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Diclofenac Toxicity Abatement in Wastewater with Solar Disinfection: A Study in the Rural Area of Brazil's Central—West Region

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Abstract: Domestic wastewater has been targeted for the presence of emerging contaminants such as antibiotics, of which diclofenac is one of the most frequently detected. Many studies have focused on the removal of these emerging pollutants. However, the legislation has focused on toxicity monitoring. In search of simplified solutions for rural areas, and to guarantee the safe reuse of effluent in agriculture, this study evaluated the efficiency of a decentralized solar disinfection (SODIS) system regarding the reduction of ecotoxicity, phytotoxicity, and pathogens in domestic wastewater after adding diclofenac potassium. For this purpose, the bioindicators *Artemia* sp., *Allium cepa* L. and *Lactuca sativa* were used, after 1, 2, and 3 h of exposure to solar radiation. After 3 h of exposure to solar radiation, toxicity was reduced and root growth inhibition was noted, which indicates low effluent toxicity after treatment by the SODIS system. It was achieved a reduction of 3 and 2 log units in the concentration of total coliforms and *Escherichia coli*, respectively.

Keywords: domestic sewage; ecotoxicity; phytotoxicity; emerging contaminants; septic tank; solar disinfection

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1. Introduction

The occurrence of emerging contaminants has become a question of global importance due to the concern they pose, regarding possible undesirable effects on the environment [1,2]. The extent of their negative effects may be affected by the type of contaminant, and its concentration, distribution, and fate in aquatic ecosystems. For instance, it may lead to hormonal disorders, chromosomal alterations, and reproductive problems in organisms [3–6]. The emerging contaminants include a range of synthetic and natural chemical compounds, such as drugs, hormones, endocrine disrupting chemicals, personal care products, pharmaceuticals, pesticides, surfactants, and flame retardants, whose occurrence and removal are often reported in the literature, although there is still a lack of knowledge concerning the various contaminants' fate [6–12]. Commonly, they are found in varied water matrices (e.g., wastewater, surface water, groundwater) in concentrations ranging from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ [1,6,13]. However, to assess the new technologies' efficiency in removing emerging pollutants, higher concentrations in the order of $mg \cdot L^{-1}$ are used [14,15]. This order of magnitude ($mg \cdot L^{-1}$) usually guarantees an effluent that has ecotoxicity. Smaller concentrations ($ng \cdot L^{-1}$ and $\mu g \cdot L^{-1}$) may not have associated ecotoxicity.

Pharmaceutical products have received more attention by virtue of their large-scale consumption, associated with self-medication practices and the correlation between life expectancy increase and the use of medicines [16]. Wastewaters are characterized as the main entry route of drugs in the aquatic environment due to their incomplete removal in conventional treatment plants [17,18]. Non-steroidal anti-inflammatory drugs (NSAIDs), widely used as analgesics and in the treatment of inflammations, stand out among those drugs, and are easily acquired without medical prescription [19]. One of the NSAIDs often found in waste and surface waters is diclofenac [20–23]. Several authors have assessed the removal of this drug in wastewaters [24–26]. Some bioindicators such as fishes, microcrustaceans, and plants have been used to evaluate its toxicity [27–30].

The presence of emerging contaminants, such as drugs, in water matrices has a toxicological effect on the environment. Thus, an integrated evaluation of the biological effects of effluent disposal in aquatic environments becomes essential. Ecotoxicological and phytotoxicological tests prove to be a viable alternative to assess the effects of substances potentially toxic to ecosystems [31–33]. In Brazil, concurrent with CONAMA's resolution 357/2005 [34], ecotoxicological assays are characterized as a legal tool for monitoring the quality of waters. As provided by the CONAMA resolution 430/2011 [35], the effluent disposed of in receiving bodies, in addition to meeting physicochemical and microbiological parameters, must not cause deleterious effects on them.

Ecotoxicological (including phytotoxicological) tests are useful instruments to assess a treatment system's efficiency in reducing toxicity in wastewater [36]. Commonly, when assessing the removal of emerging contaminants using technology such as advanced oxidative processes, bioassays are carried out to investigate the formation of toxic compounds after treatment [12,37]. However, the use of ecotoxicological and phytotoxicological assays for monitoring systems, such as for toxicity removal assessment with specific emerging contaminants and micropollutants, are little reported in the literature. Precursor studies have evaluated the wastewater toxicity reduction when considering microalgae [38], constructed wetlands [39–41], anaerobic reactors [42,43], photocatalysis [44], and activated sludge [45,46] applications.

Concerning toxicity reduction associated to water disinfection, low-cost and easy-to-control systems, such as a solar disinfection (SODIS) system, are an interesting alternative to be studied. SODIS is characterized by its low cost and is habitually used for the disinfection of water intended for human consumption by means of solar radiation [47–49]. It has also been employed for wastewater disinfection, decreasing its pathogen load and enabling its reuse for several purposes [50–52]. In addition to its germicidal potential, solar light, especially ultraviolet (UV) radiation, can lead to the breaking of chemical bonds, helping to significantly degrade some compounds, such as diclofenac, and can reduce their toxic potential. Diclofenac is removed by photodegradation (natural processes), but residues still remain in the environment as potentially toxic metabolites. [53,54]. It is recommended that effluents have a reduced load of solids, preferably with turbidity values equal to or lower than 30 NTU, to avoid possible interference with the solar radiation activity [55,56]. To be efficient with SODIS in disinfection, Santos et al. [57] used a vertical flow constructed wetland for the post-treatment of a septic tank to decrease turbidity. Oliveira et al. [58] and Souza et al. [59] observed low turbidity values in domestic wastewater after its passage through septic tanks, possibly enabling post-treatment with a SODIS. However, to our knowledge, there are no published studies on the use of UV radiation coming from natural sources, as in a SODIS, with septic tank post-treatment, especially when it comes to applying it as an ecotechnological alternative in the sustainable treatment of domestic wastewater, with focus on toxicity reduction and safe water reuse in agriculture. Similar studies are from Homlok et al. [60], Salgado et al. [61] (UV irradiation), and Michael et al. [62] (photo-Fenton), but they worked with emphasis on toxicity reduction and not on pollutant removal. Technologies based on UV, but using advanced oxidative processes, reduce emerging contaminants in less than 3 h. Therefore, it is important to reduce the time, optimizing the reactor with natural sunlight without adding chemicals to reduce toxicity, and evaluate the performance in removing pathogenicity indicators.

