using the technique suggested in the APHA Standard Methods for the Examination of Wastewater. pH and EC were measured with the WTW Multi 340i device. Total phosphorus (TP) was analyzed with Hach LCK349 TP test kits [11]. Heavy metals were analyzed with an ICP-MS instrument. Total nitrogen (TN) was determined with Hach Lange Dr 5000 TN kits.

2.3. Bioassays

Three different biotests for different trophic levels were chosen for this study. *L. sativum*, a terrestrial plant, was selected to represent the trophic level of producers. *V. fischeri*, a bacterium, was selected to represent the decomposers. The fish toxicity test was selected to represent secondary consumers. Wastewater samples were collected from the treatment plants and analyzed by the *Lepidium sativum*, *Vibrio fischeri*, and fish bioassays.

2.3.1. *Lepidium sativum*

Different dilutions of wastewater samples (6.25%, 12.5%, 25%, 50%, and 100%) were used for the *Lepidium sativum* toxicity test. A total of six control groups were prepared for each wastewater sample. Two Whatman filter papers, 90 mm in diameter, were positioned into 9 cm glass Petri dishes. Five milliliters of distilled water or five milliliters of wastewater at varying dilutions was applied to a filter paper for the control group and the experimental group, respectively. Air bubbles were removed from the filter papers carefully. Each Petri dish contained 25 *Lepidium sativum* seeds with equal spacing; the dishes were closed with their lids and incubated at 25 °C in the dark for 72 h. After 72 h, the 20 seeds in each Petri dish that grew the fastest were selected to measure their root and stem lengths. The mean values of root and stem length were determined for the control and the experimental Petri dishes and were compared. The inhibition rates in "%" and the EC_{50} values were calculated according to the measured lengths. The EC_{50} values were employed to determine the toxic unit values [12].

2.3.2. *Vibrio fischeri* Toxicity Test

The *Vibrio fischeri* toxicity test was employed to examine the influent and effluent samples taken from Konya Industrial Zone Wastewater Treatment Plant and Konya Urban Wastewater Treatment Plant. The toxicity test was carried out in the accredited Dokuz Eylul University Microbiology Laboratory using the SM 8050 A-B method.

Vibrio fischeri is a Gram-negative heterotrophic bacterium belonging to the Vibrionaceae family, a large family of gamma-proteobacteria, characterized by their pathogenic interactions and consisting of many species. The frozen bacterial strain NRRL-B-11177 of *Vibrio fischeri* was employed in the LUMIStox toxicity test to analyze the toxicity of wastewater by performing four different characterizations. According to the standard approach recommended by the Lange company, acute toxicity tests were performed by determining the decline in light transmittance with time (typically, in 5, 10, and 30 min). Standard bacteria cultures of *Vibrio fischeri* were obtained from Dr. Lange Company (Düsseldorf, Germany), and their luminescence levels were measured using the LUMIStox system [13]. The inhibition rate was calculated by comparing the inhibitory effects of the tested dilutions with those of a toxin-free control. The acquired value was plotted against the dilution factor, and the resulting curve was used to calculate the EC_{50} of the samples [14].

2.3.3. *Fish Bioassay* (TDF) Toxicity Test

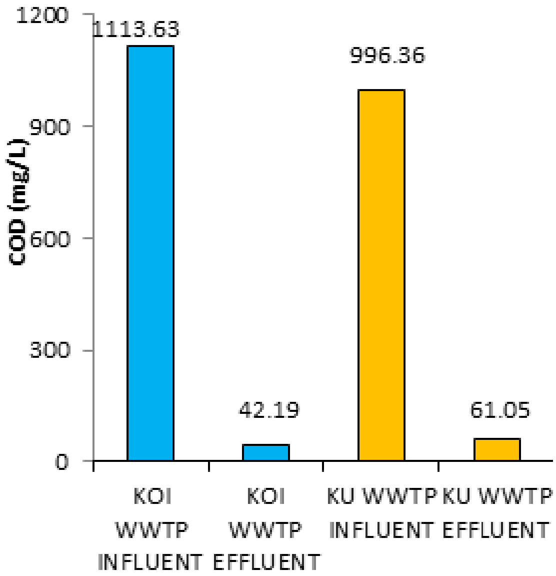
The influent and effluent samples acquired from Konya Organized Industrial Zone were subjected to the fish bioassay (TDF) toxicity test. The toxicity test was performed in the accredited Envirolab Measurement and Analysis Laboratory according to the proposed procedure of the TS 5676 WPCR Sampling and Analysis Methods Communiqué [15].

The toxic effect of wastewater on fish is expressed by a dilution factor, referring to the smallest dilution unit at which all fish is potentially alive. In other words, the smallest dilution unit at which all fish survive (no dead fish) is called TDF (toxicity dilution factor). The fish species *Lepistes reticulates* (*Poecilia reticulata*) was used in the fish bioassay. This fish is one of the most common species among aquarium fishes. The ichthyologist Wilhelm Peters, who first identified the *Lepistes* (rainbow fish) in Caracas/Venezuela in 1859, named it *Poecilia reticulata* and included it in the family *Poeciliidae* [16].

