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Integrating Ecotoxicological Assessment to Evaluate Agricultural Impacts on Aquatic Ecosystems: A Case Study of the Lage Reservoir (Mediterranean Region)

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Abstract

This study analyzed the use of a toolbox to evaluate the impact of agricultural activity on the water quality/status classification of a hydro-agricultural reservoir (Lage reservoir, Southern Portugal). The framework integrated the quantification of a group of 51 pesticides and ecotoxicological endpoints with organisms from different trophic categories (the bacterium *Aliivibrio fischeri*, the microalga *Pseudokirchneriella subcapitata*, and the crustaceans *Daphnia magna* and *Thamnocephalus platyurus*) at two sampling points in the reservoir (Lage (L) and Lage S (LS)) between 2018 and 2020. Over the three-year study, we quantified 36 of the 51 pesticides analyzed in the Lage reservoir. Total concentrations increased successively from 0.95 µg L^{-1} to 1.99 and 2.66 µg L^{-1}. Among these, the pesticides most frequently detected were terbuthylazine (100% of detection) and metolachlor (83% of detection), with maximum concentrations of 115.6 and 85.5 µg L^{-1}, respectively. Samples from the LS site showed higher toxicity, where *A. fischeri* presented 30 min EC_{50} values of 39–51%. Microalgae growth was consistently inhibited, correlating with agricultural activity, mainly the application of herbicides and insecticides, while *D. magna* feeding rates revealed no inhibitory effects in the Lage samples. The results highlight that although the detected pesticide levels were below regulatory limits, they still induced toxic effects in the tested organisms. The potential ecological status of the reservoir was classified as moderate, and the integration of the proposal toolbox allowed refinement of the classification of water status. The results demonstrated that this integrated approach, combining multiple assessment methods, establishes a more robust water quality evaluation methodology, allowing it to be used as a tool complementary to the WFD methodology. This proposal not only identified existing pollution impacts but also enabled (1) early detection of the toxic effects of emerging contaminants to prevent ecological damage; (2) proactive management through specific actions to restore water status; and (3) improved sustainable water use.

Keywords: Alqueva project; bioassays; irrigation agriculture; water status classification; Water Framework Directive

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1. Introduction

Nowadays, achieving sustainable water resource management while maintaining agricultural productivity represents a critical challenge worldwide, as recognized by the United Nations [1]. In agricultural areas, aquatic ecosystems face a stress increment from multiple factors, such as (1) extensive diversion of freshwater for irrigation [2]; (2) changes in hydrological regimes due to climate change [3]; (3) land-use changes associated with agricultural expansion that have substantially modified global hydrological systems, frequently resulting in negative impacts on both water quality and availability [4]; and (4) the direct input of agricultural runoff and untreated wastewaters [5]. In fact, current perspectives of agricultural water have shifted from a narrow view focused only on irrigation to a holistic paradigm that encompasses environmental functions, including sustaining ecological flows and improving habitat quality [6]. Effective water management in hydro-agricultural systems relies on the integration of sustainable farming practices with comprehensive and robust water quality control programs [7]. However, with agricultural intensification, irrigation water is increasingly exposed to contaminants such as pesticides, potentially toxic metals, and emerging pollutants [8], which can accumulate in soils [9], crops [10], and waters [11], posing risks to ecosystems and human health [12].

Aquatic ecosystems face continuous exposure to complex contaminant mixtures, requiring advanced and integrative methodologies to evaluate bioavailable pollutants and their interactions [13]. Chemical analyses provide an incomplete picture of water quality due to some limitations, such as the inability to quantify all existing substances, the frequent failure to detect hazardous compounds at trace concentrations, and the excessive time required for processing [14,15]. To overcome these limitations, researchers advocate the use of integrative toolboxes that combine chemical, ecological, and ecotoxicological data [16–18]. In fact, detection of ecotoxicological effects provides rapid, real-time, sensitive, and cost-effective water quality data, enabling rapid and targeted management responses. Early biological assessments identify harmful samples even before chemical confirmation, significantly reducing delays in interventions. Furthermore, the application of this type of framework is particularly valuable for substances present in trace concentrations that escape current analytical methods, but that (in mixtures) can cause negative effects on the ecosystem's trophic groups [13,19]. By linking chemical exposure to functional ecological consequences [19], these tools bridge the gaps left by conventional monitoring and can support a more robust water body classification, more comprehensive risk assessments, and sustainable water management policies. In this study, we tested how the use of a set of integrative tools, including hazardous substances, and rapid ecotoxicological bioassays could refine knowledge about the impacts of intensive agriculture in water bodies of agroecosystems. It examined how ecotoxicological variables can enhance our comprehension of agriculture's effects on reservoir ecosystems. To our knowledge, this is the first study to relate agricultural practices to pesticide and nutrient levels in water reservoirs in irrigated agroecosystems, employing a rapid ecotoxicological toolbox based on multitrophic groups. Hence, the investigation focuses on three key objectives: (1) analyze the correlation of pesticides with agricultural practices; (2) assess the aquatic ecosystem impacts of hazardous substances through multi-trophic group ecotoxicological bioassays; and (3) assess the feasibility of integrating ecotoxicological data to enhance the potential ecological status classification framework for water bodies in agroecosystems. This study focused on a reservoir within a hydro-agricultural system in a Mediterranean climate, where seasonal water scarcity and agricultural pressures impact water quality [20], and assessed how these factors influence the selection of the most sensitive water quality endpoints.

2. Materials and Methods

2.1. Study Area

The study area is integrated into the Alqueva reservoir, Southern Alentejo (Portugal), within the Guadiana basin. The Alentejo region comprises multiple sub-basins, with the transboundary Guadiana River basin being the most extensive. The Guadiana basin extends across 67,026 km^2, distributed between Spain (55,492 km^2, 83%) and Portugal (11,534 km^2, 17%), ranking among the five largest river systems on the Iberian Peninsula. The hydrological regime of this region was significantly altered by the construction of the Alqueva reservoir in Southern Portugal's Alentejo region. As part of the Alqueva Multipurpose Development Project (AMDP), this infrastructure gave rise to three irrigation subsystems (Alqueva, Ardila, and Pedrogão), which together constitute the Alqueva Global Irrigation System (AGIS) [21]. The Ardila irrigation subsystem, located downstream of the Alqueva reservoir within the Brinches-Enxoé Hydroagricultural Area (BEHA), contains the study site, the Lage reservoir (Figure 1: L; LS).

Portugal has around 3,641,592 ha of agricultural production, of which 14.3% (2,146,508 ha) is in Alentejo (Southern Portugal) [22]. Land use/land cover (LULC) analysis of its drainage basin reveals the following distribution: holm oak forest (25.5%); irrigated–annual crops (11.6%, with predominance of corn, sunflower, onion, and garlic); irrigated–permanent crops (34.3%, with predominance of olive trees and vines); non-irrigated–annual crops (12.7%, with the predominance of alfalfa and clover); set-aside land (12%); and urban land (0.2%) [20].

The soils in the region are predominantly Vertisols, Cambisols, and Luvisols, with medium to fine soil textures [23]. The study area presents a typical Mediterranean climate (Köppen classification Csa), characterized by hot, dry summers and mild, wet winters [24]. The study analyzed the agricultural practices on six distinct farms with annual crops in rotation: A1 (sunflower—maize—sunflower), A2 (sunflower—clover—onion), and A3 (maize—sunflower—maize), and permanent crops: P1 and P3 (two different vineyards) and P2 (olive grove) during three consecutive years (2018, 2019, and 2020) (Figure 1).

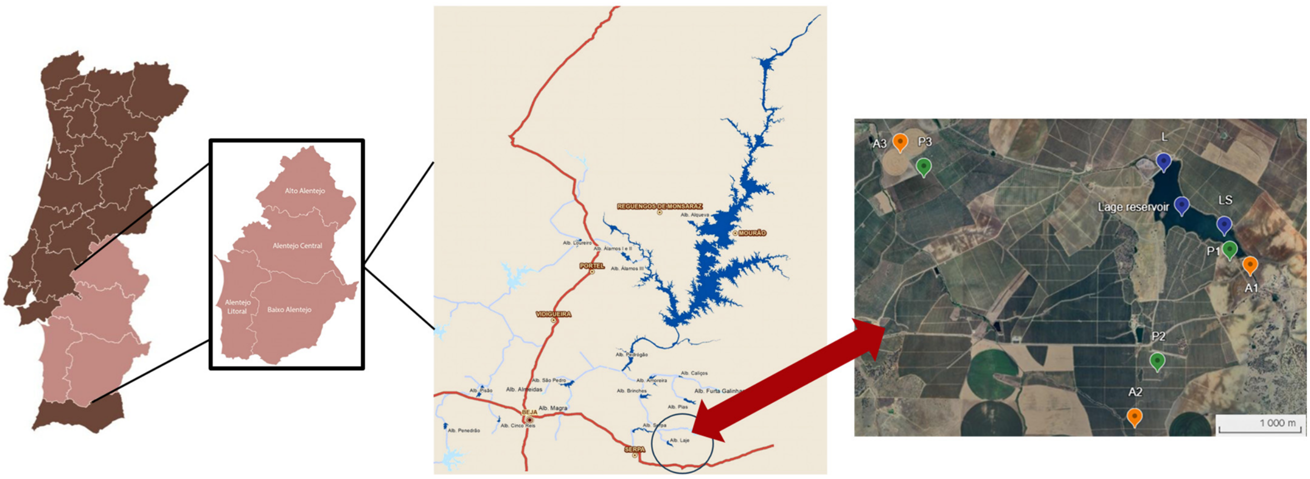


Figure 1. Map of Portugal showing the location of Baixo Alentejo, the Lage reservoir, the sampling points (Lage—L and Lage S—LS) (blue markers), and surrounding crops: permanent crops, where P1 and P3 are vineyards and P2 is an olive grove (green markers), and annual crops, where A1, A2, and A3 have crop rotation (orange markers) (Source: Google Earth and adapted from EDIA (2017) [25]).

Figure 2 displays the interannual variability of precipitation, evapotranspiration, and temperature from 2018 to 2020. The year 2018 exhibited classic Mediterranean climate characteristics—hot, dry summers combined with mild winters and low precipitation.

However, the following years showed clear deviations. Both 2019 and 2020 were warmer than usual, but 2020 represented a significant climatic anomaly: Despite being the hottest year on record with an average temperature of 17.3 °C, it also received 615 mm of rain—a clear anomaly compared to the region's typically dry summer conditions. As expected, evapotranspiration followed the temperature trend [24].