Given the abovementioned, the objective of the present work was to evaluate the reduction of ecotoxicity and phytotoxicity in a system composed of septic tank+SODIS, by treating domestic wastewater with low turbidity and the presence of diclofenac potassium, towards safe agricultural reuse.

2. Materials and Methods

2.1. Configuration of the System

The SODIS uses solar radiation to disinfect water by the germicidal action of ultraviolet A (UVA) radiation, on pathogens and other compounds [55]. The SODIS under study was set up outdoors at the Center of Technology and Agribusiness (CeTeAgro), Brazil, at ground level and receiving direct sunlight. It was comprised of three transparent plastic bottles (polyethylene terephthalate—PET), with 2 L capacity each, connected by silicone hoses (Figure 1). The bottles were installed on a metal plate solar concentrator, with the aim of reflecting solar radiation and favoring heat absorption [56]. Solar radiation (W·m^{-2}) and temperature (°C) were registered by a meteorological station at the site (Squitter, S1220, Brazil), at the surface of the experimental apparatus.

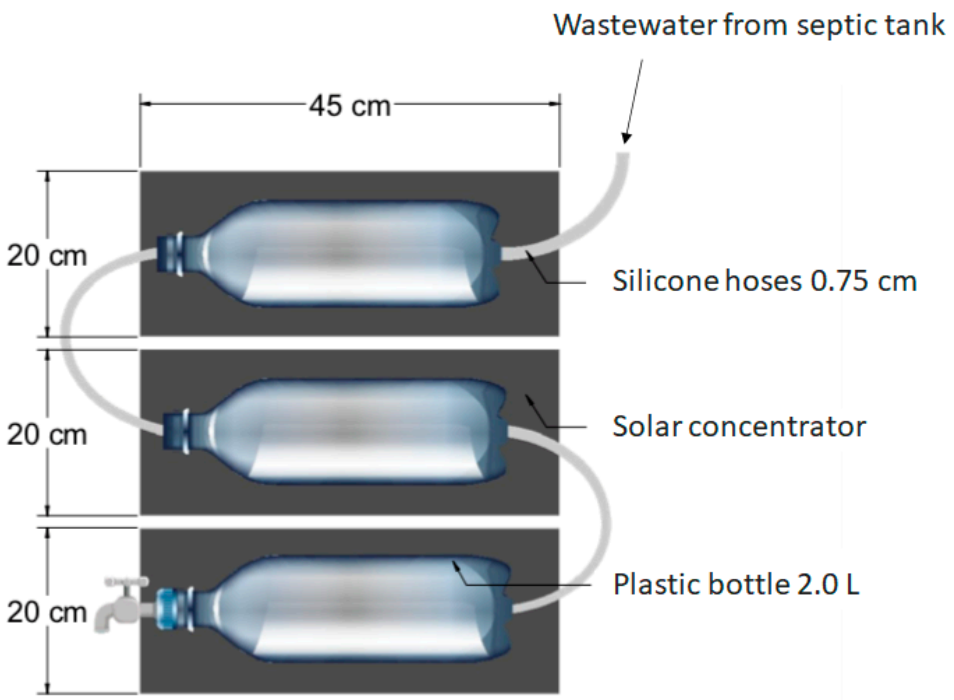

Figure 1. Solar disinfection (SODIS) system schematic representation.

The SODIS operated in batch mode, being manually fed with post-septic tank domestic wastewater, and subsequently exposed to solar radiation for 3 h. The hydraulic retention time (HRT) was chosen based on studies that evaluated the performance of the SODIS with solar concentrators, where the most appropriate HRT varied between 2 and 4 h [51,57,63]. The septic tank (38.7 m^{3}) provided primary treatment for domestic wastewater coming from a rural community of 100 population equivalent (PE).

2.2. Wastewater Analysis

Wastewater physicochemical parameters were analyzed after the septic tank following the methodology described in the Standard Methods for the Examination of Water and Wastewater [64] for: nitrite (4500 B Method), nitrate (4110 B Method), total nitrogen (4500 A Method), ammoniacal nitrogen (4500 B Method), total phosphorus (4500 B E Method), chemical oxygen demand (Closed Reflux, Colorimetric Method), and biochemical oxygen demand (BOD_5; 5−Day BOD Test). Total solids, temperature, pH, turbidity, and dissolved oxygen were measured with a multiprobe (Hanna HI9829, Hanna, São Paulo, Brazil). Five sampling campaigns took place during the period from July 2018 to July 2019.

Following previous studies, the diclofenac potassium drug (Pharmanostra, China, purity 99%) was added at a concentration of 30 mg·L^{-1} into the post-septic tank domestic wastewater, with the intent of evidencing the toxic characteristics [29,65,66], enabling the evaluation of the solar disinfection method for toxicity reduction. Samples of post-septic tank domestic wastewater with diclofenac potassium (ST), without diclofenac potassium (WD), and samples submitted to 1 h (S1), 2 h (S2), and 3 h (S3) of SODIS treatment were collected to evaluate the reduction of ecotoxicity, phytotoxicity, and fecal indicator organisms. Following the methodology proposed by Klauck [67] to carry out ecotoxicity and phytotoxicity assays, the samples ST, S1, S2, and S3 were diluted in distilled water in 25:75, 50:50, 75:25, and 100:0 ratios, resulting in samples with diclofenac potassium concentrations equal to 7.5 mg·L^{-1}, 15.0 mg·L^{-1}, 22.5 mg·L^{-1}, and 30 mg·L^{-1}, respectively.