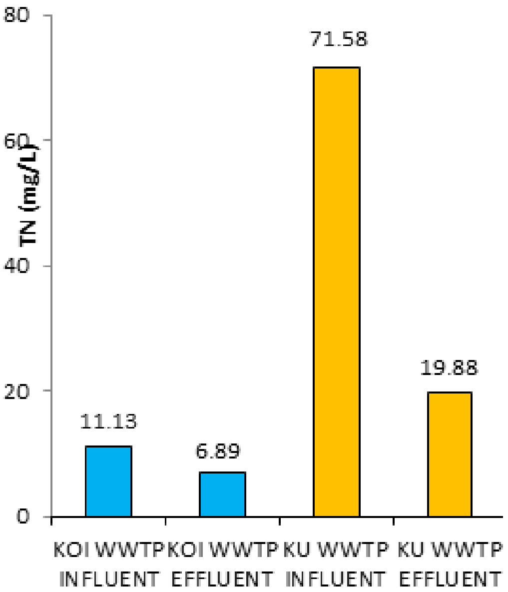
3. Results

3.1. Physical and Chemical Analysis Results

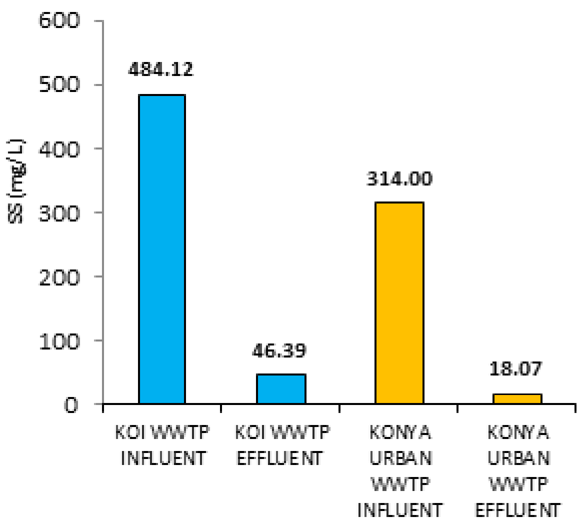
The results of the physical and chemical analyses of the wastewater samples are presented in Figure 3 and Table 1. Table 2 displays the results of the heavy metal analysis of wastewater from KOI WWTP.



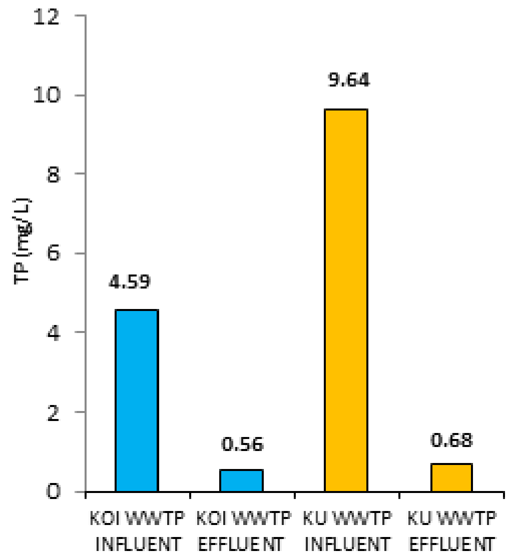
(**a**)



(**b**)



(**c**)



(**d**)

Figure 3. Physicochemical properties of KU WWTP and KOI WWTP wastewater [17]. (**a**) COD: chemical oxygen demand; (**b**) TN: total nitrogen (**c**) SS: suspended solid; (**d**) TP: total phosphorus.

Table 1. Physicochemical properties of KU WWTP and KOI WWTP wastewater [17].

Wastewater Type	pH	COD (mg/L)	SS (mg/L)	TN (mg/L)	TP (mg/L)
KOI WWTP influent	7.11 ± 0.3	1113.63 ± 100	484.12 ± 50	11.13 ± 2	4.59 ± 2
KOI WWTP effluent	7.58 ± 0.5	42.19 ± 10	46.39 ± 10	6.89 ± 0.5	0.56 ± 0.2
KU WWTP influent	7.47 ± 0.4	996.36 ± 100	314.00 ± 60	71.58 ± 5	9.64 ± 3
KU WWTP effluent	7.67 ± 0.5	61.05 ± 15	18.07 ± 7	19.88 ± 2	0.68 ± 0.3

KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant; KU WWTP: Konya Urban Wastewater Treatment Plant. COD: chemical oxygen demand; TN: total nitrogen; SS: suspended solid; TP: total phosphorus.

Table 2. Heavy metal analysis results of KOI WWTP influent and effluent wastewater [17].

Parameter	Influent Values	Effluent Values	Unit	WPCR Table 19. (2-h)
Chromium (Cr^{+6})	0.48 ± 0.4	0.14 ± 0.4	mg/L	0.5
Lead (Pb)	1.9 ± 0.1	0.57 ± 0.1	mg/L	2
Cadmium (Cd)	0.45 ± 0.1	0.34 ± 0.1	mg/L	0.1
Iron (Fe)	4.4 ± 0.3	2 ± 0.3	mg/L	10
Fluoride (F^-)	13.4 ± 0.3	6.3 ± 0.3	mg/L	15
Copper (Cu)	2.86 ± 0.2	0.42 ± 0.2	mg/L	3
Zinc (Zn)	1.99 ± 0.4	3.61 ± 0.4	mg/L	5
Sulfate ($SO_{4)}$	1000 ± 300	1000 ± 300	mg/L	1500
Color	1.9 ± 0.5	0.7 ± 0.5	(Pt-Co)	280

KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant. WPCR Table 19: Discharge standards of mixed industrial wastewaters to the receiving environment (small and large organized industrial zones and other industries in undefined sectors).