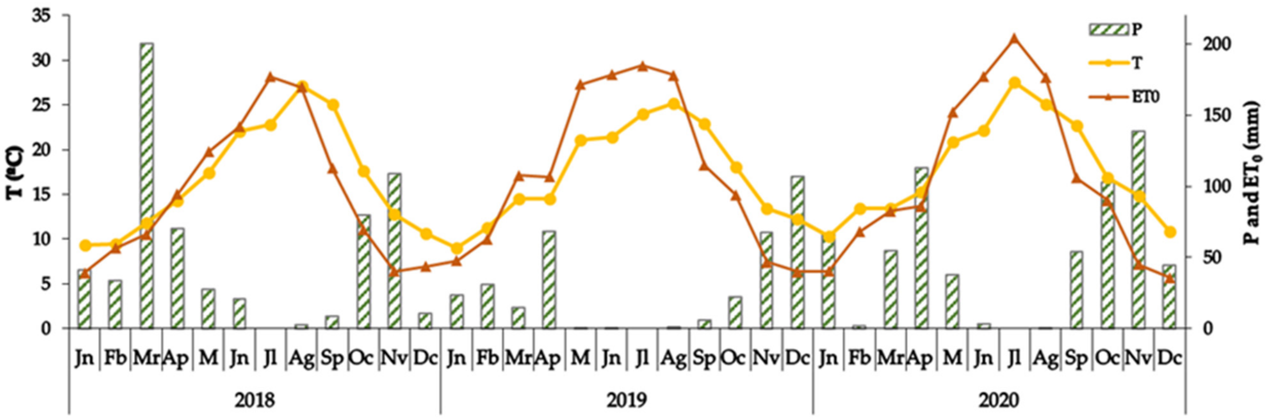


Figure 2. Variation in average monthly temperature (T; yellow line), evapotranspiration (ET_0; orange line), and average monthly precipitation (P; green bars) over the three years of study (2018–2020) in the municipality of Serpa. (January(Jn); February(Fb); March(Mr); April (Ap); May(M); June (Jn); July (Jl); August(Ag); September (Sp); October(Oc); November(Nv); December (Dc)).

This study was developed within the scope of the GOFitoFarmGest project to investigate how agricultural practices related to irrigated systems, such as fertilization and phytosanitary applications, influence water quality and the dynamics of aquatic ecosystems. The study also aimed to determine the most sensitive indicators for assessing the status of irrigated agroecosystems and the ecological condition of agricultural reservoirs.

2.2. Sampling Campaigns

Water sampling was conducted seasonally over a three-year period (2018–2020), with four campaigns representing both wet (April, December) and dry (July, September) hydrological conditions. Samples were collected from two distinct sites in the Lage reservoir: (1) at the middle of the reservoir: Lage (L: 37°58′12.64″ N, 7°31′0.06″ W); and (2) near the reservoir bank: southern Lage (LS: 37°57′44.60″ N, 7°30′26.10″ W) (WGS84 coordinate system).

At each sampling site, 3 L surface water samples were collected at 50 cm depth using a Van Dorn water sampler. All samples were immediately placed in lightproof coolers maintained at 4 °C during transport to the laboratory. Samples for physico-chemical analysis were processed within 24 hours of collection, while those for ecotoxicological testing were stored at −18 °C until analysis (maximum storage time: 1 month). Frozen samples were gradually thawed at 4 °C for 24 hours prior to bioassay initiation. The preservation of water samples for ecotoxicological analysis was based on the method used for pesticide analysis [26], preventing the degradation of pesticides before bioassay tests.

2.2.1. Surface Water Physico-Chemical Variables

Physico-chemical analysis of the Lage reservoir based on the assessment of pH, electrical conductivity, dissolved oxygen, biochemical oxygen demand, total suspended solids, total phosphorus and nitrogen, ammoniacal nitrogen, nitrates, and nitrites during the study period (2018–2020) was reported by Catarino et al. [20]. In sum, the water results revealed the following (Table S1): (1) frequent deviations of pH levels from the recommended range for irrigation water (6.5–8.4) [27] were observed, which can negatively affect soil structure and nutrient availability; (2) nutrient concentrations remained always below the regulatory limits for irrigation water despite agricultural pressure [27]; and (3) water quality-supporting variables such as total phosphorus and nitrogen, total suspended solids, and biochemical oxygen demand consistently classified the reservoir with a moderate ecological status according to the EU Water Framework Directive [20].

2.2.2. Pesticide Analysis

To reinforce our findings, the results incorporated pesticide quantification from 2018 to 2020. While pesticide dynamics during 2018–2019 were characterized by Alves-Ferreira et al. [26], the current study expands this research by incorporating pesticide quantification and dynamics of 2020. The analytical methodology used for the quantification of the target pesticides in water was performed by on-line solid phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS), as described by Alves-Ferreira et al. [26]. Pesticides were selected through a three-tier process considering (1) detection frequencies in regional water monitoring programs [26,28,29]; (2) information on pesticides applied to crops, which was recorded in the farm's agricultural practice management books (Table S2a,b); and (3) pesticides used in water status classification: specific (for the classification of potential ecological status (Directive 2000/60/EC) and priority compounds used for the classification of chemical status (Directive 2013/39/EU). During each year of the study, 51 pesticides belonging to the classes of herbicides, insecticides, and fungicides were quantified. Each of these substances has corresponding regulatory thresholds for water classification and analytical detection limits for each compound (Table S3).

2.3. Ecotoxicological Analysis

The ecotoxicological assessment was conducted using a multi-trophic approach with standardized bioassays: *Aliivibrio fischeri* luminescence inhibition (decomposer); *Pseudokirchneriella subcapitata* growth inhibition (primary producer); *Thamnocephalus platyurus* mortality; and *Daphnia magna* feeding rate inhibition (primary consumers). *A. fischeri* is widely used in ecotoxicology due to its rapid, standardized, low-cost, and highly sensitive bioassay, which detects contaminants (e.g., pesticides) effectively and complements results from other organisms such as *D. magna* [3]. *D. magna* and *T. platyurus* were chosen for their broad sensitivity to contaminants, standardized protocols, ecological relevance, and regulatory acceptance [30]. *D. magna*, a keystone species, comprises 60% of zooplankton biomass in temperate lakes and regulates algae through intense grazing (100–300% of body weight daily) [31].

The *P. subcapitata* is a standard model due to its ecological importance as a primary producer, global freshwater distribution, and role in the biomagnification of contaminants [32].

The temperature, pH, dissolved oxygen (DO), and electric conductivity (EC) of the samples were measured and were in accordance with the standard protocols used. For all bioassays, a test with a reference substance was performed as a positive control for validating the bioassay: (i) *A. fischeri*—with potassium dichromate solution ($K_2Cr_2O_7$) to verify whether the bacteria were viable and metabolically active. Several dilutions, between 0.25 and 4 mg L^{-1}, were prepared to verify if the toxic effect was within the optimal range ($0.4 < EC_{50} < 1.7$ mg L^{-1}; (ii) *T. platyurus*—with a potassium dichromate solution ($K_2Cr_2O_7$) with concentrations between 0.25 and 4 mg L^{-1} to verify whether the LC_{50} obtained was between 0.2 and 0.6 mg L^{-1}; (iii) *P. subcapitata*—with potassium dichromate solution ($K_2Cr_2O_7$) (0.5 to 4 mg L^{-1}) to verify whether the EC_{50} obtained was within the range stipulated (0.5 to 3.0 mg L^{-1}); and (iv) *D. magna*—with a potassium chloride (KCl) solution to verify that the inhibition of feeding rates was greater than 50%.

Therefore, the use of this group of bioassays is linked to the feasibility of using a battery of rapid, easy-to-perform, and cost-effective bioassays to complement chemical monitoring. Acute tests provide rapid, standardized, and reproducible results, allowing the screening of many samples across different seasons and conditions. The use of this ecotoxicological suite allows for the rapid separation of contaminated areas that induce strong ecotoxicological effects, requiring more rapid intervention, from those that exhibit less pronounced ecotoxicological responses. Furthermore, being more easily comparable with the biotic indices that have been used to determine ecological status, these rapid tests are more feasible for integration into the current water body classification framework supported by the WFD.

For an integrated assessment of water potential ecological status using ecotoxicological endpoints, we developed a toxicological classification system (TCS) based on Roig et al. [33], which was later validated and detailed by Novais et al. [17] (Table 1).

Table 1. Ecotoxicological classification system based on toxic endpoints (EC_{50} and LC_{50}; growth and feeding rate) for assessing water body status.

	Classification	**EC_{50} and LC_{50}**	**Growth and Feeding Rate**	**Color**	**Score**
Class 1	Non-toxic	EC_{50}/LC_{50} >100%	GFR > 80%	blue	0
Class 2	Slightly toxic	61% < EC_{50}/LC_{50} < 100%	50% < GFR ≤ 80%	green	1
Class 3	Marginally toxic	21% < EC_{50}/LC_{50} < 60%	20% < GFR ≤ 50%	yellow	2
Class 4	Moderately toxic	10% < EC_{50}/LC_{50} < 20%	10% < GFR ≤ 20%	orange	3
Class 5	Highly toxic	EC_{50}/LC_{50} < 10%	GFR ≤ 10%	red	4

Notes: EC_{50}: concentration that promotes an effect in 50% of the population exposure; %, (v/v). LC_{50}: concentration that promotes lethality in 50% of the population exposure; %, (v/v).

2.3.1. Luminescence Inhibition of *A. fisheri*

The luminescence inhibition bioassay was performed using freeze-dried *Aliivibrio fischeri* (strain NRRL B-11177) following the standardized DR LANGE LUMIStox protocol [34]. The reservoir water samples were diluted in 2% NaCl solution (v/v) to six test concentrations (100%, 50%, 25%, 12.5%, 6.25%, and 3.125%), with duplicate measurements at each concentration level. The test conditions were as follows: temperature of 15.0 ± 0.5 °C, exposure duration of 30 minutes, and a reference control non-toxic 2% NaCl solution (negative control). The 30 min EC_{50} (concentration that promotes an effect in 50% of the bacterial population exposure; %, v/v) was calculated.

2.3.2. Mortality Bioassay with *T. platyurus*

To determine the mortality of the crustacean *T. platyurus*, the standard operating procedure provided in the THAMNOTOXKIT FTM kit [35] was followed. The first 24 hours of the test focused on the hatching of the crustacean cysts, which were subsequently exposed to different concentrations of samples from the Lage reservoir (25, 50, 75, and 100%, v/v), with synthetic freshwater included in the test kit, in quadruplicate. At the same time, a negative control was prepared using synthetic freshwater included in the kit. The samples were then stored at 25 °C for 24 hours in the dark, and the organisms were not fed during the test. Finally, the dead organisms in each dilution were quantified, and the 24-hour LC_{50} (concentration that promotes lethality in 50% of the population exposure; %, v/v) was calculated.

2.3.3. Growth Inhibition Bioassay with the Green Microalga *P. subcapitata*

To determine the growth inhibition of the microalga *P. subcapitata*, the 201 OECD protocol was followed. Initially, samples from the Lage reservoir were diluted with MBL (25, 50, 75 and 100%, *v*/*v*) and subsequently 900 µL of these and 100 µL of suspended microalgae (prepared in laboratory) were transferred to a 24-well microplate, with 6 replicates for each concentration and 8 replicates for the negative control (MBL).

The microplates were then placed on a reciprocating shaker under continuous light for approximately 72 h. Finally, the microalgae were quantified in a Neubauer chamber, and the growth rate was calculated.

The average growth rate for a specific period was determined from Equation (1):

$$\mu_{i-j} = (\text{In } B_j - \text{In } B_i)/t_j - t_i \tag{1}$$

where μ_{i-j} is an average specific growth rate for the specific time interval, i to j; ti is the time at the start of the exposure period; t_j is the time at the end of the exposure period; B_i is the biomass concentration at time i; and Bj is the biomass concentration at time j.

The inhibition of algal growth was estimated from Equation (2):

$$\%I = [(\mu c - \mu t)/\mu c] \times 100 \tag{2}$$

where %I is the mean percentage of inhibition for a specific growth rate; µc is the mean value for the growth rate in the control; and µt is the mean for the growth rate in the Lage water samples.