To evaluate fecal indicator organisms removal by solar disinfection, the most probable number (MPN) of total coliforms and *Escherichia coli* in a wastewater sample from ST, S1, S2 and S3 were determined. The methodology followed was based on the multiple tube method described in the Standard Methods for the Examination of Water and Wastewater [64] and using Colilert chromogenic substrate (Idexx Laboratories, Inc., Westbrook, ME, USA).

2.3. Ecotoxicity Assay

The acute ecotoxicity assay consisted of the use of *Artemia* sp. cysts acquired in a fishkeeping articles specialized store. For their eclosion, aerated salt solution was used at a 38 g·L^{-1} concentration (synthetic sea salt, Sera Premium, Germany). After 48 h from the start of eclosion, the *Artemia* sp. neonates (nauplii) were exposed to the samples ST, S1, S2, and S3, in their respective dilutions, for 24 h, and the counting of living and dead organisms was performed. Aerated salt solution was also prepared for the negative control. Results obtained were expressed as percent dilution able to produce a lethality of 50% (lethal concentration, LC_{50}).

The LC_{50} values were calculated from the linear regression obtained from the ratio between the percentage of dead nauplii and the sample concentration. For this purpose, PROBIT analysis was conducted using STATPLUS software version 7.3.3.0, according Duarte et al. [68] and Faria et al. [69]. Detailed description of the assay's protocols can be found in Vanhaecke et al. [70], Meyer et al. [71], and technical standard ABNT NBR 16.530:2016 [72] from Brazilian Association of Technical Standards.

2.4. Phytotoxicity Assays

The phytotoxicity assay using *Allium cepa* L. bulbs was carried out by adapting methodologies described by Fiskesjó [73] and Cuchiara et al. [74]. After their skins and old roots were removed, the bulbs were exposed to the samples ST, WD, S1, S2, and S3, in their respective dilutions, for 72 h. The assay occurred in a semi-static way, so that samples were changed at every 24 h interval. The assay's negative control was performed with distilled water. At the end of the 72 h of exposure, the *A. cepa* L. bulb roots' size was measured with a digital caliper (Mitutoyo, Aurora, Illinois, USA). Results obtained were expressed in relation to root size and growth inhibition percentage.

The phytotoxicity assay using *Lactuca sativa* seeds with no chemical treatment and acquired in an agricultural establishment was carried out by adapting procedures described by Sobrero and Ronco [75]. The seeds were exposed to the samples ST, WD, S1, S2, and S3, in their respective dilutions, for 72 h in the absence of light. Distilled water was used as a negative control. At the end of the 72 h exposure, the roots' size was measured. Results obtained were expressed in relation to root size and growth inhibition percentage.

2.5. Toxic Units and Class Weight Score

Toxic units (TU) were estimated on the basis of acute toxicity of effluents to organisms according to Personne et al. [76]. TU values were calculated following Equation (1).

$$TU = [1/(L(E)C_{50})] \times 100 \quad (1)$$

where LC_{50} = lethal concentration at which 50% of the tested individuals die, and EC_{50} = effect concentration at which 50% of the tested effect is reached. Class weight score was calculated according to the methodology of Personne et al. [76], where no significant toxic effect = score 0 (no acute hazard), significant toxic effect but <$L(E)C_{50}$ (i.e., <1 TU) = score 1 (slight acute hazard), 1−10 TU = score 2 (acute hazard); 10−100 TU = score 3 (high acute hazard), and >100 TU = score 4 (very high acute hazard).

2.6. Statistical Analysis

All assays were carried out in triplicate, with the exception of the phytotoxicity assay with *A. cepa* L., which was carried out in quintuplicate. Results of the phytotoxicity assays were submitted to analysis using Microsoft Excel software and sizes and mean ± standard deviation growth inhibitions were obtained. Responses of the ecotoxicity assay, in terms of living and dead individuals, were used for calculating the lethal concentration LC_{50} through StatPlus 7.3.3.0 software using PROBIT analysis by Finney's method, with a 95% confidence interval.

3. Results and Discussion

3.1. Wastewater Characteristics and Disinfection Assessment

In Table 1, the physicochemical characteristics of the post-septic tank wastewater that fed the SODIS are presented. The results give rise to a typical medium-low strength by primary treatment domestic wastewater, concerning its organic contents, and low strength untreated domestic wastewater, concerning its nutrient content [77]. Regarding the concentration of ammoniacal nitrogen and total nitrogen, the systems were below the limit imposed (20 $mg \cdot L^{-1}$) by Brazilian legislation [35]. The pH also fell below the limit imposed by Brazilian legislation, between 5 and 7. However, the same is not true for BOD, which delimits the effluent release standard at 120 $mg \cdot L^{-1}$, in some cases the value is 60 $mg \cdot L^{-1}$, according to such local restrictions as in the Brazilian states of São Paulo, Minas Gerais, and Mato Grosso [78–80].

Table 1. Physicochemical characteristics of the post-septic tank domestic wastewater (n = 5).

Parameters	Post-Septic tank
pH	6.9 ± 0.2
Turbidity (NTU)	29.3 ± 0.3
Temperature (°C)	25 ± 2
Nitrite ($mgNO_2^- \cdot L^{-1}$)	0.9 ± 0.3
Nitrate ($mgNO_3^- \cdot L^{-1}$)	0.3 ± 0.1
Total nitrogen ($mg \cdot L^{-1}$)	18.0 ± 6.9
Ammoniacal nitrogen ($mg \cdot L^{-1}$)	17.5 ± 9.9
Total phosphorus ($mg \cdot L^{-1}$)	2.6 ± 0.7
Dissolved oxygen ($mg \cdot L^{-1}$)	1.1 ± 0.4
Chemical oxygen demand ($mg \cdot L^{-1}$)	167 ± 9
Biochemical oxygen demand ($mg \cdot L^{-1}$)	129 ± 27
Total solids ($mg \cdot L^{-1}$)	264 ± 70

The physicochemical characteristics of the domestic wastewater treated by a septic tank were within the range of those mentioned in the literature [81]. However, the values of total solids (264 $mg \cdot L^{-1}$) and turbidity (29.3 NTU) were low, similar to that found by Oliveira et al. [58] and Souza et al. [59], considering that it is domestic sewage, which usually has total solid values around 1000 $mg \cdot L^{-1}$ and turbidity above 30 NTU. These characteristics of wastewater allow the use of SODIS as a post treatment for a septic tank, without a secondary treatment (intermediate between septic tank and SODIS). Avoiding costs, operational and maintenance aspects with a secondary treatment that could remove the present nutrients, necessary for agricultural reuse.