The pH values of the samples varied between 7.10 and 7.70. Considering the influent wastewater COD and SS concentration, the KOI WWTP measurements were higher than those of KU WWTP. The removal efficiency according to the COD and SS parameters for KOI WWTP was 96.21% and 90.42%, respectively. On the basis of the same measurements (COD and SS) the removal efficiency was 93.87% and 94.25% for KU WWTP, respectively. As a result, a higher removal efficiency for KOI WWTP was obtained with the COD measurement, while it was obtained with the SS measurement for KU WWTP. Since Konya provincial domestic wastewater is collected in KU WWTP, the TN and TP concentrations in KU WWTP influent wastewater were greater than those in KOI WWTP wastewater. The N and P removal efficiency according to the TN and TP measurements was 72.23% and 92.94% for KU WWTP and 38.10% and 87.91% for KOI WWTP, respectively. It appeared that the utilization of the four-stage Bardenpho biological treatment method in KU WWTP led to a high N elimination efficiency.

According to the Discharge Standards of Mixed Industrial Wastewaters to the Receiving Environment (small and large organized industrial zones and other industries of undefined sectors) of the Regulation on Water Pollution and Control to which KOI WWTP effluent is subject, the set limit values were as follows: 6–9 for pH, 200 mg/L for SS, 400 mg/L for COD, 20 mg/L for TN, and 2 mg/L for TP. Accordingly, KOI WWTP effluent wastewater seemed to meet the standard values. Based on the Urban Wastewater Treatment Regulation (UWWTR) to which KU WWTP effluent is subject, it was observed that the COD and TP parameters did not exceed the limit values. It was observed that the limit values indicated in the regulation for TN were exceeded.

According to the heavy metal analysis results of KOI WWTP, the removal efficiencies were 70.83% for Cr^{+6}, 70% for Pb, 24.44% for Cd, 54.55% for Fe, 53% for F^-, and 85.31% for Cu. The color removal efficiency was 63.15%. Although Zn concentration in the effluent wastewater was higher than in the influent wastewater, it remained within the discharge limits. However, the Cd concentration failed to meet the discharge standards. The analysis results for other heavy metals in the effluent revealed that their concentrations were also within the discharge limits.

3.2. Toxicity Classification of the Samples

The 'toxic unit' value is generated based on the '%' inhibition rates or the EC_{50} figures calculated from the toxicity tests. The determination of toxic units is used to assess the acquired results. The symbol 'TU' stands for toxic unit value.

Equation (1) is employed for the EC_{50}-based toxic unit calculation.

$$TU = \lceil 1/L(E)C_{50} \rfloor \times 100, \tag{1}$$

Persoone and Janssen (1997) [12] used the following classification for the toxicity levels in their study: if the toxic unit TU is 0, the sample is non-toxic, if 0 < TU < 1, the sample is slightly toxic, if 1 < TB < 10, the sample is toxic, if 11 < TB < 100, the sample is highly toxic, and if TB > 100, the sample is extremely toxic (Table 3).

Table 3. Acute toxicity classification based on "toxic unit" values [17].

Acute Toxicity Classes	Toxic Unit	Toxicity Assessment
Class 1	No toxic effect	Non-toxic
Class 2	<1	Slightly acute toxicity
Class 3	1–10	Acute toxicity
Class 4	10–100	Highly acute toxicity
Class 5	>100	Extremely acute toxicity

According to the '%' inhibition rates, sample classification is as follows: non-toxic if the inhibition rate is between 0–10%, slightly toxic if it is between 11–49%, and toxic if it is between 50–100% (Table 4). The increase in the inhibition rate over time is an indication of rising toxicity.

Table 4. Acute toxicity classification according to "% inhibition" rates [17].

Acute Toxicity Classes	Inhibition Rates (%)	Toxicity Assessment
Class 1	0–10%	Non-Toxic
Class 2	11–49%	Slightly Acute Toxicity
Class 3	50–100%	Acute Toxicity

This study used the EC_{50} values to define the toxic unit values in the *Lepidium sativum* toxicity test, whereas it utilized '%' inhibition rates to calculate the toxic unit values in the *Vibrio fischeri* toxicity test.

3.3. Results of the Lepidium sativum Toxicity Test

The EC_{50} values for four different wastewater samples were calculated via the generation of a calibration curve corresponding to the dilution ratios of the '%' inhibition rates estimated at the end of the experiment. Accordingly, the toxic unit classification was determined by the defined EC_{50} values. Since some organic substances in Konya Urban WWTP and KOI WWTP influents had a favorable effect on the root length of *Lepidium sativum* plants, their impact on the inhibition rates seemed to be negative. Table 5 lists the EC_{50} values identified by the *Lepidium sativum* toxicity test for each of the four different wastewater samples and the toxic unit classification.

Table 5. EC_{50} and toxic unit values based on the average root length values by wastewater type [17].

Wastewater Types	EC_{50} (%)	Toxic Unit	Classification
KU WWTP influent	70.17 ± 30	1.43 ± 0.3	Toxic
KU WWTP effluent	91.05 ± 50	1.10 ± 0.5	Toxic
KOI WWTP influent	163.47 ± 30	0.61 ± 0.3	Slightly Toxic
KOI WWTP effluent	101.55 ± 10	0.98 ± 0.1	Slightly Toxic

KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant; KU WWTP: Konya Urban Wastewater Treatment Plant.