2.3.4. Feeding Rate Bioassay with *D. magna*

The organisms used for this bioassay were obtained through continuous laboratory cultures contained in American Society for Testing and Materials hard water [36], enriched with the organic additive Marine "25" (Pann Britannica Industries Ltd., Waltham Abbey, UK), an extract from the alga *Ascophyllum nodosum* [37].

The methodology of Mcwilliam and Baird [38] was adapted to perform the feeding rate bioassay with *D. magna*. Dilutions were started with samples from the Lage reservoir (0 (control), 25, 50, 75 and3 100% (*v*/*v*)) in quadruplicate, with five newborns aged 4 or 5 days, born between the 3rd and the 5th L. A volume of algae (*P. subcapitata*) was added to each 25 ml pot, corresponding to a density of 3.0×10^5 cells mL^{-1} $Daphnia^{-1}$ (equivalent to 2.65 C mL^{-1}). In parallel, a comparative control (4 replicates) was prepared with the samples and algae at a density of 3.0×10^5, without organisms, to serve as a negative control for the growth of algae under the test conditions. At the beginning of the test, the number of algae in each dilution was quantified using a Neubaeur chamber. The pots were then placed for 24 h at 20 °C in the dark to prevent algae growth. To finish the test, the organisms were removed from the pots and the algae were quantified.

The feeding rate was calculated according to Equation (3), reported by Allen et al. [39]:

$$(FR = V \times ((C_0 - C_{24}))/t) \tag{3}$$

where FR = feeding rate (cells *animal $-1 \times h^{-1}$); V = volume of the medium in the test pot (mL); C_0 = the initial cell concentration (numbers $\times$ mL^{-1}); C_{24} = the final cell concentration (numbers * mL^{-1}); t = duration of the experiment (h).

2.4. Statistical Analysis of Data

Water quality data were analyzed through descriptive statistics. Physico-chemical variables were expressed as median, minimum, and maximum values, while pesticide levels were evaluated using mean concentration, detection frequency, and range. Seasonal and annual variations were also examined.

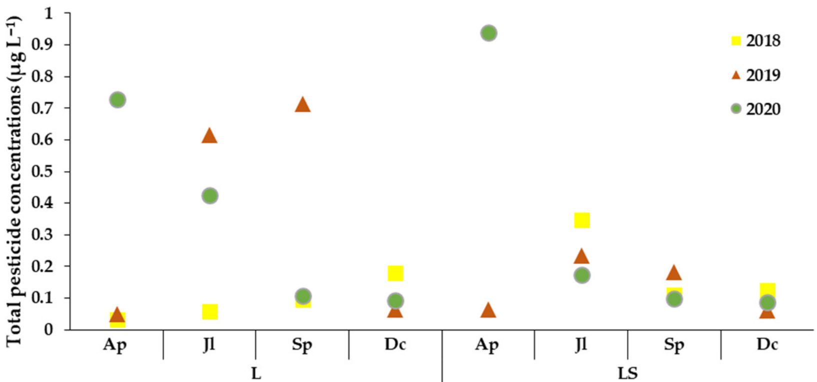
Data on ecotoxicological endpoints (growth inhibition and feeding rate) were checked for homogeneity of variance using the Kolmogorov–Smirnov test and, when possible, subjected to one-way analysis of variance (ANOVA). Data that did not satisfy the assumptions for ANOVA were analyzed using Kruskal–Wallis ANOVA by ranks test. Whenever significant differences were found ($p < 0.05$), a post hoc Dunnett's test was used to compare treatments with the control, using a 0.05 significance level [40]. Spearman's rank coefficients, as a non-parametric measure calculated on ranked data, for a confidence level of $p < 0.05$, were applied to assess the correlations of physico-chemical variables and pesticides with the ecotoxicological endpoints.

These statistical analyses were performed using STATISTICA 7.0 (Software™ Inc., Chalfant, PA, USA, 2007). For the *A. fischeri* bioluminescence inhibition test, the EC_{50} values were determined using LUMISsoft 4 Software™ (HACH-LANGE GmbH, Hørsholm, Denmark), while for the *T. platyurus* bioassay, the 24 h LC_{50} values were determined by applying the Probit analysis [41], using SigmaPlot 12.0 software (Systat Software Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Pesticide Dynamics

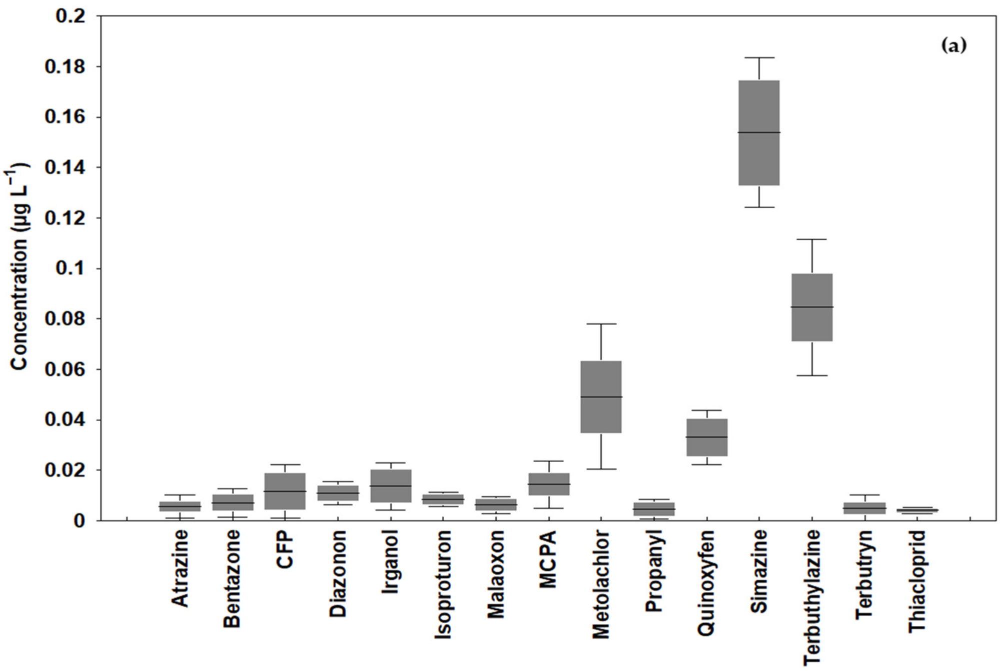
In 2020, 26 of the 36 pesticides quantified over the study period were detected in the Lage reservoir. Concentrations at both sites (L: 1.35 μg L^{-1}; LS: 1.31 μg L^{-1}) were 2.8 times higher than in 2018 (L: 0.37 μg L^{-1}; LS: 0.38 μg L^{-1}) and 1.3 times higher than in 2019 (L: 1.44 μg L^{-1}; LS: 0.54 μg L^{-1}). This peak was driven by high-concentration events in April 2020 (Figure 3; Table S4a,b). Temporally, pesticide levels peaked in April, followed by July. This pattern reflects two key factors: (1) Agricultural practices: Increased pesticide application in spring (particularly March–April; Table S2a,b); (2) Climatic conditions: higher precipitation during March–April, with 270, 82.9, and 188 mm in 2018, 2019, and 2020, respectively (Figure 2), promoting an increase in the carryover of substances [42], including those that could be adsorbed to the soil [43]. Spatial analysis revealed that during 2020, the total amount of pesticides was similar at both sampling sites (L: 1.35 μg L^{-1}; LS: 1.30 μg L^{-1}). However, analysis over the three-year study period showed that Lage (L) presented higher total pesticide concentrations than LS.



	Ap (L)	Jl (L)	Sp (L)	Dc (L)	Ap (LS)	Jl (LS)	Sp (LS)	Dc (LS)
2018	0.03	0.06	0.09	0.18	n.d.	0.35	0.11	0.13
2019	0.05	0.62	0.71	0.06	0.06	0.23	0.18	0.06
2020	0.73	0.43	0.11	0.09	0.94	0.17	0.1	0.09

Figure 3. Total pesticide concentrations (μg L^{-1}) in the study during 2018 (yellow square), 2019 (orange triangle), and 2020 (green circle) (April (Ap); July (Jl); September (Sp); December (Dc)) in L (Lage) and LS (Lage S). n.d.: sample not collected.

Figure 4 displays the most representative pesticides detected in the reservoir during 2020. Quantification revealed 21 compounds at site L and 26 at site LS. Twenty-one pesticides were common on both sites, while five additional compounds were found at LS in April.



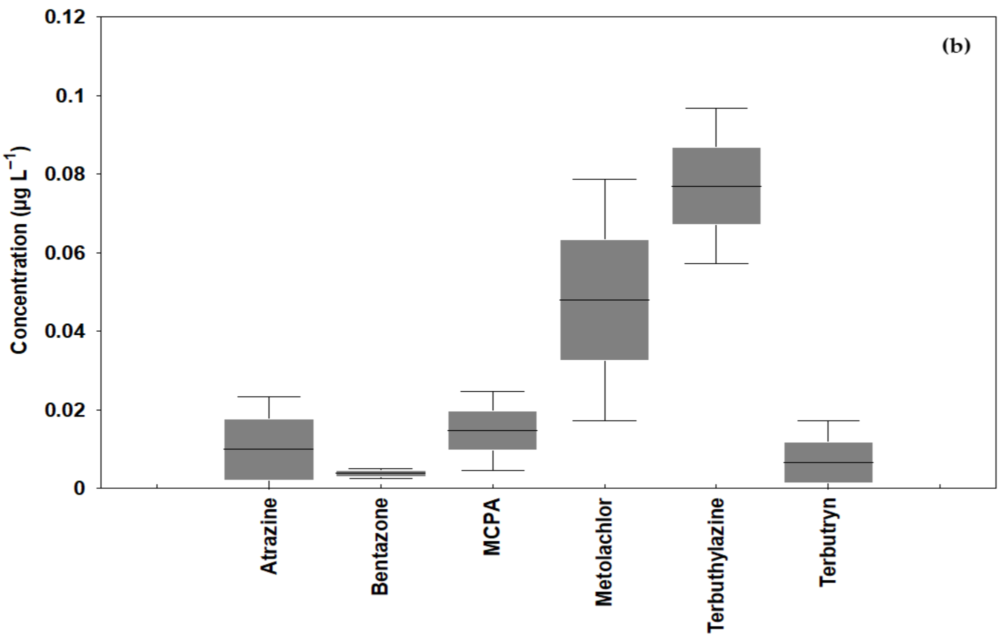


Figure 4. Concentration range (μg L^{-1}) of pesticides quantified in Lage (**a**) and Lage S (**b**) during 2020. The pesticides displayed are the most representative of the study (straight line box: median values; box: 25 % and 75 % cut of the values; whiskers: maximum and minimum. CFP: chlorfenvinphos; MCPA: 2-methyl-4-chlorophenoxyace-tic acid.

