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Use of the Pesticide Toxicity Index to Determine Potential Ecological Risk in the Santiago-Guadalajara River Basin, Mexico

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Abstract: The Santiago-Guadalajara River Basin (SGRB), located in western Mexico, is one of the most polluted rivers in the country. A pesticide monitoring program was carried out from January 2022 to September 2022, during which time water samples collected at 25 sites in the main stem and tributaries revealed the presence of 13 of the 24 pesticides analyzed, including α-BHC, β-BHC, γ-BHC (Lindane), γ-Chlordane, Hexachlorobenzene, Heptachlor, Aldrin, α-Endosulfan, DDT, -4,4, Methoxychlor, Chlorpyrifos-methyl, Endosulfan sulfate, and Dicofol. A high level of correlation was found between the identified pesticides, which could mean that several of these pesticides reached a stable level within the monitored water bodies. Most of the identified pesticides are classified as high environmental risk according to the Stockholm Convention because of their persistence and high degree of toxicity to the environment and human health. A Pesticide Toxicity Index (PTI) was applied to identify the streams considered to be of concern due to the presence of pesticides exceeding the threshold limits established by national and international guidelines. Performing a calculation for the entire Santiago-Guadalajara River Basin, the PTI reached a value of 0.833, which, according to the criteria of this method, classifies it as a “Moderate” level of risk for aquatic life. Increased regulatory and surveillance measures by state and federal authorities are required to prevent the use of these pesticides, which have been restricted globally.

Keywords: organochlorines; organophosphates; pesticide toxicity index; Santiago-Guadalajara River

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1. Introduction

Pesticides are defined as a broad spectrum of chemical mixtures including insecticides, fungicides, molluscicides, nematicides, herbicides, rodenticides, and plant growth regulators, among other compounds [1]. The main sources of pesticides in ecosystems are agriculture and forestry [2]. Pesticides are also applied intensively in urban areas, such as urban gardens and parks [3]. In the past few years, it has been observed that pesticide residues have spread throughout the environment, contaminating ecosystems, food, and water resources [4]. This overall pesticide contamination is derived from human population growth, since such growth would not be possible without an increase in food production, which is closely linked to the use of pesticides [5].

Since the end of the 20th century, there has been a growing concern in the scientific community in Mexico about the presence of pesticides in several waterbodies throughout the country, and the potential implications for human health and biodiversity [6–16]. High levels of toxic contaminants have been found in soil, water, and plant and animal species in some locations in Mexico, and adverse effects on human health, especially in children, have been identified [17,18]. According to Bejarano-González [19], from 2000 to 2014, the use of pesticides in Mexico rose by 59.2%. Fungicides and bactericides were the most widely used types of pesticides in 2014, at 40,016 tons (40.5%), followed by insecticides, at 32,406 tons (32.8%), and herbicides, at 26,392 tons (26.7%). This increase in pesticide use occurred because Mexico does not have an overarching national policy on pesticides, nor does it have a policy that favors the transition to more sustainable agriculture in the country [20,21]. The National Development Plan, which is the country's highest-level policy, does not specifically address pesticides [22]. Instead, since the 1990s, goals and objectives have been included in various policy and regulatory tools dealing with these substances [23,24].

This work presents the first results of an extensive monthly pesticide monitoring effort carried out from January 2022 to September 2022 at 25 sampling sites located in the main stem of the river and tributary streams of the Santiago-Guadalajara River Basin (SGRB). The main objective of this monitoring work was to provide a first approach on the status of the SGRB in terms of the presence of pesticides in the main stem of the river basin. For this purpose, the analytical results obtained in the monitoring campaign were compared with the threshold limits established by national and international environmental regulations, and a method based on a Pesticide Toxicity Index (PTI) was used to assess the potential risks to aquatic life in the monitored streams [25,26]. Likewise, with this information, we identified the streams that represent the greatest risk to aquatic life. This work also investigated whether there was a relationship between the precipitation regime in the basin and the level of toxicity of surface waters due to the presence of pesticides.

Santiago-Guadalajara River Basin (SGRB)

The SGRB is located in the western part of Mexico (Figure 1). The main stem of the river is the Río Grande de Santiago, or simply the Santiago River, which originates in Lake Chapala and empties into the Pacific Ocean. It is 433 km long with an average flow of 320 m^3/s. The upper basin, known as Santiago-Guadalajara, with a catchment area of 10,016.78 km^2, is characterized by extensive agricultural and industrial development that extends from the tributary basin of the Zula River, located near Lake Chapala, to the Metropolitan Area of Guadalajara (MAG) [27,28]. The MAG had a population of 5 million inhabitants in 2017 and is the second largest metropolitan area in Mexico [29].

The predominant land use in the SGRB is agriculture, with 454,619 hectares (45.05%) of the total area of the SGRB (Figure 2). Rainfed agriculture occupies 36.49% of the surface, followed by irrigated agriculture at 8.37%, and finally gravity irrigation agriculture at 0.19% [28,30] (Figure 2). The second largest land use by area (20.09%) is forested land (primary and secondary growth), including oak forest (13.71%), oak–pine forest (4.76%), and pine–oak forest (1.62%) [28,30] (Figure 2). The third largest area is occupied by low deciduous forest (14.86%), but it should be noted that most of that forest is secondary growth (9.96%). The deciduous forest is the most widely distributed, since it extends from the north of the basin, where it crosses from west to east in a very thin strip, and from there down to the central and southern parts, where it is mostly surrounded by agriculture [28,30] (Figure A1 in Appendix A). Grasslands are in fourth place for land use (10.6%), where pastureland predominates at 10.5%, and the rest (0.1%) is natural, with secondary shrub vegetation [28,30] (Figure 2). Lastly, there is urban land (7.62%) (Figure A1 in Appendix A). Despite being the land use with the smallest surface area, it is the one with the greatest negative environmental impact on the river, since the MAG and other cities in the basin discharge most of their sewage untreated or partially treated directly into the main stem of the river or indirectly through tributary streams [31].