The toxic unit values were calculated based on the defined EC_{50} values and Equation (1). Figure 4 displays the toxic unit graph according to the wastewater type.

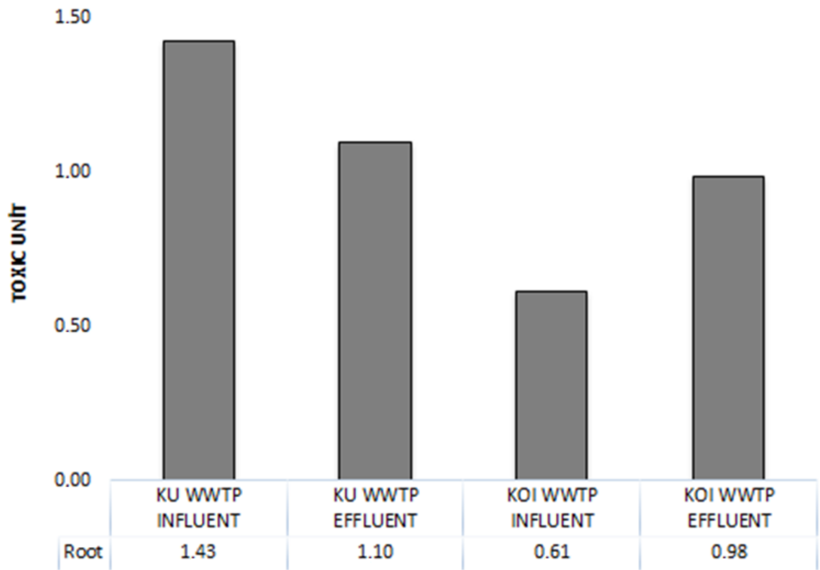


Figure 4. Toxic unit values by wastewater type [17]. KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant; KU WWTP: Konya Urban Wastewater Treatment Plant.

This study analyzed the correlation between the average COD, SS, TN, and TP physicochemical measurements and the inhibition of root growth measured for *Lepidium sativum* treated with influent and effluent wastewater from KU WWTP and KOI WWTP. The COD, AKM, TN, and TP values explicitly indicated toxic effects in the *Lepidium sativum* toxicity test, since the correlation coefficient p was >0.5 for the four different wastewater parameters.

3.4. Results for the Vibrio fischeri Toxicity Test

The influent and effluent wastewater samples taken from KU WWTP and KOI WWTP were analyzed; their '%' inhibition rates were determined at 6.25%, 12.5%, 25%, and 50% dilution rates and classified according to their toxic unit values, based on the determined inhibition rates. Considering the inhibition rates for KU WWTP and KOI WWTP, the influent wastewater values were higher than those determined for the effluent wastewater. As a result, the influent wastewater from both WWTPs had a higher level of toxicity than the effluent wastewater (Table 6).

Table 6. Inhibition rates and toxicity classification of wastewater types according to the Vibrio Fischeri toxicity test [17].

Wastewater Type	Test Period	Inhibition Rate (%)	Classification
KU WWTP influent	For 30 min	79–100%	Toxic
KU WWTP effluent	For 30 min	6–9%	Non-toxic
KOI WWTP influent	For 30 min	70–100%	Toxic
KOI WWTP effluent	For 30 min	30–48%	Slightly Toxic

KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant; KU WWTP: Konya Urban Wastewater Treatment Plant.

The correlation between the physical and chemical parameters of wastewater and the root inhibition rates measured in the *Lepidium sativum* toxicity test was calculated.

The correlation coefficient (p) for the physicochemical parameters measured for the inlet and outlet samples taken from KOI WWTP was determined to be $p > 0.5$. This revealed that there was a relationship between the physicochemical parameters and root growth inhibition for KOI WWTP influent and effluent wastewater.

3.5. Results for the Fish Bioassay (TDF) Toxicity Test

The current study employed the fish bioassay (TDF) toxicity test to determine the toxicological effect of the influent and effluent samples from KOI WWTP. Industrial wastewater discharges are toxic to aquatic organisms living in the receiving environment, causing damage to their ecological features [18]. The WPCR (Discharge Standards of Mixed Industrial Wastewaters to the Receiving Environment, small and large organized industrial zones and other industries of undefined sectors) contains sector-specific discharge limits used to control industrial wastewater discharges in Turkey. Yet, the fish bioassay (TDF) analysis provides criteria to be satisfied, reported in pertinent tables, in the evaluation of particular industrial sectors. In this context, the WPCR, reporting the Discharge Standards of Mixed Industrial Wastewaters to the Receiving Environment (small and large organized industrial zones and other industries of undefined sectors), was used to compare the influent and effluent wastewater analysis data from KOI WWTP. Table 7 displays the fish bioassay (TDF) analysis results for the influent and effluent samples of KOI WWTP. As indicated by the analysis results, the values determined with the fish bioassay (TDF) analysis for the effluent wastewater remained below the WPCR limits, satisfying the standard discharge values.

Table 7. Fish bioassay (TDF) analysis results for KOI WWTP influent and effluent wastewater [17].