The data revealed an increasing trend in pesticide water contamination, driven by increasing concentrations of the herbicides terbuthylazine, simazine, metolachlor, and MCPA, as well as the organophosphate fenthion sulfoxide. Terbuthylazine was the most consistently detected pesticide [26,44], appearing in 100% of the 2020 samples (Figure 4), with an increase over time (2018: L—0.073, LS—0.056 μg L^{-1}; 2019: L—0.11, LS—0.086 μg L^{-1}; 2020: L—0.34, LS—0.31 μg L^{-1}). Furthermore, the presence of this herbicide in the reservoir followed a seasonal pattern, with peak concentrations occurring during dry periods, reaching the highest concentration in the reservoir of 0.12 μg L^{-1} in April 2020 at the Lage sampling point. Terbuthylazine is commonly applied to annual crops like maize [29]. The farmers' records (from agricultural practice registers) confirm its use on maize crops in 2020 (Table S2b). The concentrations of metolachlor, MCPA, and terbutryn increased over the three years, being detected in 100% of the samples from 2020, with total concentrations of 0.12, 0.39, and 0.05 μg L^{-1}, respectively. In general, our results are in line with those of Mugudamani et al.'s study [45] conducted in Southern Africa (Mediterranean climate), reporting (1) 100% detection of terbuthylazine and metolachlor, (2) the highest concentrations of terbuthylazine and simazine, and (3) the greater concentration observed in the dry season. MCPA and metolachlor are predominantly used in soybean, potato, cotton, and maize crops [26], in the present study reports indicated their application in maize (Table S2b). Terbutryn, an herbicide of the triazine class, has been banned in the European Union since 2003 (Regulation EC no 2076/2002). However, its detection in hydro-agricultural water reservoirs persists, which can be attributed to its high affinity for organic carbon particles (K_{oc} = 2432 mg L^{-1}), facilitating strong adsorption in soil and aligning with its prolonged half-life in soil (DT_{50}_soil = 74 days) [46]. Despite that, the concentrations in the water reservoir were always below the maximum allowable Environmental Quality Standards (EQS) levels (EQS: 0.34 μg L^{-1}; Directive 2013/39/EU). In contrast to the consistently detected pesticides, several compounds punctually exhibited elevated concentrations in 2020, namely, (i) diflufenican (L_Ap: 0.085 μg L^{-1}; LS_Ap: 0.075 μg L^{-1}), with levels similar to those in surface waters influenced by agriculture activities (Germany [47]); (ii) fenthion sulfoxide (L_Ap: 0.080 μg L^{-1}; LS_Ap: 0.050 μg L^{-1}), consistent with findings in rivers of agricultural fields [48]; (iii) quinoxyfen (L_Ap and L_Jl: 0.066 μg L^{-1}; LS_M: 0.030 μg L^{-1}), similar to concentrations reported in Germany vineyards streams [49]; and (iv) simazine (L_Ap and L_Jl: 0.31 μg L^{-1}; LS_M: 0.082 μg L^{-1}), aligning with the data of water reservoirs with agricultural influence in Southern Africa [45]. The occasional detection of these pesticides may be correlated with a group of factors, such as their historical application, their properties, and the specific meteorological conditions of 2020. Agricultural records indicate that none of them were applied during the study period (diflufenican and quinoxyfen remain permitted; otherwise, fenthion sulfoxide (a metabolite of fenthion) and simazine are prohibited [26]. These compounds are characterized by high soil half-life (DT_{50}-Soil (days): diflufenican = 94.5; quinoxyfen = 308; simazine = 60) [46] and high organic carbon partition coefficient (K_{oc} (mg L^{-1}): diflufenican = 5504; quinoxyfen = 23; fenthion sulfoxide = 183; simazine = 130) [46], factors that promote a high tendency to persist in and adsorb to soil, limiting the leaching processes. Consequently, their transport into surface water can be dependent on high-intensity precipitation events that facilitate the desorption process and transport. This mechanism explains their detection following the short-duration, high-intensity rainfall that occurred in March and April 2020 (Figure 2).

In addition to terbutryn, a group of 10 banned pesticides and their metabolites were quantified during the study. These compounds, whose use is prohibited by various EU regulations, include atrazine, simazine, diazinon, malaoxone, diuron, isoproturon, alachlor, CFP, irgarol, and propanil. Their maximum detected concentrations are listed below, along with the EU legislation that withdrew their authorization: CFP: 49.9 μg L^{-1} (Regulation 1107/2009/EU); Irgarol: 28.4 μg L^{-1} (Regulation 2016/107/EU); Propanil: 18.9 μg L^{-1} (Decision No. 2008/769/EC); Simazine: 0.39 μg L^{-1} (Decision No. 2004/247/EC); Diazinon: 0.046 μg L^{-1} (Decision No. 2007/393/EC); Isoproturon: 0.027 μg L^{-1} (Regulation 2016/872/EU); Atrazine: 0.026 μg L^{-1} (Decision No. 2004/248/EC); Malaoxone: 0.016 μg L^{-1} (Decision No. 2007/389/EC); Diuron: 0.016 μg L^{-1} (Regulation 2019/707/EU); and Alachlor: 0.014 μg L^{-1} (Decision No. 2006/966/EC).

The continued detection of banned pesticides has been frequently reported in other Mediterranean studies [50,51]. Despite regulatory bans, these pesticides persist in hydro-agricultural reservoirs at detectable concentrations. This phenomenon can be attributed to their environmental persistence (e.g., diuron with a DT_{50} in soil = 146.6 days [46] exceeding the European Union regulatory limit of 100 days for persistent organic pollutant classification (POPs) [46], their lipophilic properties (log $K_{ow} > 3$; e.g., alachlor, CFP, diazinon, irgarol, and terbutryn), and historical accumulation in watersheds. Furthermore, the results also suggest ongoing contamination, either through illegal use or through the remobilization of residues from soil during extreme hydrological events [26,29].

Among the analyzed pesticides, five (2,4-D, bentazone, linuron, mecoprop, and terbuthylazine) were used to classify the potential ecological status of the reservoir, as they are integrated in the group of specific pollutants (Figure 5). 2.4-D (LS_Jl 2019 with 0.0125 μg L^{-1}) and mecoprop (LS_Ap 2020 with 0.025 μg L^{-1}) were detected punctually. The concentrations quantified in the Lage reservoir were all below the threshold, contributing to the potential ecological status classification of the Lage reservoir as "Good" (Table S3; Annex VII; Directive 2000/60/EC). Despite regulatory compliance, the ubiquitous detection of terbuthylazine (100% detection frequency) and bentazone (70% detection frequency) suggests continuous input from agricultural runoff [20].

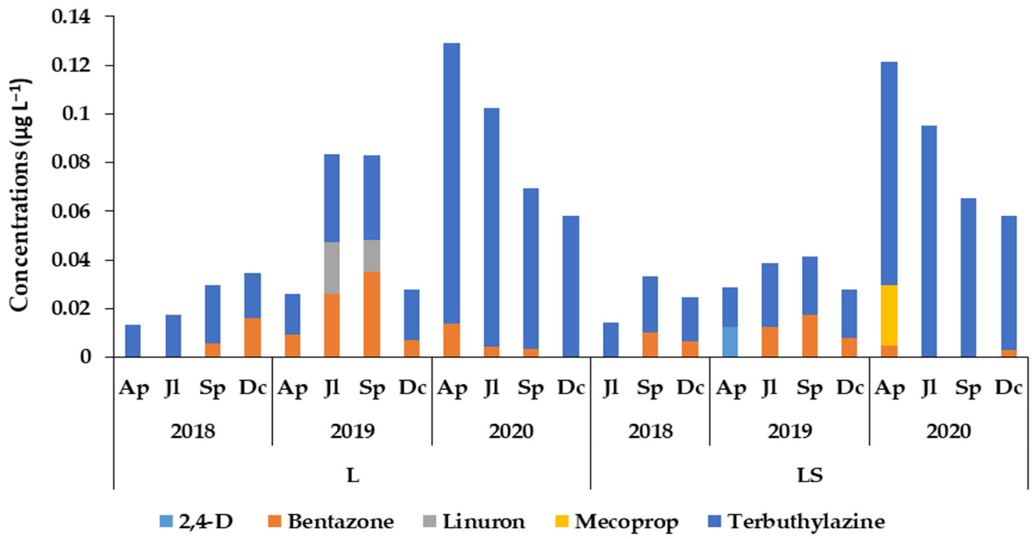


Figure 5. Total pesticide concentrations (μg L^{-1}) of quantified specific pollutants in the Lage reservoir during the study (2018, 2019, and 2020) at Lage (L) and Lage S (LS). April (Ap); July (Jl); September (Sp); December (Dc).

Alachlor, atrazine, CFP, diuron, and simazine were analyzed for chemical status classification. These compounds are classified as hazardous under EU Directive 2013/39/EU and were selected based on their (1) banned status, (2) environmental persistence, (3) toxicity, and (4) historical detection patterns in the study region. Several studies reported that these compounds represent key pollutants of concern for Mediterranean agricultural watersheds [29,50]. The present results consistently measured concentrations below regulatory thresholds (MAC, Table S3; Figure 6), supporting the classification of the reservoir with a "Good Chemical Status". Although the detected pesticide concentrations did not affect the classification of the reservoir's chemical status, they can still be toxic to aquatic species and harm ecosystem functioning [52]. This finding suggests that current classifications of water ecological status should be based on more robust tools incorporating indicators capable of assessing sub-lethal toxicological effects or the disruption of interspecific interactions within the ecosystem.

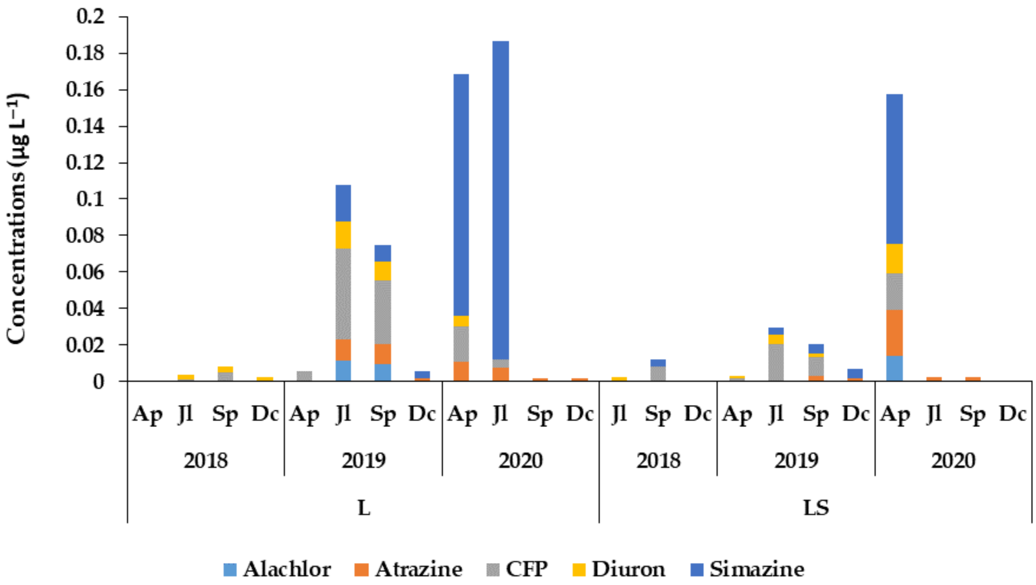


Figure 6. Total pesticide concentrations (μg L^{-1}) of quantified priority substances in the Lage reservoir during the study (2018, 2019 and 2020) at Lage (L) and Lage S (LS). April (Ap); July (Jl); September (Sp); December (Dc).

3.2. Ecotoxicological Analysis

For the Lage reservoir, ecotoxicological risk was screened through bioassays using bacterial, algae, and crustacean species. The results showed that the Lage water samples did not induce toxicity in *T. platyurus*. The observed response differs from the recognized sensitivity of this species to water reservoirs [53]; however, it agrees with Szklarek et al. [54], who reported no toxicity detected in the Pilica River (Poland). Furthermore, *T. platyurus* has demonstrated sensitivity to contaminants present in eluates of sediments [28] and in soil leachates [55]. Additionally, studies have highlighted its sensitivity in reservoirs with bentazone, dimetoathe, linuron, mecoprop, and terbuthylazine [56] and in a temporary stream with bentazone, mecoprop, metolachlor, MCPA, and terbuthylazine [57]. These previous works suggest that the current results likely reflect key differences in contaminant concentrations, bioavailability, and environmental matrices. These results highlight the critical importance of matrix-specific considerations when employing bioindicators for environmental assessments.