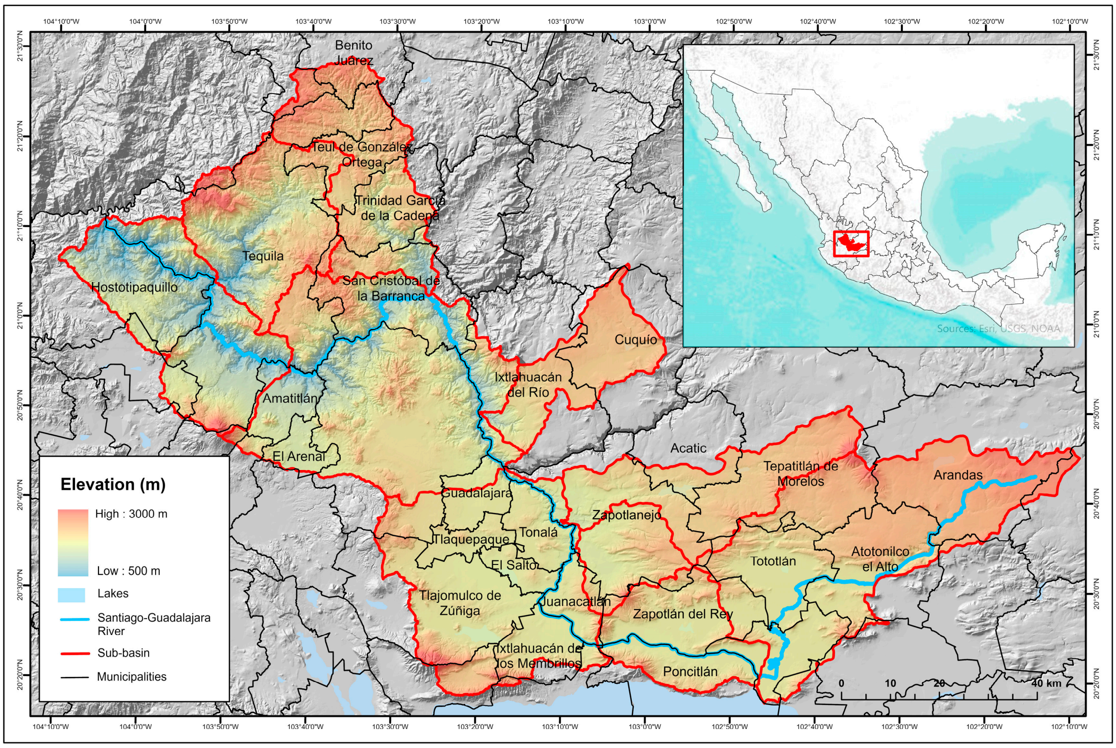

Figure 1. Geographic location of the SGRB, sub-basins, and municipalities.

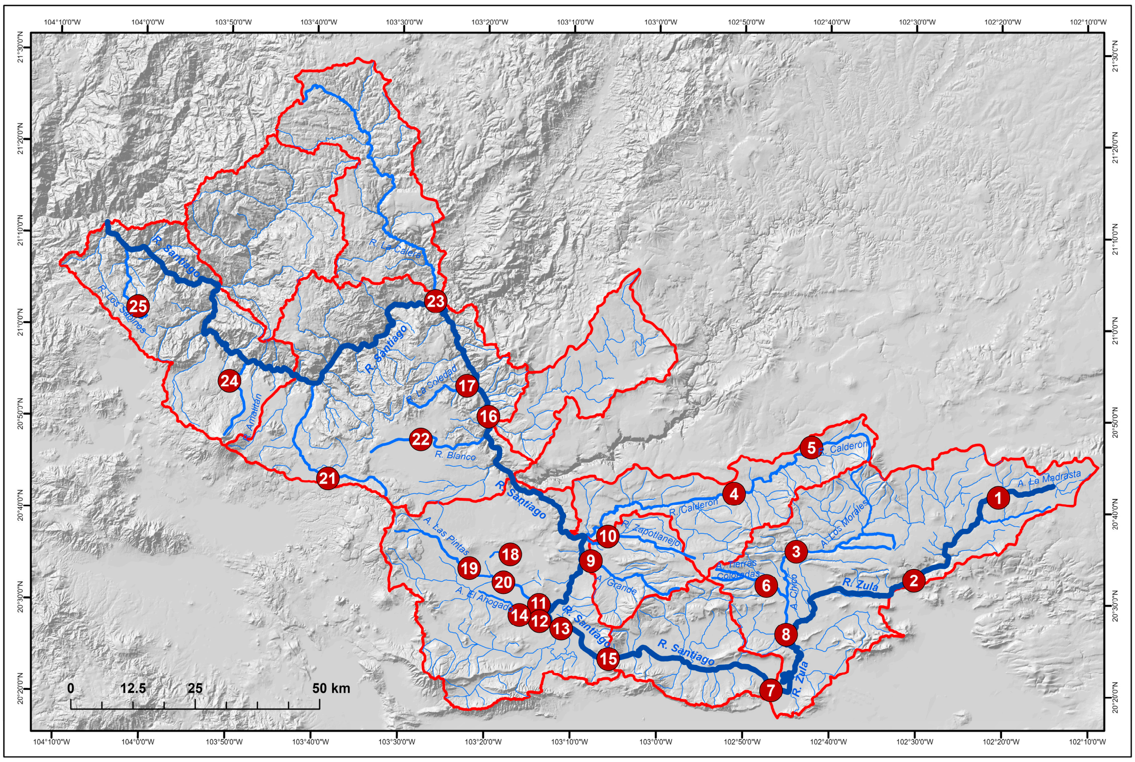

Figure 2. Location of the 25 monitoring stations located in the main stem and tributaries of the SGRB.

Municipal and industrial wastewater discharges, wastewater discharges from agricultural activities, the infiltration of pollutants into the subsoil, the infiltration of leachate from municipal landfills, air pollution due to atmospheric emissions from industry, motor vehicles, and brick factories, soil erosion due to deforestation, and the increasing expansion of the agricultural sector have accelerated the process of environmental degradation in the SGRB [32]. Adding to this problem is the limitation in the treatment and operation capacity of the sanitation infrastructure for municipal, industrial, and agricultural wastewater, and the overexploitation of water resources in rural and urban areas [28,33–35].

The highest degree of environmental deterioration in the basin has been observed around the municipalities of El Salto and Juanacatlán, and in the El Ahogado watershed. Pollution of the river has caused serious health problems in the local population, including kidney disease, cancer, and respiratory problems [36]. These diseases have led to the filing of lawsuits in state, national, and international human rights commissions by organizations at the local, national, and international levels to address these environmental problems [3]. Because river recovery efforts have been limited, the population of the basin is gradually losing one of the most important ecosystems in the region and one of the main sources of drinking water supply for the MAG. Riverine communities are becoming more vulnerable to waterborne diseases, water shortages due to the overexploitation and contamination of ground and surface waters, droughts, and the effects associated with land-use change such as deforestation and the expansion of the agricultural sector [37–39].

2. Materials and Methods

2.1. Sampling Sites

The selection of the sampling sites was determined by considering the following criteria: the accessibility, hydrological regime of waterway, inhabitants of the watershed, and socioeconomic level of the local population. The proposed stations were verified in the field and 25 points were chosen for monitoring. Table A1 in Appendix B describes the names and geographic coordinates of each monitoring station, and Figure 2 shows their geographic location. Table A2 in Appendix B shows the sampling stations located in each sub-basin. These stations were also divided into clusters for statistical analysis.

2.2. Analytical Methods

There are several factors that significantly influence the identification and quantification of pesticides in aquatic environments [40,41]. Some of these factors include (1) the crop rotation and consequent changes in the type and quantity of pesticides applied, (2) the changes in the timing of the pesticide application, (3) the interannual variability in the hydrological conditions in the basin, (4) modifications in the hydrological regime of the monitored streams, and (5) modifications in crop irrigation systems, among others [40–42]. In this research, 24 organochlorine and organophosphate pesticides considered dangerous to aquatic life were selected due to their probability of occurrence in the study area (see Table A3 in Appendix B). A monthly monitoring campaign was carried out from January to September 2022 at the 25 selected stations (Figure 3). Water samples were placed in dark glass containers, labeled, placed in a plastic bag, and sealed with a safety seal. According to the Mexican standard [43], the samples were transported in a cooler at 4 °C and were kept refrigerated at the same temperature prior to analysis [43]. The chromatographic analysis for the determination of the pesticides was carried out using a Perkin Elmer model Clarus 680 gas chromatograph with an autosampler and electron capture detector. A 30 m, 0.32 mm, and 0.25 µm, a DB-5 column was used at temperatures of 200 °C (injector) and 260 °C (detector). The analysis was carried out isothermally at 200 °C for 30 min with an injection volume of 1 µL of sample to reduce the detection limits established in the current Mexican regulations, which are in accordance with EPA method 8081 and the Association of Official Agricultural Chemists (AOAC) Official Method 970.52. The modified methods for the determination of pesticides to reduce the detection limits established by the official Mexican standards were previously tested with control samples to assure reproducibility, repeatability, and recovery (see Table A3 in Appendix B). To ensure quality assurance and quality control, standard solutions were used. Calibration curves were prepared for each analyte, and a record was kept of the percentage of recovery and the coefficient of variation was estimated. The limits of detection (LOD) and quantification (LOQ) were determined to define the lowest concentration of each analyte that could be reliably detected and quantified, respectively, assuring a 95% probability of obtaining a correct result (see Table A4 in Appendix B).