Concerning fecal indicator organisms, the SODIS method in relation to the total coliforms and *E. coli* removal was evaluated, being the results shown in Table 2.

Table 2. Results for solar radiation and most probable number (MPN) of total coliforms and *Escherichia coli*.

Parameters	Samples			
	ST	**S1**	**S2**	**S3**
Solar radiation ($W{\cdot}m^{-2}$) *	–	768	781	800
Temperature (°C) *	24 ± 2	31 ± 3	36 ± 4	40 ± 5
Total coliforms ($MPN{\cdot}100mL^{-1}$)	1.3×10^8	2.4×10^7	2.4×10^5	2.3×10^5
Escherichia coli ($MPN{\cdot}100mL^{-1}$)	8.0×10^6	1.7×10^6	8.0×10^4	5.0×10^4

Note: (ST) post-septic tank domestic wastewater with diclofenac; post-septic tank domestic wastewater with diclofenac, treated with solar radiation for 1 h (S1), 2 h (S2), and 3 h (S3). * data from the meteorological station.

A reduction was noted of one logarithmic place in total coliforms after 1 h of exposure to solar radiation (S1) when compared to the post-septic tank domestic wastewater sample (ST). After 2 and 3 h of exposure (S2 and S3), the reduction was of three logarithmic places. As for *E. coli*, the reduction of two logarithmic places after 2 and 3 h of solar exposure (S2 and S3) was observed. Similar results were shown in the research of Dababneh et al. [50], which used a SODIS for treating turbid wastewater, obtaining the reduction of one logarithmic place in the number of total coliforms in samples with turbidity equal to 135 and 160 NTU after 3 h of exposure. However, better results in pathogen removal have been obtained with greater exposure time to solar radiation. One example is the research of Giannakis et al. [52], which evaluated the removal of *E. coli* in a synthetic secondary effluent by the SODIS method and observed its complete inactivation (10^6 $CFU.mL^{-1}$) at 4 h of exposure in samples whose radiation was equal to 800−1200 $W{\cdot}m^{-2}$ and at a temperature of 60 °C.

Results obtained in the removal of total coliforms and *E. coli* evidenced the need for greater exposure times, such as 4−6 h intervals, in the case that the system is also used to inactivate pathogens present in the post-septic tank domestic wastewater. Wegelin et al. [82] and Bitton [83] recommend a temperature of 50 °C or higher, so that, within 2 h, the pathogens' reduction is sufficient [82,83]. However, even with the solar concentrator, the maximum temperature reached was 40 °C. Thereby, the longer the sun exposure was, the greater the removal rates of microorganisms, requiring 4 h for agricultural reuse and crop irrigation, and 6 h was sufficient time for reduction to below the limit of detection [57].

The results of *E. coli* from sample S3, according to World Health Organization [84], fit in category C. Which allows for reuse for the irrigation of cereals, industrial crops, fodder, pastures, and trees, with the recommendation to avoid exposure to workers and the general public, that is, it demands mechanization and/or personal protective equipment. In relation to Brazilian legislation [85], which is more restrictive (value must be less than 1000 $MPN{\cdot}100\ mL^{-1}$), one option would be the post-treatment of the septic tank effluent, as performed by Santos et al. [57], with vertical flow constructed wetlands, or increased time in the SODIS. Risk assessment studies in this case are recommended.

3.2. Ecotoxicity Assay

Results obtained in acute ecotoxicity assays, using the microcrustacean *Artemia* sp., are presented in Figure 2. The LC_{50} evidence the toxicity reduction in domestic wastewater with diclofenac potassium after treatment with the SODIS, by means of a gradual increase of LC_{50} during the hours of exposure to solar radiation (101% for S1, 146% for S2, and 206% for S3). It should be known that the lower the LC_{50}, the more toxic the sample is in relation to the assessed bioindicator.