The *A. fischeri* bioluminescence inhibition assay evidenced a higher sensitivity in detecting toxicity in 11% of samples (4 out of 36), with EC_{50} values ranging from 39% (v/v) to 51% (v/v) (Table S5). Notably, 75% of toxic events occurred during the spring–summer period, which may be correlated with the reduction of water volume, lower dilution capacity, and increased agricultural runoff. The *Spearman* correlation analysis revealed that the toxicity of the bacteria is mostly correlated with nutrients and ions, showing significant associations with NH_4^+ (R = 0.466, p < 0.05), Cl^- (R = 0.538, p < 0.05), and NO_3^- (R = 0.537, p < 0.05) (Table S6). Studies suggest that NH_4^+ itself has minimal toxicity to *A. fischeri,* implying that its correlation may stem from co-occurring pesticides [57]. Similarly, studies have reported that Cl^- toxicity typically occurs at concentrations higher than 500 mg L^{-1} [58], the maximum level detected in the Lage reservoir being 95.66 mg L^{-1}, suggesting an indirect link to other contaminants or ionic compounds. The observed negative correlation between manganese and *A. fischeri* toxicity (R = −0.486) suggests a protective effect. This effect can be attributed to the biological functions of manganese; it acts as an essential cofactor for microbial enzymes [59], and at low concentrations, it exhibits antioxidant properties that can neutralize contaminant-induced oxidative stress [60]. These results are consistent with previous studies on manganese's protective capacity in aquatic systems [58].

Figure 7 shows the growth rates of the microalga *P. subcapitata* when exposed to the Lage samples. A significant reduction in the growth rate of *P. subcapitata* was observed in 89% of the samples. The green microalgae demonstrated greater sensitivity than *A. fischeri* in detecting toxicity in 89% vs. 11% of the samples analyzed, in accordance with Canova et al.'s study [61].

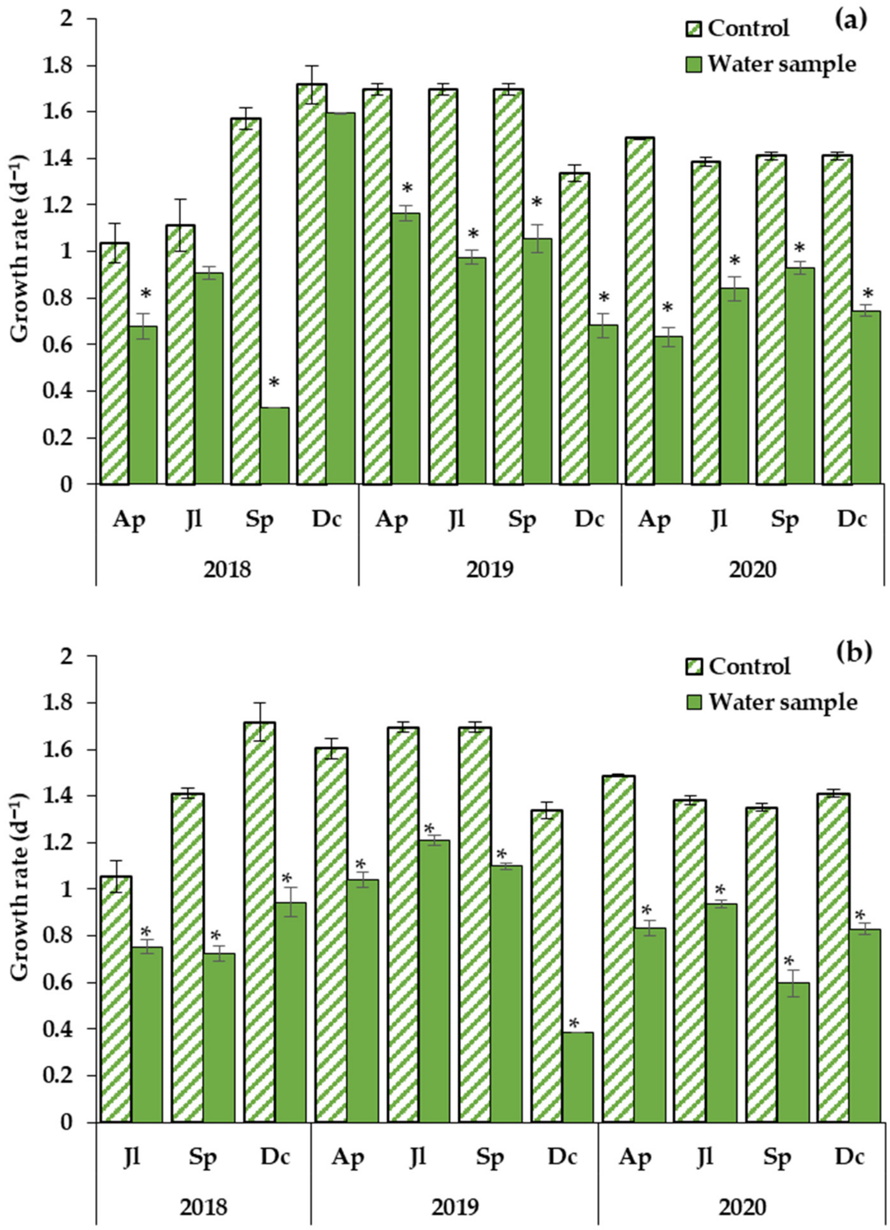


Figure 7. Growth rate (d^{-1}) of the microalga *P. subcapitata* after 72 h exposure to water samples (100% of water sample) from the Lage reservoir: Lage (L, (**a**)) and Lage S (LS, (**b**)) in April (Ap), July (Jl), September (Sp), and December (Dc) 2018, 2019, and 2020. Mean ± standard deviation (n = 6), * $p < 0.05$, using the *Dunnet* post hoc comparison test with the control MBL test.

Microalgae growth inhibition is positively correlated with precipitation (R = 0.457, $p < 0.05$), EC (R = 0.495, $p < 0.05$), and atrazine (R = 0.428, $p < 0.05$) (see Table S6). The correlation with precipitation may reflect the effects of first rains, runoff dynamics, and particle-facilitated transport [62]. The correlation with EC indicates co-transport mechanisms [63].

The significant positive correlation between microalgae growth inhibition and atrazine (R = 0.4276, $p < 0.05$) confirms its direct phytotoxic effect on *P. subcapitata*. The results are in agreement with previous studies conducted in Dutch surface waters [64] and in Mediterranean reservoirs [65]. The consistent sensitivity of *P. subcapitata* across diverse ecosystems [18], its reliability in complementing physico-chemical analysis [17], and its usefulness as an early warning indicator for herbicide impacts are well established. It is important to emphasize that even if the correlations obtained in the present study are not strong enough to demonstrate associations with other pesticides, several studies have reported toxicity to microalgae of other pesticides, such as diuron [66], terbuthylazine and metolachlor [67], and bentazone [68].

The FR results of *D. magna* showed a general increase in crustacean feeding when exposed to Lage water samples (non-filtered) during the study period (Figure 8). Furthermore, crustacean feeding rates reached their highest levels in the July samples at both sampling sites. The results corroborate the low sensitivity of this bioassay to water samples from Lage, contrasting with the results reported by Pinto et al. [69] for the Aguieira reservoir (Portugal). Otherwise, these results are consistent with those reported by Rodrigues et al. [70] for unfiltered water samples from the Pocinho reservoir and for filtered samples from the Miranda reservoir (Portugal). The observed results may indicate low contamination in the Lage water samples; however, other factors may also play a role. These include the influence of planktonic communities on *D. magna*'s feeding (since some species are more palatable than others) [70], phosphorus (P) concentrations in both phytoplankton and water samples [70], and potential dynamics related to the homeostatic P requirements of *D. magna*. Indeed, the significant increase in FR compared to the control highlights that water samples may present more palatable phytoplankton species with higher phosphorus concentrations than the control, which may mask the effect induced by hazardous substances. This finding can be supported by *Spearman* correlations, which reveal that all tested pesticides negatively affect the feeding rate of the crustacean (Table S6), with a significant effect observed for the herbicides chlortoluron ($R = -0.501$, $p < 0.05$), diuron ($R = -0.467$, $p < 0.05$), and propanyl ($R = -0.427$, $p < 0.05$) and insecticides diazinon ($R = -0.426$, $p < 0.05$) and thiacloprid ($R = -0.415$, $p < 0.05$). Although the results did not show a decrease in feeding rate, an effect likely masked by the levels of nutrients and organic matter in the reservoir water, as previously reported by us, the variability of this toxicological outcome is clearly associated with the presence of pesticides. Feeding rates showed significant spatial and temporal variations between sampling sites and months, highlighting the sensitivity of feeding behavior assays as a diagnostic tool to detect water quality degradation. These results demonstrate that assessment of *D. magna* feeding activity can effectively discriminate between indirect and site-specific ecological impacts [70]. Furthermore, daphnia populations in aquatic ecosystems exhibit seasonal peaks, reaching the highest densities in early spring and late winter. These fluctuations are driven by increased light availability and nutrient levels, which boost phytoplankton growth and subsequently enhance herbivorous zooplankton productivity, as demonstrated by Castro & Gonçalves [71]. Recent studies in a Portuguese reservoir reported comparable results, demonstrating that increasing abiotic factors (e.g., temperature and organic matter) reduces the sensitivity of bioassays, as observed by Pinto et al. [69] and Rodrigues et al. [70]. This trend may be linked to elevated levels of organic matter (in this case, measured in the form of BOD_5, which reached its maximum in Sp_18, with 9.3 mg O_2 L^{-1} in L and 10 mg O_2 L^{-1} in LS, and temperature, with 26 and 28 °C in L and LS_Jl19 (Table S1)), which often contribute to the moderate water quality classification of the reservoir.