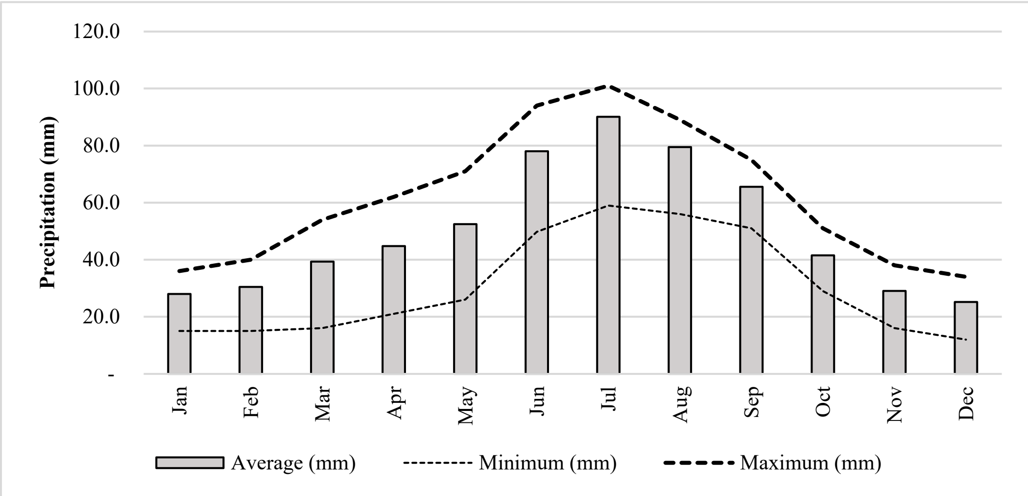

Figure 3. Records of monthly average historical precipitation in the Metropolitan Area of Guadalajara (1882–2019) [27].

2.3. Statistical Methods

Due to the complex mobility of pesticides in the environment and the numerous practices used to apply these compounds on crops, information from at least five (5) years of monitoring must be available to evaluate the behavior of pesticides in aquatic ecosystems to carry out a reliable trend analysis [40,41]. The sampling regime described in this paper was intended to provide a first approach to determining the pesticide concentrations in the monitored surface water streams of the SGRB. A basic statistical analysis was performed in grouped sub-basins and at the basin level to have an overview of the general behavior of the data. The clustering criteria in which the sampling stations are grouped are shown in Table A2 in Appendix B. In the statistical analysis, the dataset used removed only extreme outliers. A statistical cluster analysis was performed to verify if there were any significant differences between cluster groups. An analysis of the correlation coefficient was also carried out between the data on pesticides that accounted for 95% of the data. Through tests performed with curve-fitting functions, those functions that provided the best correlation coefficients were identified. The months of the year representing the highest concentrations of pesticides in the basin were deduced through an analysis of the hydrological regime in the basin and a monthly analysis of the data behavior.

2.4. Criteria for Fish Toxicity Assessment Due to Pesticides

Pesticides have been shown to affect birds, fish, plants, or other non-human organisms when they are exposed to such stressors [42–46]. The different complex routes by which pesticides are transported within the hydrological system have been widely discussed by several authors [47–49]. The environmental fate of pesticides depends on the physical and chemical properties of the pesticide, as well as the environmental conditions. The physical and chemical properties of the pesticide determine how likely it is to be transported through soil (soil mobility), how well it dissolves in water (water solubility), how well it can be adsorbed onto sediment (sediment retention), and how likely it is to be released into the air (volatility) to be deposited elsewhere with air currents or precipitation [50–52].

Since 2009, the Federal Rights Law (LFD by its acronym in Spanish) has considered the Santiago River and its direct and indirect tributaries as a type "C" [53]. A type "C" waterbody must comply with the permissible limits of contaminants for fresh waterbodies where the protection of aquatic life is set as a priority [53]. Therefore, the strategy to assess the toxicity risk of pesticides found in the SGRB considered criteria based on the maximum permissible limits established in the Federal Rights Law in Mexico for the protection of aquatic life in freshwater bodies [53], the maximum permissible thresholds of acute and chronic toxicity for the protection of freshwater vertebrate and invertebrate organisms established by the U.S. Environmental Protection Agency [45,46,53], the Water Quality Guidelines for the Protection of Aquatic Life established by the Canadian Council of Ministers of the Environment [54], and the Australian and New Zealand Guidelines for Freshwater and Marine Water Quality [55].

2.5. Pesticide Toxicity Index (PTI)

The Pesticide Toxicity Index (PTI), developed by Munn and Gilliom [25], and later improved by Munn et al. [26], was used for each pesticide as a criterion to assess which of the monitored streams could be considered of concern in relation to the presence of pesticides and their potential adverse effects on aquatic life in the river. The PTI has been widely used as a screening tool to assess the potential aquatic toxicity of complex pesticide mixtures [56–59]. According to Munn et al. [26], the PTI is defined as "the sum of toxicity quotients (measured concentration divided by the median toxicity concentration from bioassays) for each detected pesticide" and is represented by Equation (1) as follows:

$$PTI_x = \sum_{i=1}^{n} \frac{E_i}{MTC_{x,i}}. \quad (1)$$

where
E_i = the concentration of pesticide i;
$MTC_{x,i}$ = the median toxicity concentration for pesticide i for taxonomic group x;
n = the number of pesticides;
E and MTC are expressed in the same units.

When the values reported in the literature are not enough to estimate the median toxicity concentration (*MTC*), Nowell et al. [56] suggest the use of a minimum toxicity (*MinTC*) value of the reported threshold limits in the literature to protect aquatic life. But, as explained by Yadav et al. [57], there is still no agreement on the permissible pesticide concentration limits for aquatic life protection. The threshold values established for aquatic life protection by national and international environmental agencies for each pesticide continue to have significant discrepancies [58–60]. For this reason, the decision was made to take the average value reported by the selected agencies as the reference value for estimating the PTI. With this criterion, Equation (1) was transformed to Equation (2) as follows:

$$PTI = \sum_{i=1}^{n} \frac{E_i}{TC_{Avg,i}} \quad (2)$$

where $TC_{Avg,i}$ is the average toxicity concentration for pesticide *i*.

Regarding risk classes, the classification of some criteria has been established for the PTI values. Battaglin and Fairchild [61] propose the following classes to determine the level of toxicity risk according to the values calculated for the PTI: "Probable" if PTI > 1, "Potential" if PTI > 0.5, and "Limited" if PTI > 0.1. On the other hand, Anderson [62] proposes the following classification: "High" if PTI > 1, "Low" if PTI < 0.01, and "Moderate" if there are samples ranking in-between ($0.01 \leq \text{PTI} \leq 1$). In this work, the PTI criterion will be extended to the basin level to evaluate the risk of toxicity to fish due to the presence of pesticides in the different parts of the river basin.