Wastewater Type	Analysis Result for *Fish Bioassay* (TDF)	WPCR (Discharge Standards of Mixed Industrial Wastewaters to the Receiving Environment, Small and Large Organized Industrial Zones and Other Industries of Undefined Sectors)	Classification
		Composite Sample 2-h	
Influent Wastewater	13	10	Toxic
Effluent Wastewater	4		Non-toxic

3.6. Evaluation of the Toxicity Tests by Toxic Unit Classes

Different toxicity tests were utilized in this study in consideration of their growing validity and availability in the market. among wastewater tests based on diverse characterizations. The analysis of the test results indicated different sensitivities, depending on the wastewater type. The primary reason for these variations in sensitivity related to the test organisms used in the toxicity tests was the different chemical compositions of the examined wastewater samples.

The toxic unit values were calculated and then used for the acute toxicity classification shown in Tables 3 and 4 of the wastewater samples. Additionally, Table 8 lists the four different wastewater samples categorized based on their “toxic unit” and “% inhibition” values.

Table 8. Acute toxicity classification of the examined wastewater samples [17].

Wastewater Type	*Lepidium sativum*	*Vibrio fischeri*	*Fish Bioassay*
KU WWTP influent	Class 3	Class 3	*
KU WWTP effluent	Class 3	Class 1	*
KOI WWTP influent	Class 2	Class 3	Class 3
KOI WWTP effluent	Class 2	Class 2	Class 1

KOI WWTP: Konya Organized Industrial Zone Wastewater Treatment Plant; KU WWTP: Konya Urban Wastewater Treatment Plant. * The meaning can be given as not analyzed or not measured.

Table 8 indicates that the different acute toxicity tests of the wastewater samples led to potentially different toxic unit-based classifications. When KU WWTP influent wastewater was analyzed, it displayed acute toxicity in the *Lepidium sativum* and *Vibrio fischeri* toxicity tests; accordingly, it was categorized as Class 3. The *Lepidium sativum* toxicity test also revealed acute toxicity of the KU WWTP effluent, which led to its classification as Class

3. However, the *Vibrio fischeri* toxicity test for the same effluent samples resulted in no toxicity effect; as a result, the sample was categorized in Class 1. Similar to the experiment mentioned above, the KOI WWTP influent revealed slightly acute toxicity in the *Lepidium sativum* toxicity test and was classified in Class 2; however, the same influent sample displayed acute toxicity in the *Vibrio fischeri* and fish bioassay tests and was classified in Class 3 in this case. Strikingly, the same effluent of KOI WWTP revealed different sensitivities of the three toxicity tests. While the *Lepidium sativum* toxicity test and the *Vibrio fischeri* toxicity test revealed slightly acute toxicity of the sample (Class 2), the *Fish bioassay* found no toxicity (Class 1).

Toxicity analyses were carried out by Aydın et al. using the *Lepidium sativum* toxicity test for the inlet and outlet wastewater of Konya First Organized Industrial Zone [9]. Accordingly, the *Lepidium sativum* test showed a greater germination rate in the KOI WWTP effluent wastewater than in the influent wastewater. As a result, the influent wastewater of KOI WWTP appeared as more toxic than the effluent wastewater. Studies in the literature reported that the *Vibrio fischeri* toxicity test is more sensitive than other toxicity assays [19]. Similarly, studies emphasized that the *Vibrio fischeri* luminescence inhibition test, one of the many bioassays available, has been acknowledged to offer significant advantages over other toxicity tests in terms of sensitivity, speed, and reproducibility [20–22]. Sponza used test microorganisms such as protozoa (*Vorticella campanula*), algae (*Chlorella vulgaris*), fish (*Lebistes—Poecilia reticulata*), and bacteria (coliform, *Escherichia coli* and floc, *Zoogloea ramigera*) to determine the toxicity of wastewater from chemical dye plants [23,24]. The results showed that bacteria and fish were the most susceptible organisms.

In conclusion, considering the sensitivity levels, this study found that the *Vibrio fischeri* toxicity test, employing bacteria, was the most sensitive approach among the three toxicity tests used to determine the toxicity levels of wastewater. Nevertheless, the *Vibrio fischeri* toxicity test appears to have a disadvantage, albeit its best suitability and efficacy, as it is the most expensive test method. Among the toxicity test assays used in this context, the *Lepidium sativum* test was the least costly. The current study also established that the fish bioassay (TDF) test has low sensitivity, requires experienced personnel, large sample volumes and special equipment, and is labor-insensitive [25,26]. In the overall assessment, however, all three toxicity tests utilized in this study were shown to possess the potential to analyze wastewater samples by performing different characterizations.