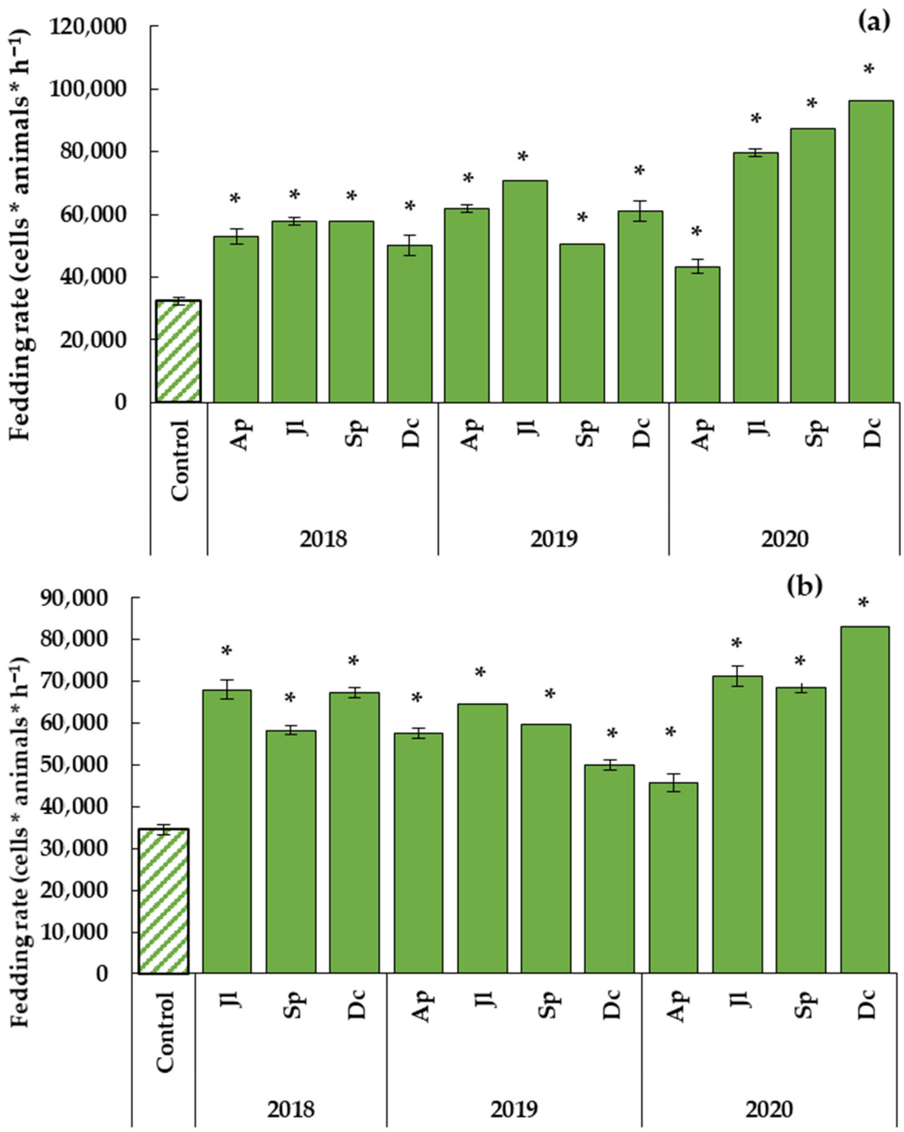


Figure 8. *D. magna* feeding rate after 24 h exposure to water samples (100% of water sample) from the Lage reservoir: Lage (L, (**a**)) and Lage S (LS, (**b**)) in April (Ap), July (Jl), September (Sp), and December (Dc) 2018, 2019, and 2020. Mean ± standard deviation ($n = 6$), * $p < 0.05$, *Dunnett's* post hoc comparison test with the control ASTM.

Integration of Ecotoxicological Bioassays in the Assessment of Water Quality Status

After using the specific chemical and ecotoxicological effects to analyze the status of the water reservoir, we integrated these results with the ecological potential assessed by Catarino et al. [20] to develop a more integrative classification of the reservoir.

As shown in Table 2 (using the scoring system from Table 1), the ecotoxicological assessment classified the samples as follows: 4.4% as non-toxic, 34.8% as marginally toxic, and 60.9% as slightly toxic. The classification as marginally toxic was mainly driven by responses from *A. fischeri* (samples L_Ap18, L_Dc18, L_Jl20, and LS_Ap20) and *P. subcapitata* (samples L_Sp18, L_Ap20, LS_Dc19, and LS_Sp20). Despite these findings, the chemical status of the reservoir remained "Good" throughout the three-year study period, as concentrations of specific pollutants and priority substances (Table S3) remained consistently below regulatory thresholds. Consequently, while chemical monitoring indicated compliance with water quality standards, ecotoxicological assessments revealed some biological impacts, reinforcing the need to integrate a greater number of biological approaches into environmental assessments.

The previous study classified the status of the Lage reservoir as “Moderate”, mainly due to elevated levels of nutrients (nitrogen and phosphorus), organic matter (BOD_5), and suspended solids (SST), which affected 91% of all samples [20]. Table 2 presents the classification of the reservoir considering the ecotoxicological outcomes, with 35% of the samples being classified as marginally toxic and 65% as slightly toxic. The integrative analysis (Table 3) allowed refinement of the classification, demonstrating that (i) the ecotoxicological component provides important complementary information to the traditional water status classification, and (ii) its integration can improve, in some situations, the accuracy of the potential ecological status classification, as reported in previous studies developed in Alqueva, with an improvement in classification sensitivity of around 20% [57]. The study highlights how the sensitivity of ecotoxicological tools can vary, along with the presence of confounding factors, emphasizing the importance of repeated testing to obtain a reliable water quality classification. This reinforces the idea that when chemical stressors affect aquatic life, appropriately chosen ecotoxicological tools can accurately detect these effects [72].

A well-designed set of ecotoxicological tests offers direct information on how organisms respond functionally, providing decision-makers with more actionable insights than relying solely on chemical thresholds or physicochemical variables [33]. For example, functional-based tools have been particularly useful in detecting subtle but ecologically relevant impacts, supporting the objectives of the Water Framework Directive (WFD), as compounds below regulated limits in composite samples can still promote toxicity.

Table 2. Table with the ecotoxicological final classification, according to Roig et al. [33] and Novais et al. [17], for Lage (L) and Lage S (LS) during the three years of study (2018–2020).

Water Sample	L												LS										
	2018				2019				2020				2018			2019				2020			
	Ap	Jl	Sp	Dc	Ap	Jl	Sp	Dc	Ap	Jl	Sp	Dc	Jl	Sp	Dc	Ap	Jl	Sp	Dc	Ap	Jl	Sp	Dc
A. fisheri (EC_{50} %)	39	>100	>100	51	>100	>100	>100	>100	>100	46	>100	>100	>100	>100	>100	>100	>100	>100	>100	41	>100	>100	>100
T. platyurus (EC_{50} %)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
P. subcapitata (% Growth)	65	81	21	93	69	58	62	51	42	61	66	53	71	51	55	65	71	65	29	56	68	44	59
D. magna (% feeding)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ecotoxicological Final Classification	M	NT	M	M	S	S	S	S	M	M	S	S	S	S	S	S	S	S	M	M	S	M	S

Note: Ap—April; Jl—July; Sp—September; Dc—December. Ecotoxicological Final Classification: M—Marginally toxic (yellow); S—Slightly toxic (green); NT—Non-toxic (blue).

Table 3. Tables with the surface water status classification based on the WFD parameters (including the ecological and chemical status) and the ecotoxicological classification in Lage (L) and Lage S (LS).

Water Sample	L												LS										
	2018				2019				2020				2018			2019				2020			
	Ap	Jl	Sp	Dc	Ap	Jl	Sp	Dc	M	Jl	Sp	Dc	Jl	Sp	Dc	Ap	Jl	Sp	Dc	M	Jl	Sp	Dc
Potential Ecological status																							
GPC																							
SP																							
Chemical Status																							
PS																							
Ecotoxicological Classification																							
WFD Classification																							
	M	M	M	G	M	M	M	G	M	M	M	G	M	M	M	M	M	M	M	M	M	M	M
WFD + Ecotoxicological Classification																							
	M	M	M	M	M	M	M	G	M	M	M	G	M	M	M	M	M	M	M	M	M	M	M

Notes: GPQ: General chemical and physico-chemical elements; SP: Specific pollutants; PS: Priority substances; WFD: Water Framework Directive. General physico-chemical classification (GPC): Moderate (yellow) and Good (green); Specific pollutants (SP) and chemical status (PS): Good (blue); Ecotoxicological classification: Marginally toxic (yellow); Non-toxic (blue); Slightly toxic (green); WFD classification: Moderate (yellow) and G—Good (green); WFD + Ecotoxicological classification: Moderate (yellow) and G—Good (green). Ap: April; Jl: July; Sp: September; Dc: December; ↑ cases where the final classification was influenced by the ecotoxicological classification.

4. Conclusions

The main source of contamination in the Lage reservoir is linked to intensive agricultural practices in the surrounding watershed, particularly the widespread use of fertilizers and pesticides in olive groves, vineyards, and annual crops such as sunflower, maize, and onion. Herbicides were the most prevalent pesticides in the Lage reservoir in 2020, with metolachlor, terbuthylazine, and MCPA showing 100% detection frequency.

In the present study, a group of 11 banned pesticides was quantified: terbutryn, atrazine, simazine, diazinon, malaoxone, diuron, isoproturon, alachlor, CFP, irgarol, and propanil. Concentrations of specific pollutants (2,4-D, bentazone, linuron, mecoprop, and terbuthylazine) below the MAC contributed to the classification of the potential ecological status of the Lage reservoir as "Good".

Furthermore, the quantified priority pollutants in the Lage reservoir allow the reservoir to be classified as having "Good chemical status". Although pesticide concentrations do not affect the reservoir's chemical status, ecotoxicological results showed that they can be toxic to aquatic species and impair ecosystem functioning. This finding suggests that the current water status classification, based on the WFD, should be strengthened by incorporating indicators capable of assessing toxicological effects.

This conclusion was reached through a tiered assessment framework that integrated potential ecological status classification, chemical analysis, and the development of ecotoxicological endpoints. A multi-trophic bioassay system was implemented using species representing various functional groups. Although *A. fischeri* bioluminescence inhibition was most strongly associated with nutrients (nitrogen forms) and ions, the inhibition of microalgae growth and *D. magna* feeding rate (FR) were primarily correlated with phosphorus and herbicide concentrations. Among these, *P. subcapitata* emerged as the most sensitive bioindicator, exhibiting significant growth inhibition. Notably, atrazine was identified as a particularly impactful herbicide, strongly correlating with reduced algal growth. These results highlight the broad scope of the selected ecotoxicological set, detecting toxic effects induced by fertilizers and pesticides (the most important contaminants in reservoirs of hydro-agricultural systems).

Furthermore, the ecotoxicological analysis led to the reclassification of sample LDc_18, highlighting the complementary value of this approach to conventional classification methods. Our results demonstrate that rapid and cost-effective bioassays using standard test species offer considerable advantages in reservoir monitoring. These methods can detect synergistic toxic effects not detected by chemical analyses alone, provide early warnings of ecosystem disturbances, and exhibit strong concordance (82%) with the ecological potential assessments required by the Water Framework Directive (WFD).

Hence, the results obtained allow us to support the complementary use of this type of strategy to fill some existing gaps in the classification of water bodies related to reservoirs (due to the lack of control of this type of system), namely reservoirs integrated into hydro-agricultural systems influenced by agricultural practices.

Considering these results, we propose an integrated monitoring framework that combines WFD-compliant biological and physico-chemical assessments with ecotoxicological tools for early contamination detection and a multi-metric assessment of ecosystem integrity. This synergistic approach addresses existing gaps in water quality assessment by providing a more comprehensive assessment of pollution impacts, enabling proactive management, supporting the sustainable use of water resources, and improving the detection of emerging contaminants before irreversible ecological damage occurs.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w17172642/s1: Table S1. Physico-chemical parameters obtained during the three-year study period (2018−2020) in the Lage reservoir (L—Lage and LS—Lage S) (median (minimum—maximum); n = 3) (adapted from Catarino et al. [20]); Table S2a. Active substances applied to permanent crop (vineyard and olive grove) for the 2018-2020 irrigation campaigns (data sourced from farmers, adapted from Alves Ferreira et al. [26]); Table S2b. Active substances applied to annual crops (sunflower, maize, clover, and onion) for the 2018-2020 irrigation campaigns (data sourced from farmers, adapted from Alves Ferreira et al. [26]); Table S3. Specific pollutants and priority substances used to classify the Lage reservoir and their limits for correct classification; Table S4a. Concentrations (μg L^{-1}) of pesticides quantified for 3 years (2018, 2019, and 2020) in Lage (L); Table S4b. Concentrations (μg L^{-1}) of pesticides quantified for 3 years (2018, 2019, and 2020) in Lage S (LS); Table S5. EC_{50} (%) values calculated for *A. fisheri* (mean ± SD; n = 2) bioassay after exposure to L (Lage) and LS (Lage S) samples in April (Ap) (April), July (Jl), (September (Sp), and December (Dc); Table S6. Spearman correlation coefficients between the meteorological parameters, physico-chemical variables, pesticide concentrations (μg L^{-1}), and ecotoxicological bioassays; blue bold values indicated to be significant at $p < 0.05$.