2.6. Results Representation

A bar graph was used to show the level of toxicity reached at each of the stations monitored according to the criteria established by the PTI. The same exercise was also carried out to determine the PTI value reached in each monitored month, indicating the months with the highest risk of negative effects on aquatic life due to the presence of pesticides. To identify the toxicity risk range of the calculated PTI values, a scatter plot was displayed.

3. Results and Discussion

3.1. Hydrological Regime

Figure 3 shows the precipitation regime in the MAG for the period of 1882–2019. The rainy season begins at the end of May and ends in October, reaching a maximum average precipitation of 90.09 mm in July and a minimum average precipitation of 25.15 mm in December. The months with the least rainfall are November and December, and from January to April. The driest months (low rainfall) are from March to mid-May, when the levels of rivers, lakes, and dams drop significantly [63,64].

3.2. Identified Pesticides

During the monitoring activities, the following thirteen (13) pesticides were identified in the analyzed samples: α-BHC, β-BHC, γ-BHC (Lindane), γ-Chlordane, Hexachlorobenzene, Heptachlor, Aldrin, α-Endosulfan, DDT, -4,4′, Methoxychlor, Chlorpyrifos-methyl, Endosulfan sulfate, and Dicofol. Table 1 shows that 11 of the 13 identified pesticides are classified as Persistent Organic Pollutants (POPs) in accordance with the Stockholm Convention, due either to the elimination or restriction of their use [65]. Although Chlorpyrifos-methyl and Endosulfan sulfate are not persistent pesticides, their potential toxicity to freshwater fish species has been reported in several studies [66,67]. Table 2 shows evidence that the identified pesticides have also been found in surface waterbodies in Mexico and other Latin American countries since the 1990s.

Table 1. Pesticides identified in other studies in the Latin American region.

Nr.	Pesticide	Latin America [68]	Chilean Patagonia [69]	Argentina [70]	Brazil [71]	Central America [72]	Honduras [73]	Southern Mexico [74]	Northern Mexico [14]	North-Western Mexico [13]	Mexico [74,75]
1	α-BHC	X	X	X		X			X		
2	β-BHC					X			X		
3	γ-BHC (Lindane)	X	X		X	X	X	X	X	X	
4	γ-Chlordane			X	X	X					
5	Hexachlorobenzene					X					
6	Heptachlor	X		X		X	X		X	X	
7	Aldrin	X			X	X	X		X	X	
8	α-Endosulfan		X	X		X	X	X	X	X	
9	DDT, -4,4				X	X	X	X	X	X	
10	Methoxychlor					X			X		
11	Chlorpyrifos-methyl				X						X
12	Endosulfan sulfate			X		X			X		
13	Dicofol										X

Table 2. Identified pesticides which are listed in the Stockholm Convention.

Nr	Pesticide	Formula	Nr of Analyzed Samples	Nr of Positive Samples	Positive Samples (%)	Annex A * (Elimination)	Annex B * (Restriction)
1	α-BHC	$C_6H_6Cl_6$	213	68	31.92	X	-
2	β-BHC	$C_6H_6Cl_6$	213	34	15.96	X	-
3	γ-BHC (Lindane)	$C_6H_6Cl_6$	213	60	28.17	X	-
4	γ-Chlordane	$C_{10}H_6C_{18}$	213	7	3.29	X	-
5	Hexachlorobenzene	C_6Cl_6	213	2	0.94	X	-
6	Heptachlor	$C_{10}H_5Cl_7$	213	100	46.95	X	-
7	Aldrin	$C_{12}H_8Cl_6$	213	79	37.09	X	-
8	α-Endosulfan	$C_9H_6C_{16}O_3S$	213	24	11.27	X	-
9	DDT, -4,4	$(ClC_6H_4)_2CHCCl_3$	213	2	0.94	-	X
10	Methoxychlor	$C_{16}H_{15}Cl_3O_2$	213	1	0.47	X	-
11	Chlorpyrifos-methyl	$C_7H_7Cl_3NO_3PS$	213	43	20.19	-	-
12	Endosulfan sulfate	$C_9H_6Cl_6O_4S$	213	9	4.23	-	-
13	Dicofol	$C_{14}H_9Cl_5O$	213	44	20.66	X	-

* Source: the UN [65].

From a total of 213 samples and 24 pesticides analyzed in each sample (5112 in total), 482 analytes were detected, which represents 9.43% of the total. Table 2 also shows that γ-Chlordane, Hexachlorobenzene, DDT, -4,4, Methoxychlor, and Endosulfan sulfate were encountered in less than 5% of the analyzed samples, so their presence could be considered as sporadic and ignorable. In this way, attention will be focused on only eight (8) pesticides. On the other hand, all measurements with LOQ values < 5 ng/L were considered as 0 ng/L; that is, it is assumed that the pesticide is not present in the sample.

The set of data obtained during the monitoring program was analyzed using a box and whisker plot, where two extreme atypical values (outliers) were identified and removed from the original data base. The extreme outliers that were removed corresponded to Dicofol, measured at station E13 in March, and Heptachlor, measured in January at station E20. This is because only 26.94% of the samples were positive in terms of the detection of the selected pesticides and the rest of the sample values were reported as zero (0). On the other hand, the data base generated in this 10-month monitoring campaign is still small compared to the criteria established in the literature, where at least five (5) years of monthly monitoring are required in the case of pesticides in water samples to observe reliable trends in the behavior of the data [40,41]. Table 3 shows the mean concentrations in each of the monitoring stations after the removal of outliers and those pesticides that represent less than 5% of the samples analyzed.

Table 3. Mean pesticide concentrations for each cluster of sampling sites and in the entire basin (values in ng/L).

Cluster	α-BHC	β-BHC	γ-BHC (Lindane)	Heptachlor	Aldrin	α-Endosulfan	Chlorpyrifos-methyl	Dicofol
Cluster 1	28.15	5.06	21.86	79.19	53.82	14.58	102.63	2364.97
Cluster 2	44.45	39.65	27.25	24.03	80.27	3.08	149.09	781.71
Cluster 3	30.17	10.88	51.56	114.07	35.35	5.43	7.85	2897.01
Cluster 4	56.31	17.59	27.12	37.31	71.91	29.76	22.87	1944.62
Cluster 5	24.39	6.36	71.98	63.80	14.47	11.33	17.08	256.14
Entire Basin	36.78	15.34	37.53	71.44	52.63	12.43	57.26	2006.13

Table 3 presents the mean concentrations observed for each pesticide organized into clusters according to the criteria shown in Table A2 in Appendix B to observe whether there were significant differences between the mean concentrations of each group of sub-basins. It can be observed that the pesticides found in the highest mean concentrations in the basin are Heptachlor, Aldrin, Chlorpyrifos-methyl, and Dicofol, whereas α-Endosulfan was detected as the lowest mean concentration in the basin, followed by β-BHC. It can also be observed that Dicofol was dominant in all of the clusters (Figure 4). Of particular interest is cluster 3, where the highest concentrations of Heptachlor and Dicofol were found. Of all the clusters of the monitored stations, cluster 3 appears to be the one that represents the greatest pollution problems, because it is home to a significant amount of agricultural and industrial activities, in addition to being one of the fastest growing and most concentrated urban areas in the MAG [34].