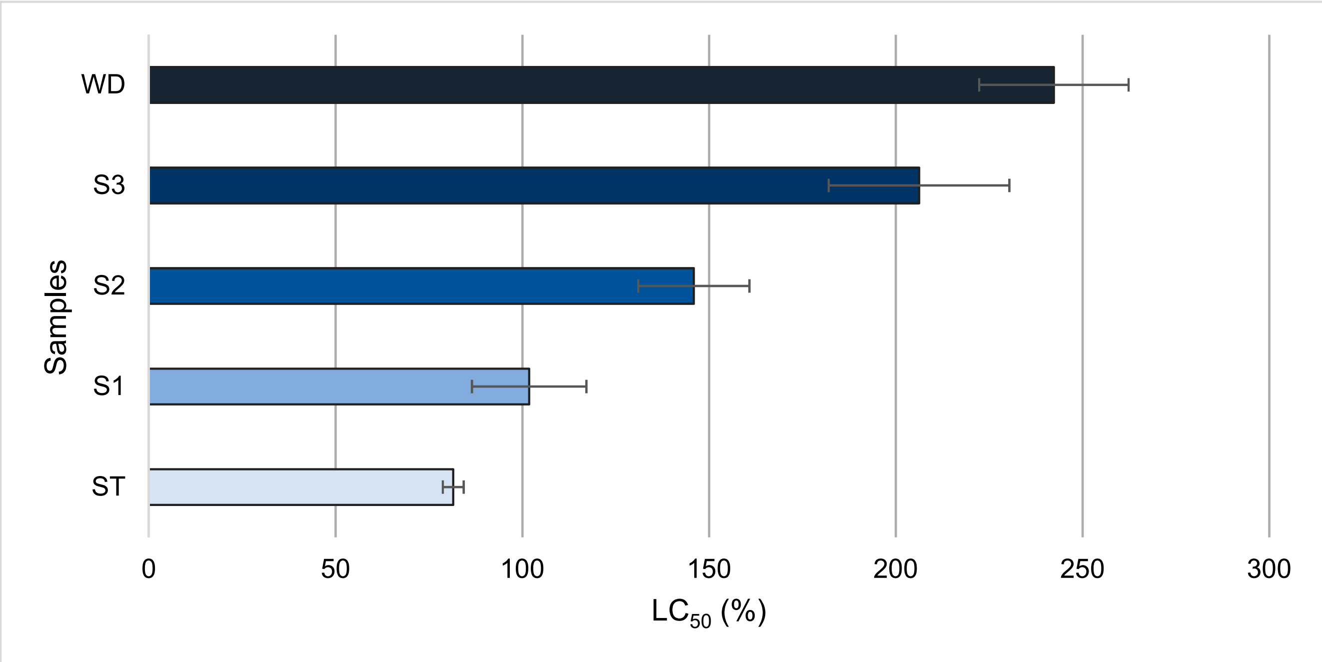

Figure 2. Lethal concentration (LC_{50}) in *Artemia* sp. for different assessed samples (n = 5). Abbreviations: wastewater without diclofenac (WD); post-septic tank domestic wastewater with diclofenac (ST); post-septic tank domestic wastewater with diclofenac, treated with solar radiation for 1 h (S1), 2 h (S2), and 3 h (S3).

The raw wastewater without diclofenac comes from a rural area and has no toxic characteristics. WD presented the highest LC_{50} (242), indicating less toxicity for the microcrustaceans, *Artemia* sp., in relation to ST and treated effluents (S1, S2, and S3). In some studies, it was shown that the raw effluents had less toxicity [86,87].

The LC_{50} before treatment with the SODIS (ST) was 82%. It was consistent with the investigations of Castro et al. [29] and Haap et al. [65], which demonstrated changes in other microcrustaceans' survival in the presence of diclofenac.

Although there are no studies on SODIS and diclofenac degradation, Homlok et al. [60], using UV at a concentration level of 0.1 mM in a 1 kGy dose, obtained a complete transformation of diclofenac molecules into products, while for mineralization and strong reduction of toxicity, a 5–10 times higher dose was needed. However, this process demands energy consumption, which does not occur with the SODIS because it uses the available solar energy, abundant in the region of the present study. Michael et al. [62], used the solar UV radiation and photo-Fenton to reduce antibiotics with the addition of ferrous solution, which significantly reduced the exposure time between 180–300 min (3–5 h). Thus, the exposure time was about the same as the values in the present study, but the ferrous solution can preclude the nutrients' reuse and recovery.

Other wastewater treatment technologies, such as a SODIS, were effective in reducing ecotoxicity in relation to microcrustaceans like *Artemia* sp. Franchino et al. [38] used microalgae for treating diluted digestate for pigsties, and their assays with *Artemia franciscana* and *Cucumissativus* confirmed the absence of significant toxic effects after treatment. Authors such as Horn et al. [39] and Lutterbeck et al. [40] used constructed wetlands to reduce ecotoxicity in wastewaters of a university campus and of a rural property, respectively. Their results revealed the wetlands' efficacy in reducing ecotoxicity to *Daphnia magna*. In turn, an integrated system composed of an anaerobic reactor, microalgae, and constructed wetlands completely reduced the acute ecotoxicity against *D. magna* in wastewater [43]. Another model of a combined system, comprised of an upflow anaerobic sludge blanket (UASB) reactor and a biofilter system, was inefficient in the detoxification of effluents from a university campus to *D. magna* and *Ceriodaphnia dubia* [42].

The post-septic tank solar disinfection design poses advantages over other technologies studied for wastewater ecotoxicity reduction since does not demand considerable areas, such as constructed wetlands; does not depend on artificial energy sources or gas emission control mechanisms; and to the detriment of some anaerobic reactors such as UASB, presents low complexity in its functioning and maintenance, if compared with integrated systems combining the use of more robust technology.

3.3. Phytotoxicity Assays

3.3.1. *Allium Cepa* L.

Results obtained in phytotoxicity assays using *A. cepa* L. bulbs, expressed in relation to root size and growth inhibition percentage, are presented in Figure 3.

No WD toxicity was observed for the root size of *A. cepa* L. (Figure 3A). All WD concentrations evaluated showed values equal to and/or greater than the control (red line). This evidences a promoting effect on root elongation.

The root size of bulbs (Figure 3A) exposed to samples not treated by the SODIS (ST) was lower than the size observed in the assay negative control, evidencing the toxicity of the post-septic tank domestic wastewater with diclofenac in all dilutions evaluated. It was verified that the lowest concentration of diclofenac tested, equal to 7.5 mg·L^{-1} (25%), was already capable of causing alterations in the *A. cepa* L. roots' growth.

After treatment with the SODIS, a toxicity reduction in all samples treated (S1, S2 and S3) was verified. It was observed that the samples, with diclofenac concentrations equal to 15 (50%) mg·L^{-1} and 7.5 mg·L^{-1} (25%), resulted in sizes of *A. cepa* L. roots equal to 2.58 cm and 2.84 cm after 3 h of exposure to solar radiation (S3), respectively, and that these were higher than the assay negative control size, which was equal to 2.20 cm. With the toxicity reduction caused by the drug, nutrients present in the sludge might have favored the *A. cepa L.* roots' growth. Such observations were carried out in the phytotoxicity assays of Silveira et al. [43], where the *A. cepa* L. root growth was greater in raw sludge samples when compared to samples of sludge treated by an integrated system, because of the higher organic load of the raw sludge.

WD showed an inhibitory effect (Figure 3B) on the growth of *A. cepa* L. roots at the highest concentrations (100, 75, and 50%). Treatments S2 and S3 showed less inhibitory effects on root elongation compared to WD. At the lowest concentration (25%), a root growth-promoting effect was observed in WD. Possibly related to nutrient levels and less organic matter with a toxic characteristic present in the effluent. This effect was also observed by Rehman et al. [88] and Alvim et al. [89], but with textile effluents.