Author Contributions: Conceptualization: P.P.; Formal analysis: P.P.; Investigation: A.C., C.M., M.C., P.A. and P.P.; Writing—original draft preparation: A.C. and P.P.; Writing—review and editing: A.C., P.A. and P.P.; Visualization: A.C. and P.P.; Supervision: P.P.; Project administration: P.P.; Funding acquisition, P.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The author Patrícia Palma is currently serving on the Board of Directors of Águas Públicas do Alentejo (AgdA) under a public interest secondment. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Derossi, F.N.; Ribeiro, P.F.; Santos, J.L. Farming System Choice Is Key to Preserving Surface Water Quality in Agricultural Watersheds. *Agronomy* **2024**, *14*, 214. [CrossRef]
2. Bănăduc, D.; Curtean-Bănăduc, A.; Barinova, S.; Lozano, V.L.; Afanasyev, S.; Leite, T.; Branco, P.; Gomez Isaza, D.F.; Geist, J.; Tegos, A. Multi-Interacting Natural and Anthropogenic Stressors on Freshwater Ecosystems: Their Current Status and Future Prospects for 21st Century. *Water* **2024**, *16*, 1483. [CrossRef]
3. Abbas, M.; Adil, M.; Ehtisham-ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Shar, G.A.; Asif Tahir, M.; Iqbal, M. Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review. *Sci. Total Environ.* **2018**, *626*, 1295–1309. [CrossRef]
4. Nascimento, T.; Proença de Oliveira, R.; de Melo, M.T. Impacts of large-scale irrigation and climate change on groundwater quality and the hydrological cycle: A case study of the Alqueva irrigation scheme and the Gabros de Beja aquifer system. *Sci. Total. Environ.* **2023**, *907*, 168151. [CrossRef]
5. Imran, U.; Ullah, A.; Mahar, R.B.; Shaikh, K.; Khokhar, W.A.; Weidhaas, J. An integrated approach for evaluating freshwater ecosystems under the influence of high salinity: A case study of Manchar Lake in Pakistan. *Environ. Monit. Assess.* **2023**, *195*, 1340. [CrossRef]
6. Santos, J.I.; Vidal, T.; Gonçalves, F.J.M.; Castro, B.B.; Pereira, J.L. Challenges to water quality assessment in Europe—Is there scope for improvement of the current Water Framework Directive bioassessment scheme in rivers? *Ecol. Indic.* **2021**, *121*, 107030. [CrossRef]
7. Teweldebrihan, M.D.; Dinka, M.O. Sustainable Water Management Practices in Agriculture: The Case of East Africa. *Encyclopedia* **2025**, *5*, 7. [CrossRef]
8. Drechsel, P.; Marjani Zadeh, S.; Pedrero, F. (Eds.) *Water Quality in Agriculture: Risks and Risk Mitigation*; FAO & IWMI: Rome, Italy, 2023. [CrossRef]

9. Fang, S.; Hua, C.; Yang, J.; Liu, F.; Wang, L.; Wu, D.; Ren, L. Combined pollution of soil by heavy metals, microplastics, and pesticides: Mechanisms and anthropogenic drivers. *J. Hazard. Mater.* **2025**, *485*, 136812. [CrossRef] [PubMed]
10. Naeem, M.; Gill, R.; Gill Sarvajeet, S.; Singh, K.; Sofo, A.; Tuteja, N. Emerging contaminants and their effect on agricultural crops. *Front. Plant Sci.* **2023**, *14*, 1296252. [CrossRef] [PubMed]
11. Adil, N.; Ashraf, K.; Munir, M.; Mohiuddin, M.; Abbasi, A.; Riaz, U.; Aslam, A.; Marey, S.A.; Hatamleh, A.A.; Zaman, Q.u. Pesticides, Heavy Metals and Plasticizers: Contamination and Risk Assessment of Drinking-Water Quality. *Sustainability* **2023**, *15*, 13263. [CrossRef]
12. Zhou, W.; Li, M.M.; Achal, V.A. Comprehensive review on environmental and human health impacts of chemical pesticide usage. *Emerg. Contam.* **2025**, *11*, 100410. [CrossRef]
13. De Baat, M.L.; Van der Oost, R.; Van der Lee, G.H.; Wieringa, N.; Hamers, T.; Verdonschot, P.F.M.; De Voogt, P.; Kraak, M.H.S. Advancements in effect-based surface water quality assessment. *Water Res.* **2020**, *183*, 116017. [CrossRef]
14. Belaid, A.; Bekir, K.; Beltifa, A.; Sedrati, M.; Santana Rodríguez, J.J.; Ben Mansour, H. Physicochemical and ecotoxicological approaches for Moknine Continental Sebkha in Tunisia. *J. Water Health* **2024**, *22*, 785–796. [CrossRef] [PubMed]
15. Martinez-Haro, M.; Acevedo, P.; Pais-Costa, A.J.; Neto, J.M.; Vieira, L.R.; Ospina-Alvarez, N.; Taggart, M.A.; Guilhermino, L.; Ribeiro, R.; Marques, J.C. Ecotoxicological tools in support of the aims of the European Water Framework Directive: A step towards a more holistic ecosystem-based approach. *Ecol. Indic.* **2022**, *145*, 109645. [CrossRef]
16. Lameira, C.M.R.; Oliveira, A.K.G.; Antes, D.C.; Souza, C.A.; Bressiani, P.A.; Silva, M.P.R.D.; Moreira, A.C.P.; Oliveira Schmitz, A.P.; Pokrywiecki, J.C.; Pokrywiecki, T.S.; et al. Integrated assessment of water quality: Influence of physicochemical parameters and ecotoxicological impacts under different flow conditions of lonqueador river, Southern Brazil. *Ecotoxicology* **2025**, *ahead of print*. [CrossRef] [PubMed]
17. Novais, M.H.; Penha, A.M.; Catarino, A.; Martins, I.; Fialho, S.; Lima, A.; Palma, P. The usefulness of ecotoxicological tools toimprove the assessment of water bodies in a climate change reality. *Sci. Total Environ.* **2023**, *901*, 166392. [CrossRef]
18. Kronberg, M.F.; Rossen, A.; Clavijo, A. Integrated water quality assessment of two Rivers Basins from a semiarid region of Argentina. *Environ. Sci. Pollut. Res.* **2024**, *31*, 2279–2296. [CrossRef]
19. Schuijt, L.M.; Peng, F.J.; van den Berg, S.J.; Dingemans, M.M.; Van den Brink, P.J. (Eco)toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. *Sci. Total Environ.* **2021**, *795*, 148776. [CrossRef]
20. Catarino, A.; Martins, I.; Mourinha, C.; Santos, J.; Tomaz, A.; Anastácio, P.; Palma, P. Water Quality Assessment of a Hydro-Agricultural Reservoir in a Mediterranean Region (Case Study—Lage Reservoir in Southern Portugal). *Water* **2024**, *16*, 514. [CrossRef]
21. EDIA. Empresa de Desenvolvimento e Infraestruturas de Alqueva: Beja, Portugal. 2024. Available online: https://www.edia.pt/pt/ (accessed on 22 March 2025).
22. INE Instituto Nacional de Estatística. Estatísticas Agrícolas. 2020. Available online: https://www.ine.pt (accessed on 22 March 2025).
23. Tomaz, A.; Martins, I.; Catarino, A.; Mourinha, C.; Dôres, J.; Fabião, M.; Boteta, L.; Coutinho, J.; Patanita, M.; Palma, P. Insightsinto the Spatial and Temporal Variability of Soil Attributes in Irrigated Farm Fields and Correlations with Management Practices:A Multivariate Statistical Approach. *Water* **2022**, *14*, 3216. [CrossRef]
24. IPMA—Instituto Português do Mar e da Atmosfera. *Normais Climatológicas 1981–2010 de Beja*; Instituto Português do Mar e da Atmosfera: Lisbon, Portugal, 2024. Available online: https://www.ipma.pt/pt/oclima/normais.clima/1981-2010/002/ (accessed on 22 March 2025).
25. EDIA. *Massas de Água de Alqueva. . . Uma Nova Realidade Territorial, 1.ª Fase*; Empresa de Desenvolvimento e Infraestruturas de Alqueva: Beja, Portugal, 2017. Available online: https://www.edia.pt/wp-content/uploads/2019/05/PublicacaoMassasAgua_07032018.pdf (accessed on 20 May 2025).
26. Alves-Ferreira, J.; Vara, M.G.; Catarino, A.; Martins, I.; Mourinha, C.; Fabião, M.; Costa, M.J.; Barbieri, M.V.; de Alda, M.L.; Palma, P. Pesticide Water Variability and Prioritization: The First Steps towards Improving Water Management Strategies in Irrigation Hydro-Agriculture Areas. *Sci. Total Environ.* **2024**, *917*, 170304. [CrossRef]
27. Ayers, R.S.; Westcot, D.W. *Water Quality for Agriculture*; FAO Irrigation and Drainage, Paper 29; Food and Agriculture Organization: Rome, Italy, 1985.
28. Palma, P.; Ledo, L.; Soares, S.; Barbosa, I.R.; Alvarenga, P. Integrated environmental assessment of freshwater sediments: A chemical and ecotoxicological approach at the Alqueva reservoir. *Environ. Geochem. Health* **2014**, *36*, 209–223. [CrossRef]
29. Palma, P.; Fialho, S.; Lima, A.; Catarino, A.; Costa, M.J.; Barbieri, M.V.; Monllor-Alcaraz, L.S.; Postigo, C.; de Alda, M. Lopez. Occurrence and risk assessment of pesticides in a Mediterranean Basin with strong agricultural pressure (Guadiana Basin: Southern of Portugal). *Sci. Total. Environ.* **2021**, *794*, 148703. [CrossRef] [PubMed]
30. Valíčková, J.; Zezulka, Š.; Maršálková, E. Potential toxicity of *Schisandra chinensis* to water environment: Acute toxicity tests with water crustaceans. *Environ. Sci. Pollut. Res.* **2023**, *30*, 112625–112630. [CrossRef] [PubMed]
31. Tian, Y.; Xia, X.; Wang, J. Chronic Toxicological Effects of Carbamazepine on *Daphnia magna* Straus: Effects on Reproduction Traits, Body Length, and Intrinsic Growth. *Bull. Environ. Contam. Toxicol.* **2019**, *103*, 723–728. [CrossRef] [PubMed]