3.3. Correlation Analysis

Table 4 shows the results obtained from the correlation analysis between the data of the pesticides that account for 95% of the data. Those correlation coefficients that are significant are marked in bold. The value of −1.000 obtained in the correlation coefficient between α-Endosulfan and Heptachlor is because they share data coinciding only in two (2) monitored months. Similarly, the high level of correlation observed between α-Endosulfan and β-BHC is because there are only four (4) data points that coincide in the same monitored months, and the coincidence between Chlorpyrifos-methyl and α-Endosulfan is in only three (3) pairs of data.

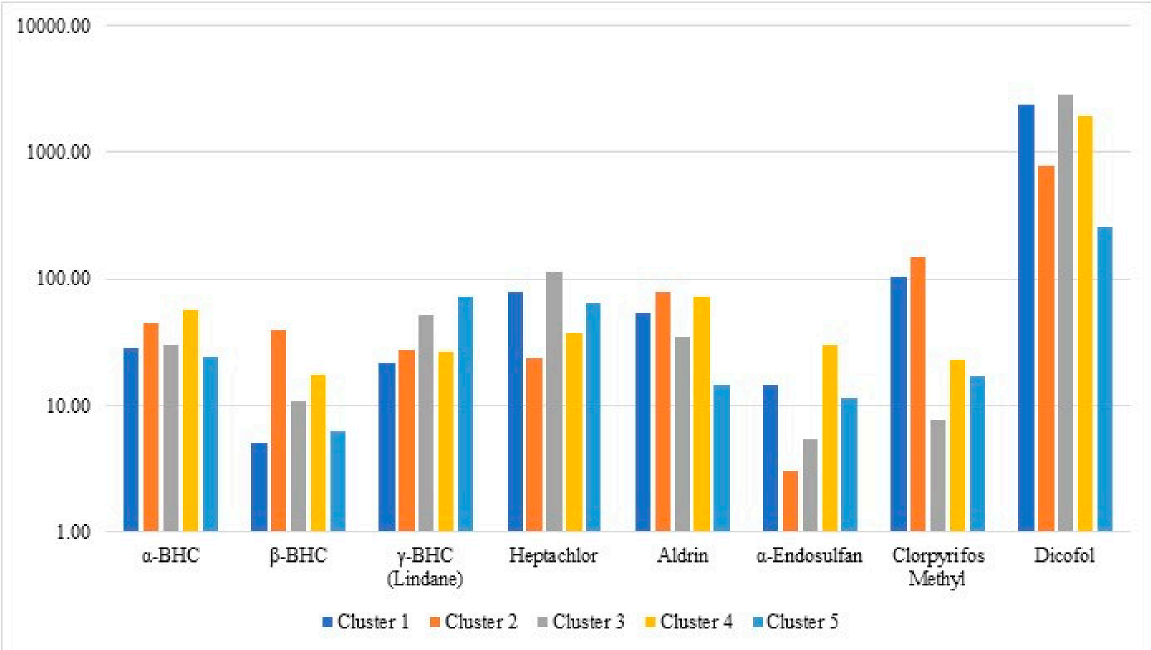

Figure 4. Mean concentrations (ng/L) of identified pesticides in each defined sub-basin cluster.

Table 4. Correlation analysis of pesticide concentrations representing 95% of the data base. High correlation values are in bold.

	α-BHC	β-BHC	γ-BHC (Lindane)	Heptachlor	Aldrin	α-Endosulfan	Chlorpyrifos-Methyl	Dicofol
α-BHC	1.000							
β-BHC	0.058	1.000						
γ-BHC (Lindane)	**0.693**	**0.834**	1.000					
Heptachlor	0.076	**0.447**	**0.451**	1.000				
Aldrin	0.253	0.453	0.520	0.567	1.000			
α-Endosulfan	−0.298	**0.959**	0.088	−1.000	**0.450**	1.000		
Chlorpyrifos-methyl	**0.832**	0.229	0.246	**0.579**	0.150	**0.921**	1.000	
Dicofol	**0.928**	**0.892**	**0.600**	0.191	**0.708**	**0.564**	**0.688**	1.000

On the other hand, as expected, a high level of correlation was found between γ-BHC (Lindane) and its isomers α-BHC and β-BHC. There are significant correlation values between the other pesticides, although their molecules are not related to each other as isomers or metabolites resulting from the decomposition of another molecule. For example, a high level of correlation is observed between Dicofol and α-BHC and β-BHC isomers. If a regression analysis is performed by modifying the x-axis to a logarithmic scale, there is a significant coincidence in the exponential behavior that occurs between Dicofol and α-BHC and β-BHC. In this way, Figure 5A,B show that the relationships between these pesticides remain relatively constant at low pesticide concentrations and show exponential behavior as the concentration of the pesticide present in higher concentrations increases. This could be interpreted as a chemical equilibrium that has been established in the aquatic system between these pesticides, which is altered when the concentration of γ-BHC (Lindane) or Dicofol increases significantly. Apparently, the same behavior was found between Dicofol and Aldrin (see Figure 5C). Although the value of the correlation coefficient between Heptachlor and γ-BHC and its isomer α-BHC is $R^2 < 0.500$, the correlation analysis shows that there is a similar trend compared to the previous cases (see Figure 5D).