As for growth inhibition results (Figure 3B), the complete toxicity removal of post-septic tank domestic wastewater samples was verified with diclofenac concentrations equal to 15 mg·L^{-1} and 7.5 mg·L^{-1}, after 3 h (S3) and 1 h (S1) exposures to solar radiation, respectively, considering that the growth inhibition was null. Similar results were reported by Lutterbeck et al. [40] when they evaluated the toxicity reduction of rural effluents to *A. cepa* L. after treatment by an integrated system with UASB, subsurface constructed wetlands, and UV radiation from photoreactors. Berberidou et al. [41] also observed reductions in wastewater phytotoxicity with the herbicide Clopyralid to *Sorghum saccharatum*, *Sinapisalba*, and *Lepidiumsativum* after treatment with solar photocatalysis combined with horizontal flow constructed wetlands.

However, results obtained in the present research evidence the efficacy of using solar radiation directly after anaerobic treatment with a septic tank for the toxicity reduction in domestic wastewater with a low load of solids and with turbidity values lower than 30 NTU, as recommended by Luzi et al. [55] and Khedikar and Tembhurkar [56], without combined use with constructed wetlands or other technology.

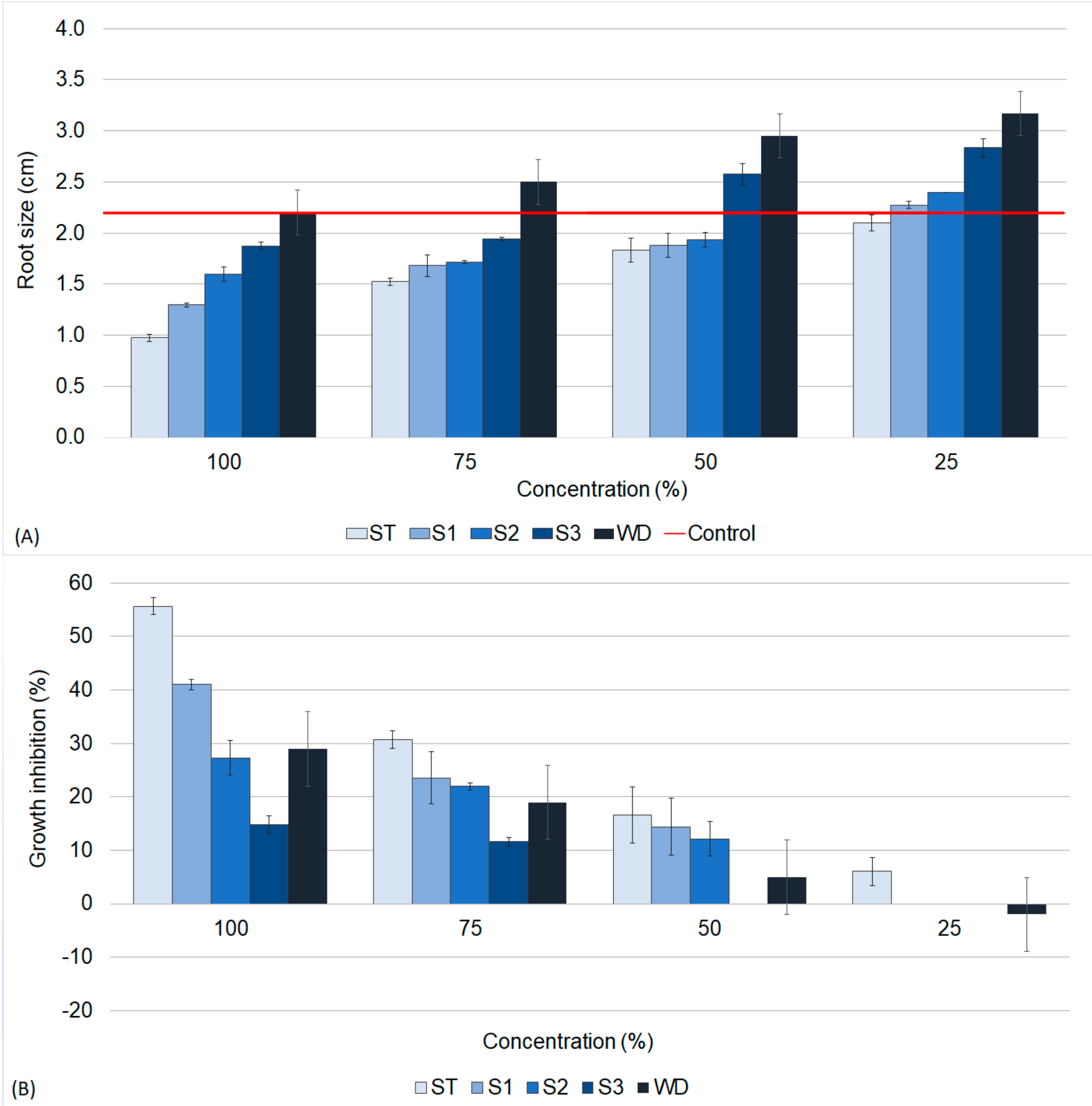

Figure 3. Root size (**A**) and growth inhibition (**B**) of *Allium cepa* L. bulbs exposed to different evaluated samples. Abbreviations: wastewater without diclofenac (WD); post-septic tank domestic wastewater with diclofenac (ST); post-septic tank domestic wastewater with diclofenac, treated with solar radiation for 1 h (S1), 2 h (S2), and 3 h (S3). Diclofenac concentration of 100%: 30 mg·L^{-1}; 75%: 22.5 mg·L^{-1}; 50%: 15 mg·L^{-1}; 25%: 7.5 mg·L^{-1}.

3.3.2. *Lactuca Sativa*

Results obtained in phytotoxicity assays using *L. sativa* seeds, expressed in root size and growth inhibition percentage, are presented in Figure 4. WD, instead of being toxic, stimulated root size (Figure 4A), revealing the potential of rural sewage in agricultural production.