4. Discussion

The escalating economy is causing an annual rise in municipal wastewater discharge volumes, resulting in substantial water source contamination and exacerbating water pollution. The sustainable discharge of wastewater without harming the environment aims to ensure the safety and protection of the aquatic ecosystems [27,28]. Pollutants in wastewater are generally complex and include pathogens and microorganisms and chemicals, such as those from personal care products, pharmaceuticals, polycyclic aromatic hydrocarbons, pesticides, phenolic compounds, steroids, and hormones [29,30]. Industrial pollutants are xenobiotic compounds that occur in the environment but are not routinely monitored, and their fate, behavior, and ecological toxic effects are not fully understood [31]. Additionally, the presence of drug and personal care product residues, food additives, and endocrine disruptors pose challenges and responsibilities for wastewater treatment [32]. Removing these toxic and persistent pollutants from urban wastewater is a priority for the stability, safety, and sustainable development of the aquatic ecosystems. Therefore, the analysis of water quality and organic pollutants in urban wastewater treatment plants is of great importance. Traditional parameters are commonly evaluated to assess the pollution status of municipal wastewater treatment plants. However, the analysis of toxic compounds in wastewater is often expensive and time-consuming due to the complexity of municipal wastewater and the presence of low concentrations of unknown chemicals in wastewater [33]. Instead of testing each parameter individually, obtaining information about wastewater properties through acute toxicity testing is a more economical and practical approach [34]. Therefore, the use of acute toxicity tests is becoming more important day by day. Recent studies showed that the Vibrio fischeri toxicity test has high sensitivity compared to other toxicity tests. Erbe et al. (2011) highlighted the superiority of the Vibrio fischeri luminescence inhibition test compared to other toxicity tests, citing sensitivity, speed, and reproducibility evaluations [20]. The Vibrio fischeri toxicity test was found to be more sensitive and yield faster results compared to the Lepidium Sativum and fish bioassay (TDF) toxicity tests. In another study, Arienzo et al. (2009) attempted to measure the phytotoxicity of winery wastewater using the Lepidium sativum toxicity test [35]. Lepidium sativum toxicity testing was found to be applicable for wastewater toxicity assessment in the wine industry. Our study also found that Lepidium sativum toxicity testing is applicable to wastewater. When compared with other toxicity tests used in this study, it was found to be advantageous in terms of availability and cost-effectiveness. In the past, fish bioassays conducted on inlet and outlet samples from the KOI wastewater treatment plant showed that the toxicity levels met the discharge standards [9]. Approximately 15 years later, a survey at the same sampling site showed that the influent wastewater values exceeded the accepted limits. This highlights the importance of the long-term monitoring of wastewater through toxicity testing. In the same study, Aydın et al. (2015) evaluated the ThamnotoxF^{TM} and DaphtoxF^{TM} toxicity tests in comparison to the fish bioassay (TDF), considering factors such as cost, labor, and sample volume [9]. Sponza (2003) used a variety of test organisms, including protozoa (*Trigana*), algae (*Chlorella vulgaris*), fish (guppies—*Poecilia reticulata*) and bacteria (coliforms—*Escherichia coli*) to improve the production of chemical dyes. They evaluated the toxicity of wastewater and found that the bacterium *Zoogloea ramigera* and some fish were the most susceptible organisms [7]. This study found that the fish bioassay (TDF) has drawbacks, as it requires large sample volumes, qualified personnel, special equipment, and extensive labor. This study suggests that although the fish toxicity bioassay (TDF) is recommended to test the emission limits in certain industrial sectors in our country, alternative toxicity tests should also be applied. The study by Häder Friedrich (2018) focused on domestic and industrial wastewater. Samples were taken from the inlet and outlet wastewater of a treatment equipment, and toxicity tests were conducted. Measurements with ECOTOX showed a significant decrease in toxicity after each pre-treatment step. This result highlighted the effectiveness of pre-treatment processes for industrial wastewater before its transfer to municipal wastewater treatment facilities [18,36,37]. Similarly, in this study, a decrease in toxicity levels was observed between inlet and outlet samples, indicating the effectiveness of the treatment. However, this also suggests the need for advanced treatments for some parameters that cannot be addressed by conventional treatments. Treatment remains a challenge around the world, as the substances contained in both municipal and industrial wastewater are highly toxic to all life forms. This situation has led to the search for new sustainable and energy-efficient treatments [38–40]. Toxicity control with these new methods is also important. In this study, similar to the literature, we found that assays commonly used in toxicity studies differ in sensitivity.

5. Conclusions

Wastewater released by various production-based and other activities in industries retains hazardous chemicals depending on the manufacturing processes. Without any treatment, discharging wastewater directly into the receiving environment restricts aquatic organisms' ability to perform their ecological functions and worsens the quality of surface waters. Considering that water is an indispensable resource, it is critical to properly treat and dispose of the domestic and industrial wastewater generated by various usages appropriately. As described in the literature and underlined in this study, the tests typically utilized in toxicity experiments have varying sensitivities. The current study showed that the fish bioassay (TDF) toxicity test provided values among the discharge limits for particular industrial sectors in Turkey; however, alternative toxicity assays should also be considered for the evaluation of common toxicity parameters. This study emphasizes the potential of bacteria and fish toxicity tests as promising approaches to enhance the application of advanced treatment technologies in wastewater treatment plants to avoid toxic effects in the receiving environment and control domestic/industrial wastewater discharges.