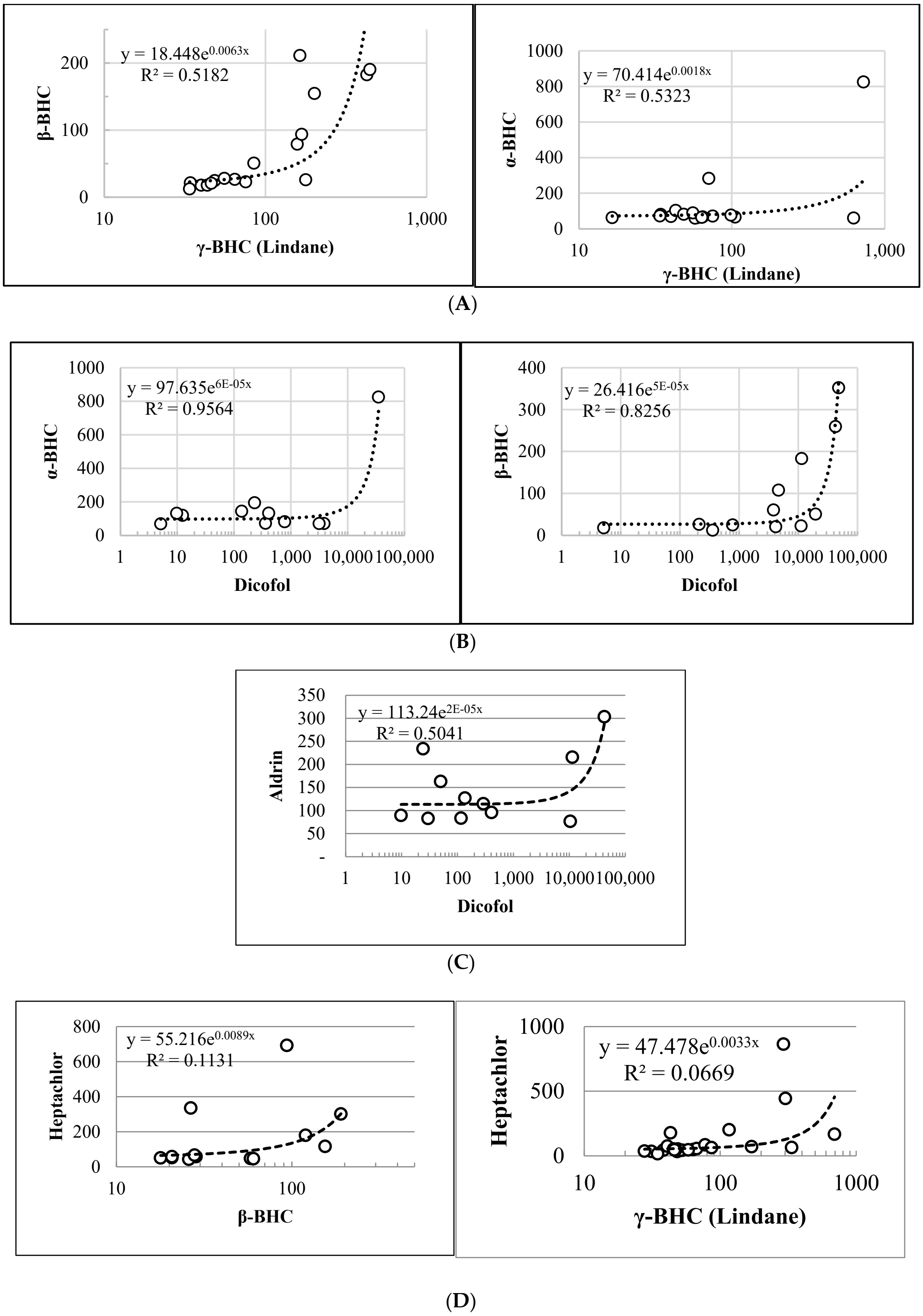

Figure 5. (**A**) Regression analysis between γ-BHC (Lindane) and its isomers α-BHC and β-BHC. (**B**) Regression analysis between Dicofol and α-BHC and β-BHC. (**C**) Regression analysis between Dicofol and Aldrin. (**D**) Regression analysis between Heptachlor and β-BHC and γ-BHC.

3.4. Estimation of the Pesticide Toxicity Index

Table A5 in Appendix B shows the pesticide threshold values established by the different national and international regulations for the protection of freshwater organisms. The threshold value for the toxicity of the organochlorine pesticides α-BHC and β-BHC was found only in the Mexican legislation [52]. In general, Table A5 in Appendix B shows that several of the identified pesticides have limited information regarding the concentration thresholds allowed for the protection of aquatic life. For the samples identified as positive in the pesticide analyses, the Pesticide Toxicity Index (PTI) was calculated according to Equation (2).

Figure 6 shows the mean values of the pesticide concentrations reached in each of the sampling stations. In this figure, there are seven sampling sites—stations E1, E8, E11, E16, E19, E20, and E22—where the average value of their PTI is > 1.0, which can be considered as a "High" toxicity risk. These stations correspond to the Zula River (cluster 1), El Ahogado watershed (cluster 3), Paso de Guadalupe (E16) (the last station of the MAG), and Blanco River (E22) (cluster 4). The latter is a small urban river that collects urban and industrial sewage and the drainage of agricultural lands that still exist within this urban watershed (see Figure 2). The mean PTI values reached by month in the SGRB are shown in Figure 7. It is clear from this figure that the highest PTI values were measured during the dry season, with some reaching values of PTI > 1.0, which places them in the classification of the "High" risk range. After May, the rainfall caused a significant dilution effect on the pesticide concentrations, reducing the risk level to "Moderate". The scatter plot of Figure 8 shows that most of the calculated PTI values are in the range of "Moderate" ($0.01 \leq$ PTI ≤ 1.0). From the 183 analyzed data points, 18 (9.9%) fell into the "Low" risk range classification, 133 (73.5%) fell into the "Moderate" classification, and 30 (16.4%) fell into the "High" classification. The overall average value of the Pesticide Toxicity Index for the SGRB was 0.833, which classifies it in the "Moderate" risk level according to Anderson et al. [63].

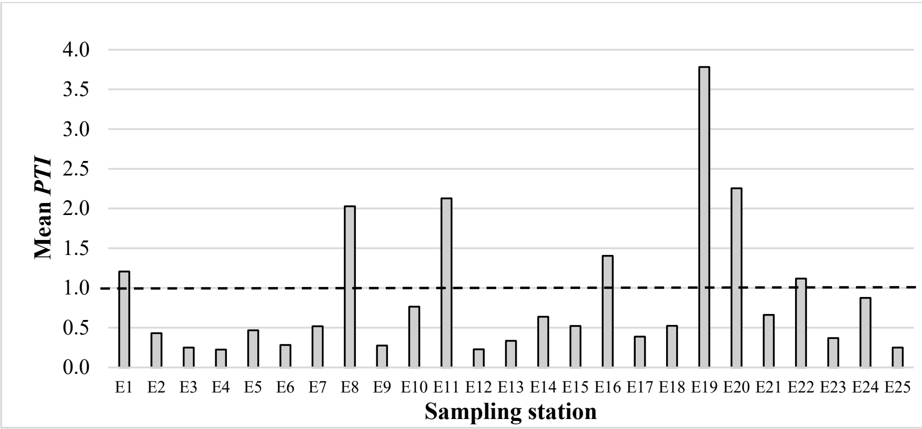

Figure 6. Mean *PTI* values reached in each sampling station of the SGRB.

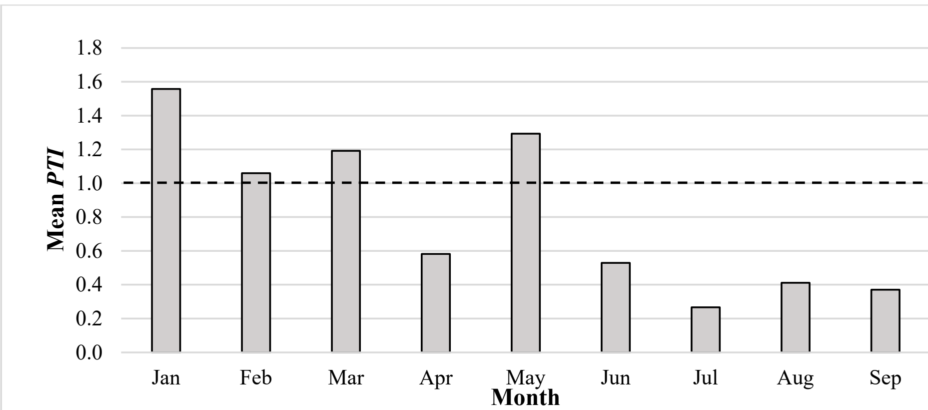

Figure 7. Mean *PTI* values reached by month in the SGRB.

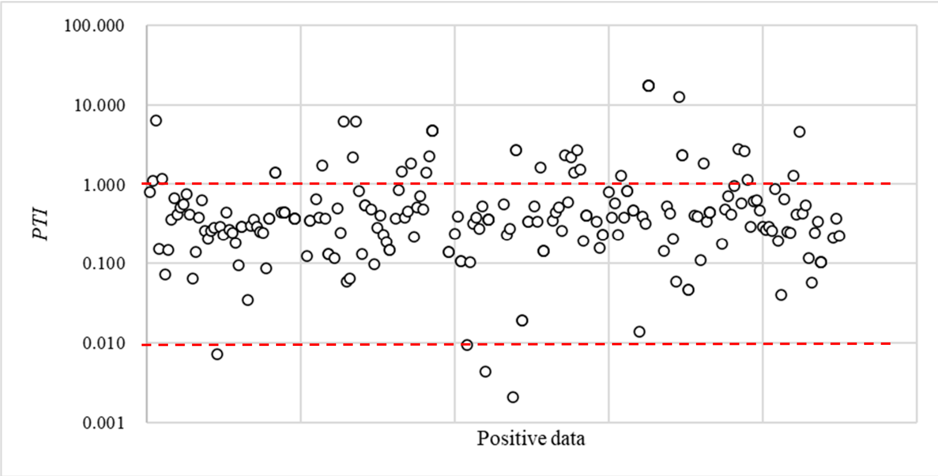

Figure 8. Scatter diagram showing the PTI estimated values.