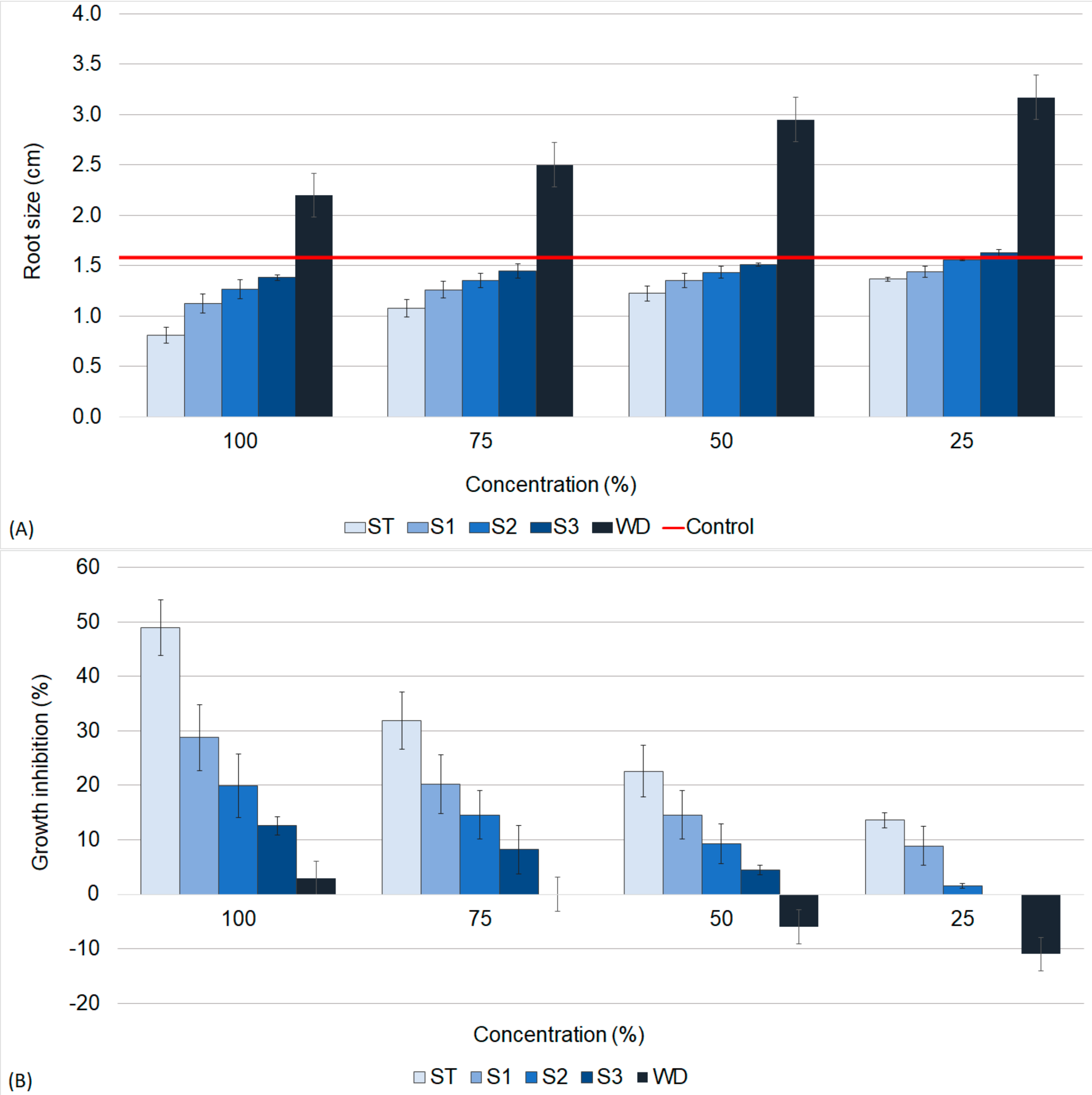

Figure 4. Root size (**A**) and growth inhibition (**B**) of *Lactuca sativa* seeds exposed to different evaluated samples. Abbreviations: wastewater without diclofenac (WD); post-septic tank domestic wastewater with diclofenac (ST); post-septic tank domestic wastewater with diclofenac, treated with solar radiation for 1 h (S1), 2 h (S2), and 3 h (S3). Diclofenac concentration of 100%: 30 mg·L^{-1}; 75%: 22.5 mg·L^{-1}; 50%: 15 mg·L^{-1}; 25%: 7.5 mg·L^{-1}.

As observed in the *A. cepa* L. bulbs assay, the root sizes of *L. sativa* seeds (Figure 4A) exposed to samples not treated by the SODIS (ST) were lower than the size observed in the assay negative control, evidencing the toxicity of post-septic tank domestic sewage with diclofenac in all dilutions evaluated.

After hours of exposure to solar radiation, a rise in root size was verified in all samples treated (S1, S2, and S3), demonstrating toxicity reduction and the efficacy of the employed treatment. After a 3 h solar exposure, the sample with 7.5 mg·L^{-1} diclofenac concentration obtained a root size equal to 1.63 cm, which was greater than the assay negative control's 1.58 cm.

In evaluating the results expressed by the growth inhibition percentage (Figure 4B) according to the toxicity scale proposed by Bagur−González et al. [90] (0 to 25% low toxicity, 25 to 50% moderate toxicity, 50 to 75% very toxic, and 75 to 100% highly toxic), it was found that domestic wastewater samples, whose diclofenac concentrations were 30 mg·L^{-1} and 22.5 mg·L^{-1}, were from moderately toxic before treatment (ST), to low toxicity at the end of the 3 h exposure treatment (S3).