Author Contributions: Conceptualization, S.T. and H.A.; methodology, H.A.; software, H.A.; validation, S.T., H.A.; formal analysis, H.A.; investigation, H.A.; resources, S.T.; data curation, S.T.; writing—original draft preparation, S.T. and H.A.; writing—review and editing, S.T.; visualization, S.T. and H.A.; supervision, S.T.; project administration, S.T.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data are included in the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mustafa, E.; Valente, M.J.; Vinggaard, A.M. Complex chemical mixtures: Approaches for assessing adverse human health effects. *Curr. Opin. Toxicol.* **2023**, *34*, 100404. [CrossRef]
2. Zeyad, M.T.; Kumar, M.; Malik, A. Mutagenicity, genotoxicity and oxidative stress induced by pesticide industry wastewater using bacterial and plant bioassays. *Biotechnol. Rep.* **2019**, *24*, e00389. [CrossRef] [PubMed]
3. Välitalo, P.; Massei, R.; Heiskanen, I.; Behnisch, P.; Brack, W.; Tindall, A.J.; Du Pasquier, D.; Küster, E.; Mikola, A.; Schulze, T.; et al. Effect-based assessment of toxicity removal during wastewater treatment. *Water Res.* **2017**, *126*, 153–163. [CrossRef] [PubMed]
4. Yang, B.; Cui, H.; Gao, J.; Cao, J.; Klobučar, G.; Li, M. Using a Battery of Bioassays to Assess the Toxicity of Wastewater Treatment Plant Effluents in Industrial Parks. *Toxics* **2023**, *11*, 702. [CrossRef] [PubMed]
5. Han, X.; Zuo, Y.-T.; Hu, Y.; Zhang, J.; Zhou, M.-X.; Chen, M.; Tang, F.; Lu, W.-Q.; Liu, A.-L. Investigating the performance of three modified activated sludge processes treating municipal wastewater in organic pollutants removal and toxicity reduction. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 729–737. [CrossRef] [PubMed]
6. Rodrigues, A.S.; Souiad, F.; Fernandes, A.; Baía, A.; Pacheco, M.J.; Ciríaco, L.; Bendaoud-Boulahlib, Y.; Lopes, A. Treatment of fruit processing wastewater by electrochemical and activated persulfate processes: Toxicological and energetic evaluation. *Environ. Res.* **2022**, *209*, 112868. [CrossRef] [PubMed]
7. Sponza, D.T. Application of toxicity tests into discharges of the pulp-paper industry in Turkey. *Ecotoxicol. Environ. Saf.* **2003**, *54*, 74–86. [CrossRef]
8. Ahmed, Z.F.R.; Kaur, N.; Hassan, F.E. Ornamental Date Palm and Sidr Trees: Fruit Elements Composition and Concerns Regarding Consumption. *Int. J. Fruit Sci.* **2022**, *22*, 17–34. [CrossRef]
9. Aydin, M.E.; Aydin, S.; Tongur, S.; Kara, G.; Kolb, M.; Bahadir, M. Application of simple and low-cost toxicity tests for ecotoxicological assessment of industrial wastewaters. *Environ. Technol.* **2015**, *36*, 2825–2834. [CrossRef]
10. Zgórska, A.; Arendarczyk, A.; Grabińska-Sota, E. Toxicity assessment of hospital wastewater by the use of a biotest battery. *Arch. Environ. Prot.* **2011**, *37*, 55–61.
11. APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association; American Water Works Association; Water Environment Federation: Washington, DC, USA, 2005.
12. Persoone, G.; Janssen, C.R. Freshwater Invertebrate Toxicity Tests. In *Handbook of Ecotoxicology*; Blackwell Science Ltd.: Hoboken, NJ, USA, 1997; pp. 51–65. [CrossRef]
13. Sponza, D.T.; Oztekin, R. Removals of PAHs and acute toxicity via sonication in a petrochemical industry wastewater. *Chem. Eng. J.* **2010**, *162*, 142–150. [CrossRef]
14. Tusek, A.J.; Samec, D.; Salic, A. Modern Techniques for Flavonoid Extraction-To Optimize or Not to Optimize? *Appl. Sci.* **2022**, *12*, 11865. [CrossRef]
15. *TS 5676*; WPCR Sampling and Analysis Methods Communiqué Annex-1. Volume Annex-1, Turkish Standards Institute, 1998. Available online: https://intweb.tse.org.tr/Standard/Standard/Standard.aspx (accessed on 24 November 2023).
16. Dogan, N.; Yazıcı, Z.; Sisman, T.; Askin, H. Acute toxic effects of fenpyroximate acaricide on Guppy (*Poecilia reticulata* Peters, 1859). *Toxicol. Ind. Health* **2012**, *29*, 716–721. [CrossRef] [PubMed]
17. Atmaca, H. *Determination of Toxicological. Effects of Konya Urban Wastewater Treatment Plant and Organized Industrial Zone Wastewater*; Konya Technical University: Konya, Türkiye, 2021.
18. Cēbere, B.; Faltiņa, E.; Zelčāns, N.; Kalniņa, D. Toxicity Tests for Ensuring Succesful Industrial Wastewater Treatment Plant Operation. *Environ. Clim. Technol.* **2010**, *3*, 41–47. [CrossRef]
19. Yu, X.; Zuo, J.; Li, R.; Gan, L.; Li, Z.; Zhang, F. A combined evaluation of the characteristics and acute toxicity of antibiotic wastewater. *Ecotoxicol. Environ. Saf.* **2014**, *106*, 40–45. [CrossRef] [PubMed]
20. Erbe, M.C.; Ramsdorf, W.A.; Vicari, T.; Cestari, M.M. Toxicity evaluation of water samples collected near a hospital waste landfill through bioassays of genotoxicity piscine micronucleus test and comet assay in fish Astyanax and ecotoxicity Vibrio fischeri and Daphnia magna. *Ecotoxicology* **2011**, *20*, 320–328. [CrossRef] [PubMed]