3.5. Suggested Measures to Reduce Ecological Risks

The wide presence of pesticides with moderate-to-high levels of risk to ecosystem health and particularly to aquatic life in the SGRB requires several of the following measures to reduce their use and risks: (1) biocontrol and the use of natural pesticides; (2) the implementation of integrated pest management strategies; (3) the improvement in agronomic practices by implementing the principles of agroecology, organic agriculture, and/or regenerative agriculture [76–78]. In addition to these measures, the current national regulatory framework must be improved to guarantee more effective control over the use of pesticides restricted in international agreements to which Mexico is a signatory [78].

4. Conclusions

The results of this study revealed the presence of 13 pesticides of the 24 analyzed at 25 monitoring stations located in the tributaries of the Santiago-Guadalajara River system. The monthly monitoring events carried out from January to September 2022 were sufficient to identify eight (8) pesticides that gave a positive result in the analyzed samples, representing 95% of the total: α-BHC, β-BHC, γ-BHC (Lindane), Heptachlor, Aldrin, α-Endosulfan, Chlorpyrifos-methyl, and Dicofol. In 8 of the 25 monitored stations, the presence of pesticides exceeded the value of the Pesticide Toxicity Index (PTI), established as a "High" risk (PTI > 1.0). It is noteworthy that, at stations E1 and E8, located in the Zula River Basin, and stations E11, E19, and E20, located in the El Ahogado watershed, the PTI values exceed the value of 2.0, which can be classified as a "Very high" risk of contamination due to the presence of high pesticide concentrations. These monitoring stations coincide with the El Ahogado watershed, which is the urban basin where the greatest human health risks have been identified due to the environmental pollution of the river. Technical and governmental measures are necessary for an effective reduction in the presence of pesticides in the SGRB. It is necessary to expand the current monitoring network in the SGRB monitoring campaigns carried out by both state and federal agencies. It is also relevant to consider the permanent monthly monitoring of pesticides classified as persistent organic pollutants, especially those listed in the Stockholm Convention due to the high level of risk they pose to ecosystems and human health. Likewise, there is a need for public policies with state and federal regulatory agencies to restrict the importation and use of these pesticides into the country. The introduction of new forms of land management to preserve food production in a sustainable manner, avoiding the use of toxic substances, is an effort that will yield dividends in the future.

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review and editing, H.S.; visualization, J.d.A. and L.A.O.-V.; supervision, H.S.; project administration, J.d.A.; funding acquisition, J.d.A. All authors have read and agreed to the published version of the manuscript.

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Appendix A

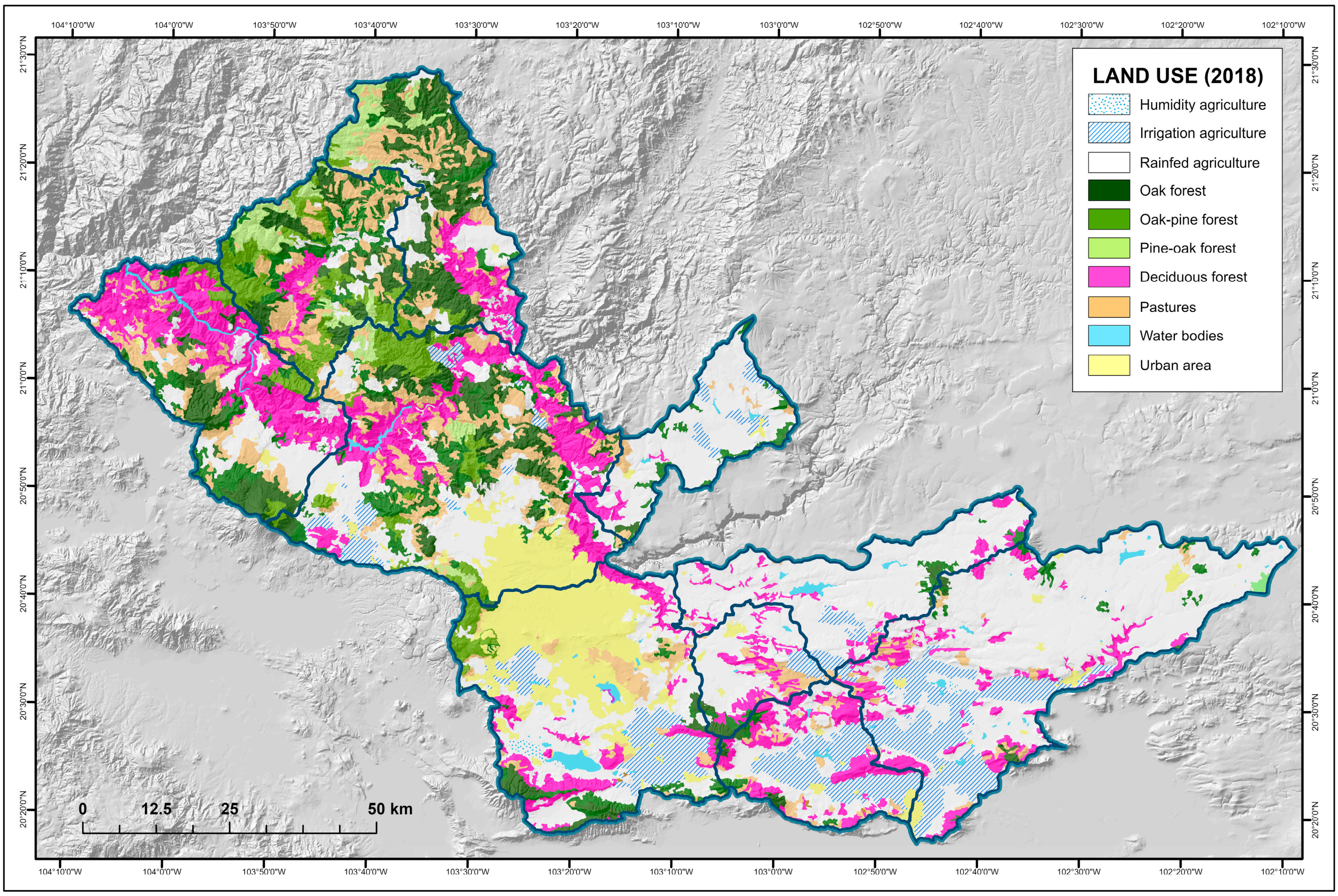

Figure A1. Land-use and vegetation type in the study area.

Appendix B

Table A1. Description of the geographical location of the monitoring stations in the SGRB.