Silveira et al. [91] used assays with *L. sativa* to evaluate the toxicity reduction of wastewater produced in a university campus, after treatment with microalgae and vertical flow constructed wetlands, in conjunction with *A. cepa* L. and *D. magna* bioassays. Their results evidenced the complete elimination of wastewater ecotoxicity and genotoxicity after treatment, and demonstrated the absence of phytotoxicity both in raw effluents and in treated effluents. WD showed low growth inhibition of *L. sativa* roots (3%) in the highest concentration of effluent (100%). In the lowest concentrations (50 and 25%) it had a growth-promoting effect in the tests carried out with the lettuce seeds, with no toxic effect. These results showed that *L. sativa* was not sensitive to the rural sewage. Organic matter and nutrients present in raw sewage can contribute to stimulating the growth of roots, without presenting a possible toxic effect [92]. Barszcz et al. [93], evaluating different types of effluents and bioindicators, also found that the raw domestic effluent did not present toxicity in relation to the tests with *L. sativa*, despite having presented toxicity in relation to other bioindicators.

When operating with solar radiation exposure times of up to 3 h, the evaluated system was capable of substantially reducing the toxicity of the post-septic tank domestic wastewater with diclofenac for all tested organisms. In Brazil, when evaluating the legislation, it is established in CONAMA's Resolution 430/2011 [35], according to Art. 18, that the effluent released in the receiving bodies should not cause, or have the potential to cause, deleterious effects in the receiving body, according to ecotoxicity criteria to be established by the competent environmental agency. However, there are no standards for agricultural reuse.

Other studies have only achieved similar results in less time using advanced oxidative processes in addition to UV. Vogna et al. [94] studied the advanced oxidation of diclofenac and observed that a solution of 296 mg·L^{-1} was degraded by approximately 45% in 1.5 h by means of UV radiation using a 17 mm low-pressure mercury lamp. Bartels and Vontumpling [53] evaluated the effects of the influence of solar radiation on the degradation of diclofenac in surface waters and found that the photochemical decomposition induced by sunlight is significant for this drug. It was found that in a day of intense solar radiation during the European summer, up to 83% of the degradation of diclofenac in the surface layer of the water occurred.

3.4. TU and Class Weight Score

As the percentage of class weight score of ST is very high (83%), Table 3, this sample can be considered seriously dangerous and acutely toxic (TU between 1 and 10). According to the classification system of Persoone et al. [76], samples S2 and S3 belong to the category of mild acute risk (TU < 1), and according to their class weight scores, there was a 33% decrease (83 to 50) in toxicity after 3 h in the SODIS. It can be concluded that S2 and S3 contain low amounts of toxic substances, as confirmed by the low values of the TU.

Table 3. Results of Toxic Unit (TU) and Class Weight Score.

Samples	*Artemia* sp.		*Allium cepa* L.		*Lactuca sativa*			
	TU	Test Score	TU	Test Score	TU	Test Score	Class Weight Score	Class Weight Score (%)
ST	1.2	2	1	2	0.8	1	1.7	83
S1	1	2	0.9	1	0.6	1	1	67
S2	0.7	1	0.7	1	0.4	1	1	50
S3	0.5	1	0.6	1	0.3	1	1	50
WD	0.4	1	0.7	1	0	0	0.7	67

Note: Wastewater without diclofenac (WD); post-septic tank domestic wastewater with diclofenac (ST); post-septic tank domestic wastewater with diclofenac, treated with solar radiation for 1 h (S1), 2 h (S2), and 3 h (S3).

Lu et al. [95], using advanced technologies in the degradation of diclofenac such as the UV/PS (UV activated persulfate) process, reduced the toxicity of diclofenac. Fischer et al. [96] showed that addition of polyvinylidene difluoride (PVDF) during UVA irradiation decreases the toxicity towards *Vibrio fischeri* from a TU value of 10.2 to 0 after 18 h of treatment. The addition of PVDF during UVA irradiation increases the transformation of diclofenac and adsorbs phototransformation products, but not diclofenac itself. In the ozonation process, Coelho et al. [97] slightly reduced the toxicity of diclofenac in *V. fischeri*. Schmid et al. [98] evaluated the transfer of organic substances from PET to water under SODIS conditions. Toxicological risk assessment of maximum concentrations revealed a minimum safety factor and a negligible carcinogenic risk. This study demonstrates that the SODIS procedure is safe with respect to human exposure to chemicals released from the PET bottle.

This evidences the importance of this study, because, using simple and low-cost technology with plastic bottles, it was possible to decrease the toxicity and phytotoxicity of diclofenac in the effluent, especially if the goal is safe agricultural reuse. Furthermore, PET has shown a considerable lifetime in outdoors use, based on its machining characteristics and chemical resistance, being adequate to integrate a SODIS [99]. In general, studies do not address the bottles' replacement necessity, but references such as Ubomba-Jaswa et al. [100] that addressed the genotoxicity of drinking water by a SODIS system, recommended that the bottles may be replaced after every 6 months to minimize the effects of bottle ageing, although also highlighting that there is a need to deepen the studies in this field.

4. Conclusions

The post-septic tank domestic wastewater with diclofenac potassium caused adverse effects in *Artemia* sp., *A. cepa* L., and *L. sativa* organisms in all dilutions evaluated. However, after treatment with solar disinfection, a toxicity reduction was observed, with better results after 3 h of exposure, which evidenced the viability of using UV radiation in the degradation of chemical compounds, such as diclofenac, and a reduction of its toxic potential.

This pioneering design of solar disinfection after anaerobic treatment has shown promising results in low turbidity effluents, without the combination with secondary treatment technology, facilitating the safe water reuse in agriculture, and the meeting of ecotoxicological standards for the disposal of effluents in Brazil. This solution is low-cost and does not depend on external sources of energy, this being advantageous in relation to other technologies.

Furthermore, it was verified that the 3 h solar exposure resulted in a significant reduction of three total coliforms' logarithmical houses and two *E. coli* logarithmical houses. However, the complete inactivation of total coliforms and *E. coli* was not reached, which evidenced the need for greater treatment times to this end, such as 4–6 h exposure intervals, if the objective is an effluent with a lower pathogens load to comply with local legislation. The findings from this study sets a base of knowledge for the further development of the SODIS as a decentralized system in rural areas towards diclofenac toxicity abatement in wastewaters.

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