21. Gutiérrez, M.; Etxebarria, J.; de las Fuentes, L. Evaluation of wastewater toxicity: Comparative study between Microtox® and activated sludge oxygen uptake inhibition. *Water Res.* **2002**, *36*, 919–924. [CrossRef]
22. Parvez, S.; Venkataraman, C.; Mukherji, S. A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals. *Environ. Int.* **2006**, *32*, 265–268. [CrossRef]
23. Sponza, D.T. Toxicity studies in a chemical dye production industry in Turkey. *J. Hazard. Mater.* **2006**, *138*, 438–447. [CrossRef]
24. Sponza, D.T. Incorporation of Toxicity Tests into the Turkish Industrial Discharge Monitoring Systems. *Arch. Environ. Contam. Toxicol.* **2002**, *43*, 186–197. [CrossRef]
25. Rodrigues de Souza, I.; de Oliveira, J.B.V.; Sivek, T.W.; de Albuquerque Vita, N.; Canavez, A.D.P.M.; Schuck, D.C.; Cestari, M.M.; Lorencini, M.; Leme, D.M. Prediction of acute fish toxicity (AFT) and fish embryo toxicity (FET) tests by cytotoxicity assays using liver and embryo zebrafish cell lines (ZFL and ZEM2S). *Chemosphere* **2024**, *346*, 140592. [CrossRef] [PubMed]
26. Xiong, Y.; Chen, X.; Li, F.; Chen, Z.; Qin, Z. Zebrafish larvae acute toxicity test: A promising alternative to the fish acute toxicity test. *Aquat. Toxicol.* **2022**, *246*, 106143. [CrossRef] [PubMed]
27. Li, M.; Chen, Z.; Wang, Z.; Wen, Q. Investigation on degradation behavior of dissolved effluent organic matter, organic micropollutants and bio-toxicity reduction from secondary effluent treated by ozonation. *Chemosphere* **2019**, *217*, 223–231. [CrossRef] [PubMed]
28. Schaider, L.A.; Rodgers, K.M.; Rudel, R.A. Review of Organic Wastewater Compound Concentrations and Removal in Onsite Wastewater Treatment Systems. *Environ. Sci. Technol.* **2017**, *51*, 7304–7317. [CrossRef] [PubMed]
29. Wang, J.; Tian, Z.; Huo, Y.; Yang, M.; Zheng, X.; Zhang, Y. Monitoring of 943 organic micropollutants in wastewater from municipal wastewater treatment plants with secondary and advanced treatment processes. *J. Environ. Sci.* **2018**, *67*, 309–317. [CrossRef] [PubMed]
30. Süheyla Tongur, S.Y. Toxicological evaluation of carbamazepine active pharmaceutical ingredient with Lepidium sativum, Daphnia magna and Vibrio fischeri toxicity test methods. *Desalination Water Treat.* **2020**, *201*, 438–442. [CrossRef]
31. Gros, M.; Blum, K.; Jernstedt, H.; Renman, G.; Rodríguez-Mozaz, S.; Haglund, P.; Andersson, P.; Wiberg, K.; Ahrens, L. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. *J. Hazard. Mater.* **2016**, *328*, 37–45. [CrossRef]
32. Hashmi, M.A.K.; Escher, B.I.; Krauss, M.; Teodorovic, I.; Brack, W. Effect-directed analysis (EDA) of Danube River water sample receiving untreated municipal wastewater from Novi Sad, Serbia. *Sci. Total Environ.* **2018**, *624*, 1072–1081. [CrossRef]
33. Daughton, C.G. Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM). *Sci. Total Environ.* **2018**, *619–620*, 748–764. [CrossRef]
34. Shama Adam, W.A. In vitro Antimicrobial Assessment of Lepidium sativum L. Seeds Extracts. *Asian J. Med. Sci.* **2011**, *3*, 261–266.
35. Arienzo, M.; Christen, E.W.; Quayle, W.C. Phytotoxicity testing of winery wastewater for constructed wetland treatment. *J. Hazard. Mater.* **2009**, *169*, 94–99. [CrossRef] [PubMed]
36. Häder, D.-P. 18—Ecotoxicological monitoring of wastewater. In *Bioassays*; Häder, D.-P., Erzinger, G.S., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 369–386. [CrossRef]
37. Ciccotelli, M.; Crippa, S.; Colombo, A. Bioindicators for toxicity assesment of effluents from a wastewater treatment plant. *Chemosphere* **1998**, *37*, 2823–2832. [CrossRef]
38. De La Cruz-Noriega, M.; Benites, S.M.; Rojas-Flores, S.; Otiniano, N.M.; Sabogal Vargas, A.M.; Alfaro, R.; Cabanillas-Chirinos, L.; Rojas-Villacorta, W.; Nazario-Naveda, R.; Delfín-Narciso, D. Use of Wastewater and Electrogenic Bacteria to Generate Eco-Friendly Electricity through Microbial Fuel Cells. *Sustainability* **2023**, *15*, 10640. [CrossRef]
39. Wang, W.; Zhang, J.; Hu, M.; Liu, X.; Sun, T.; Zhang, H. Antidepressants in wastewater treatment plants: Occurrence, transformation and acute toxicity evaluation. *Sci. Total Environ.* **2023**, *903*, 166120. [CrossRef] [PubMed]
40. Zhang, Y.; Sun, Q.; Zhou, J.; Masunaga, S.; Ma, F. Reduction in toxicity of wastewater from three wastewater treatment plants to alga (*Scenedesmus obliquus*) in northeast China. *Ecotoxicol. Environ. Saf.* **2015**, *119*, 132–139. [CrossRef]