32. Tongo, I.; Onokpasa, A.; Emerure, F.; Balogun, P.; Enuneku, A.; Erhunmwunse, N.; Osaro, A.; Ogbomida, E.; Ogbeide, O.; Lawrence Ikechukwu, E. Levels, bioaccumulation and biomagnification of pesticide residues in a tropical freshwater food web. *Int. J. Environ. Sci. Technol.* **2021**, *19*, 1467–1482. [CrossRef]
33. Roig, N.; Sierra, J.; Nadal, M.; Moreno-Garrido, I.; Nieto, E.; Hampel, M.; Gallego, E.P.; Schuhmacher, M.; Blasco, J. Assessment of sediment ecotoxicological status as a complementary tool for the evaluation of surface water quality: The Ebro river basin case study. *Sci. Total Environ.* **2015**, *503–504*, 269–278. [CrossRef]
34. *ISO 11348-2*; Determination of Inhibitory Effect of Water Samples on the Light Emission of *Vibrio fischeri* (Luminescent bacteria Test). Part 2: Method Using Liquid- Dried Bacteria. International Organization for Standardisation: Geneve, Switzerland, 1998.
35. Persoone, G. THAMNOTOXKIT FTM—Crustacean toxicity screening test for Freshwater. In *Standard Operational Procedure*; MicroBioTests: Mariakerke, Belgium, 1999.
36. *ASTM E729-90*; Standard Practice for Conducting Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. ASTM: Philadelphia, PA, USA, 1998; pp. 271–296.
37. Baird, D.J.; Barber, I.; Bradley, M.; Calow, P.; Soares, A.M.V.M. The Daphnia bioassay: A critique. *Hydrobiologia* **1989**, *188*, 403–406. [CrossRef]
38. McWilliam, R.A.; Baird, D.J. Postexposure feeding depression: A new toxicity endpoint for use in laboratory studies with *Daphnia magna*. *Environ. Toxicol.* **2002**, *21*, 1198–1205. [CrossRef]
39. Allen, Y.; Calow, P.; Baird, D.J. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. *Environ. Toxicol. Chem.* **1995**, *14*, 1625–1630. [CrossRef]
40. Zar, J.H. *Biostatistical Analysis. Prentice-Hall International*; Englewood Cliff: Washington, DC, USA, 1996.
41. Finney, D.J. *Probit Analysis, Journal of Pharmaceutical Sciences*; Cambridge University Press: New York, NY, USA, 1971.
42. Tachie-Menson, J.W.; Owusu, C.; Yeboah, F.K.; Odoi-Yorke, F.; Abbey, A.A.; Ackah, F.K.; Darko, R.O. Environmental impact of pesticide runoff in irrigated agriculture: Evidence from Gomoa West field, Ghana. *Discov. Environ.* **2025**, *3*, 105. [CrossRef]
43. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* **2021**, *9*, 42. [CrossRef] [PubMed]
44. Khatib, I.; Rychter, P.; Falfushynska, H. Pesticide Pollution: Detrimental Outcomes and Possible Mechanisms of Fish Exposure to Common Organophosphates and Triazines. *J. Xenobiotics* **2022**, *12*, 236–265. [CrossRef] [PubMed]
45. Mugudamani, I.; Oke, S.A.; Gumede, T.P.; Senbore, S. Herbicides in Water Sources: Communicating Potential Risks to the Population of Mangaung Metropolitan Municipality, South Africa. *Toxics* **2023**, *11*, 538. [CrossRef]
46. PPDB. Pesticide Properties DataBase. Available online: https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (accessed on 30 March 2025).
47. Tauchnitz, N.; Kurzius, F.; Rupp, H.; Schmidt, G.; Hauser, B.; Schrödter, M.; Meissner, R. Assessment of pesticide inputs into surface waters by agricultural and urban sources—A case study in the Querne/Weida catchment, central Germany. *Environ. Pollut.* **2020**, *267*, 115186. [CrossRef]
48. Díaz, G.; Cabanillas, G.; López Soto, M.; Ortiz, J.M. Determination of fenthion and fenthion-sulfoxide, in olive oil and in river water, by square-wave adsorptive-stripping voltammetry. *Talanta* **2008**, *76*, 809–814. [CrossRef]
49. Bereswill, R.; Golla, B.; Streloke, M.; Schulz, R. Entry and toxicity of organic pesticides and copper in vineyard streams: Erosion rills jeopardise the efficiency of riparian buffer strips. *Agric. Ecosyst. Environ.* **2012**, 146. [CrossRef]
50. Peris, A.; Soriano, Y.; Picó, Y.; Bravo, M.A.; Blanco, G.; Eljarrat, E. Pesticides in water and sediments from natural protected areas of Spain and their associated ecological risk. *Chemosphere* **2024**, *362*, 142628. [CrossRef]
51. Melendez-Pastor, I.; Hernández, E.I.; Navarro-Pedreño, J.; Almendro-Candel, M.B.; Gómez Lucas, I.; Jordán Vidal, M.M. Occurrence of Pesticides Associated with an Agricultural Drainage System in a Mediterranean Environ. *Appl. Sci.* **2021**, *11*, 10212. [CrossRef]
52. Abaineh, A.; Ejigu, D.; Atlabachew, M.; Dejen, E.; Desta, G. Risks of pesticides on aquatic ecosystems and pesticide management effectiveness in Ethiopia: Review. *Int. J. Environ. Sci. Technol.* **2024**, *21*, 8833–8848. [CrossRef]
53. Palma, P.; Alvarenga, P.; Palma, V.; Matos, C.; Fernandes, R.; Soares, A.; Barbosa, R. Evaluation of surface water quality using an ecotoxicological approach: A case study of the Alqueva Reservoir (Portugal). *Environ. Sci. Pollut. Res.* **2010**, *17*, 703–716. [CrossRef]
54. Szklarek, S.; Kiedrzyńska, E.; Kiedrzyński, M.; Mankiewicz-Boczek, J.; Mitsch, W.; Zalewski, M. Comparing ecotoxicological and physicochemical indicators of municipal wastewater effluent and river water quality in a Baltic Sea catchment in Poland. *Ecol. Indic.* **2021**, *126*, 107611. [CrossRef]
55. Alvarenga, P.; Ferreira, C.; Mourinha, C.; Palma, P.; de Varennes, A. Chemical and Ecotoxicological Effects of the Use of Drinking-Water Treatment Residuals for the Remediation of Soils Degraded by Mining Activities. *Ecotoxicol. Environ. Saf.* **2018**, *161*, 281–289. [CrossRef] [PubMed]
56. Palma, P.; Ledo, L.; Alvarenga, P. Ecotoxicological endpoints, are they useful tools to support ecological status assessment in strongly modified water bodies? *Sci. Total Environ.* **2016**, *541*, 119–129. [CrossRef] [PubMed]

57. Palma, P.; Matos, C.; Alvarenga, P.; Kock-Schulmeyer, M.; Simões, I.; Barceló, D.; López de Alda, M.J. Ecological and ecotoxicological responses in the assessment of the ecological status of freshwater systems: A case-study of the temporary stream Brejo of Cagarrão (South of Portugal). *Sci. Total Environ.* **2018**, *634*, 394–406. [CrossRef]
58. Popick, H.; Brinkmann, M.; McPhedran, K. Traffic-derived contaminant loading in snow storage facilities during spring melt. *Environ. Sci. Pollut. Res.* **2022**, *29*, 27875–27893. [CrossRef]
59. Zhu, H.; Pan, J.; Wei, Y.; Lan, H.; Yang, S.; Li, X.; Tang, X. Manganese toxicity suppressing nitrogen-fixing bacteria growth and impairing nitrogen uptake and utilization in sugarcane. *Front. Microbiol.* **2025**, *16*, 1548896. [CrossRef]
60. Si, M.; Zhao, C.; Burkinshaw, B.; Zhang, B.; Wei, D.; Wang, Y.; Dong, T.; Shen, X. Manganese scavenging and oxidative stress response mediated by type VI secretion system in *Burkholderia thailandensis*. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E2233–E2242. [CrossRef]
61. Canova, L.; Sturini, M.; Maraschi, F.; Sangiorgi, S.; Ferri, E.N. A Comparative Test on the Sensitivity of Freshwater and Marine Microalgae to Benzo-Sulfonamides, -Thiazoles and -Triazoles. *Appl. Sci.* **2021**, *11*, 7800. [CrossRef]
62. Shi, P.; Zhu, M.; You, R.; Li, H.; Zou, W.; Xu, H.; Zhu, G. Rainstorm events trigger algal blooms in a large oligotrophic reservoir. *J. Hydrol.* **2023**, *622*, 129711. [CrossRef]
63. Lee, J.; Hong, S.; An, S.A.; Khim, J.S. Methodological advances and future directions of microalgal bioassays for evaluation of potential toxicity in environmental samples: A review. *Environ. Int.* **2023**, *173*, 107869. [CrossRef]
64. Booij, P.; Sjollema, S.; van der Geest, H.; Leonards, P.; Lamoree, M.; Voogt, W.; Admiraal, W.; Laane, R.; Vethaak, A. Toxic pressure of herbicides on microalgae in Dutch estuarine and coastal waters, *J. Sea Res.* **2015**, *102*, 48–56. [CrossRef]
65. Castro, M.S.; Silva, J.C.; Machado, B.R.; Guimarães, P.S.; Lombardi, A.T.; Martins, C.D.M.G.; Zanette, J. Differential Effects of Atrazine on Chlorophyceae Species and Association with Morphology, Photosynthesis, Chlorophyll Content, and Glutathione-*S*-Transferase Activity. *Environ. Toxicol.* **2022**, *41*, 1675–1685. [CrossRef]
66. Tasmin, R.; Shimasaki, Y.; Tsuyama, M.; Qiu, X.; Khalil, F.; Okino, N.; Yamada, N.; Fukuda, S.; Kang, I.; Oshima, Y. Elevated water temperature reduces the acute toxicity of the widely used herbicide diuron to a green alga, *Pseudokirchneriella subcapitata*. *Environ. Sci. Pollut. Res.* **2014**, *21*, 1064–1070. [CrossRef]
67. Huang, J.; Piao, X.; Zhou, Y.; Li, S. Toxicity Assessment of 36 Herbicides to Green Algae: Effects of Mode of Action and Chemical Family. *Agrochemicals* **2024**, *3*, 164–180. [CrossRef]
68. Queirós, L.; Bouguerra, S.; Pereira, R.; Macário, I.P.E.; Santos, J.I.; Veloso, T.; Gonçalves, F.J.M.; Pereira, P.; Pereira, J. Improved efficiency of an herbicide combining bentazone and terbuthylazine—Can weeds be controlled with better environmental safety? *Environ. Sci. Adv.* **2022**, *1*, 342–355. [CrossRef]
69. Pinto, I.; Rodrigues, S.; Lage, O.M.; Antunes, S.C. Assessment of water quality in Aguieira reservoir: Ecotoxicological tools in addition to the Water Framework Directive. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111583. [CrossRef] [PubMed]
70. Rodrigues, S.; Pinto, I.; Martins, F.; Formigo, N.; Antunes, S.C. An ecotoxicological approach can complement the assessment of natural waters from Portuguese reservoirs? *Environ. Sci. Pollut. Res.* **2022**, *29*, 52147–52161. [CrossRef]
71. Castro, B.B.; Gonçalves, F. Seasonal Dynamics of the Crustacean Zooplankton of a Shallow Eutrophic Lake from the Mediterranean Region. *Fundam. Appl. Limnol.* **2007**, *169*, 189–202. [CrossRef]
72. Rodrigues, S.; Pinto, I.; Nogueira, S.; Antunes, S.C. *Perspective Chapter: Daphnia Magna as a Potential Indicator of Reservoir Water Quality—Current Status and Perspectives Focused in Ecotoxicological Classes Regarding the Risk Prediction*; Intechopen Limited: London, UK, 2023. [CrossRef]