Station	Sampling Station (Official Name)	Name of the Monitored Stream	Longitude	Latitude
1	Arandas	Arroyo La Madrastra	−102.33959	20.69607
2	Atotonilco el Alto	Arroyo El Taretán	−102.50246	20.54540
3	La Ladera	Arroyo Los Morales	−102.73044	20.59711
4	Gaviotas	Río Calderón	−102.85156	20.70156
5	San José de Gracia	Río Calderón	−102.70205	20.78619
6	San Miguel	Arroyo Tierras Coloradas	−102.78823	20.53503
7	Ocotlán Centro	Río Zula	−102.77800	20.34467
8	Los Cerritos	Arroyo Chico	−102.74937	20.44643
9	La Laja	Arroyo Grande	−103.12778	20.57804
10	Río Zapotlanejo	Río Zapotlanejo	−103.09545	20.62301
11	La Azucena	Arroyo El Ahogado	−103.22780	20.49743
12	La Noria	Río Santiago	−103.22635	20.46763
13	Río Santiago (before El Ahogado WWTP discharges)	Río Santiago	−103.18452	20.45462
14	Carretera Guadalajara—Chapala	Arroyo Las Pintas	−103.26535	20.47816
15	Presa Corona	Río Santiago	−103.09304	20.40014
16	Paso a Guadalupe	Río Santiago	−103.32881	20.83900
17	Rancho La Soledad	Río La Soledad	−103.37082	20.89438
18	Plan de Oriente	Arroyo El Ahogado	−103.28403	20.58856
19	Villa Fontana	Arroyo Las Pintas	−103.36321	20.56268
20	San José del Quince	Arroyo El Ahogado	−103.29677	20.53781
21	El Arenal	Río Arenal	−103.63856	20.72332
22	San Isidro	Río Blanco	−103.45939	20.79633
23	San Cristóbal de la Barranca	Río La Calera	−103.43309	21.04759
24	Tequila	Río Amatitán	−103.83174	20.89847
25	Hostotipaquillo	Río Los Sabinos	−104.01113	21.03211

Table A2. Location of each sampling station in each sub-basin.

Sub-Basin Official SPANISH Name	Sampling Stations	Cluster	Dominant Land Use [(1)]
Río Zula	E1, E2, E3, E6, E7, E8	1	Rainfed and irrigation agriculture
Lago de Chapala-Río Corona	None		Irrigated agriculture
Río La Laja	E9, E10	2	Rainfed agriculture and urban and industrial areas
Río Calderón	E4, E5	2	Rainfed agriculture
Río Corona-Río Verde	E11, E12, E13, E14, E15, E18, E19, E20	3	Urban and industrial areas, and irrigated agriculture
Río Gigantes	E16	4	Urban and forest areas
Río Verde-Presa Santa Rosa	E17, E21, E22,	4	Forest
Río Cuixtla	E23	4	Forest
Río Chico	None		Forest
Presa de Santa Rosa-Río Bolaños	E24, E25	5	Urban and industrial areas, agriculture, and forest

[(1)] According to Figure A1 in Appendix A.

Table A3. Analytical methods used for the identification and quantification of pesticides in the analyzed water samples (detection limit < 5 ng/L).

Nr.	Pesticide	Chemical Formula	Analytical Method Applied (1)
1	α-BHC	$C_6H_6Cl_6$	Modified EPA Method 8081
2	β-BHC	$C_6H_6Cl_6$	Modified EPA Method 8081
3	γ-BHC (Lindane)	$C_6H_6Cl_6$	Modified EPA Method 8081
4	γ-Chlordane	$C_{10}H_6C_{l8}$	Modified EPA Method 8081
5	Hexachlorobenzene	C_6Cl_6	Modified EPA Method 8081
6	Heptachlor	$C_{10}H_5Cl_7$	Modified EPA Method 8081
7	Aldrin	$C_{12}H_8Cl_6$	Modified EPA Method 8081
8	α-Endosulfan	$C_9H_6C_{l6}O_3S$	Modified EPA Method 8081
9	β-Endosulfan	$C_9H_6C_{l6}O_3S$	Modified EPA Method 8081
10	4,4′- DDE	$C_{14}H_8Cl_4$	Modified EPA Method 8081
11	Dieldrin	$C_{12}H_8Cl_6O$	Modified EPA Method 8081
12	Endrin	$C_{12}H_8Cl_6O$	Modified EPA Method 8081
13	DDD-4,4′	$C_{14}H_{10}Cl_4$	Modified EPA Method 8081
14	Methoxychlor	$C_{16}H_{15}Cl_3O_2$	Modified AOAC Official Method 970.52
15	Heptachlor epoxide	$C_{10}H_5C_{l7}O$	Modified EPA Method 8081
16	DDT-2,4′	$C_{14}H_9Cl_5$	Modified EPA Method 8081
17	DDT-4,4′	$(ClC_6H_4)_2CHCCl_3$	Modified EPA Method 8081
18	Chlorpyrifos-methyl	$C_7H_7Cl_3NO_3PS$	Modified AOAC Official Method 970.52
19	Diazinon	$C_{12}H_{21}N_2O_3PS$	Modified AOAC Official Method 970.52
20	Ethion	$C_9H_{22}O_4P_2S_4$	Modified AOAC Official Method 970.52
21	Malathion	$C_{10}H_{19}O_6PS_2$	Modified AOAC Official Method 970.52
22	Methamidophos	$C_2H_8NO_2PS$	Modified EPA Method 8081
23	Endosulfan sulfate	$C_9H_6Cl_6O_4S$	Modified EPA Method 8081
24	Dicofol	$C_{14}H_9Cl_5O$	Modified EPA Method 8081

(1) The methods used for the determination of pesticides were modified to reduce the detection limits established by the official Mexican standards. The test quality of reproducibility, repeatability, and recovery were carried out for each of the analyzed pesticides.

Table A4. Limits of detection and quantification of identified pesticides.

Pesticide	Working Solution Concentration (ng/L)	% Recovery (1)	Coefficient of Variation (2) (%)	LOD (3) (ng/L)	LOQ (4) (ng/L)
α-BHC	250	90.40–103.20	24	2.5	5
β-BHC	250	89.70–116.10	28	2.5	5
γ-BHC (Lindane)	250	95.20–116.90	10	2.5	5
γ-Chlordane	250	97.50–107.30	15	2.5	5
Hexachlorobenzene	250	97.10–113.60	19	2.5	5
Heptachlor	250	97.80–111.40	12	2.5	5
Aldrin	250	97.40–103.30	12	2.5	5
α-Endosulfan	250	97.10–109.20	25	2.5	5
β-Endosulfan	250	97.50–113.30	19	2.5	5
4,4′-DDE	250	98.30–106.90	10	2.5	5
Dieldrin	250	93.70–115.60	34	2.5	5
Endrin	250	78.50–108.70	10	2.5	5
DDD-4,4′	250	98.70–100.70	15	2.5	5
Methoxychlor	250	95.60–104.30	34	2.5	5
Heptachlor epoxide	250	96.00–103.40	15	2.5	5
DDT-2,4′	250	94.80–101.20	14	2.5	5
DDT-4,4′	250	85.80–100.8	26	2.5	5
Chlorpyrifos-methyl	250	88.50–111.70	30	2.5	5
Diazinon	250	88.70–115.20	25	2.5	5
Ethion	250	98.70–113.40	19	2.5	5
Malathion	250	95.70–103.90	23	2.5	5
Methamidophos	250	98.40–106.20	28	2.5	5
Endosulfan sulfate	250	90.50–102.90	18	2.5	5
Dicofol	250	95.40–113.50	32	2.5	5

(1) Recovery of waste and contaminants in food and water. Analyte concentration <1 μg/kg or μg/L (ppb) acceptance criteria: 50–120%. (2) Repeatability and intermediate precision for residues and contaminants in food and water. Analyte concentration <1 μg/kg or μg/L acceptance criteria: CV $\leq$ 35%. (3) LOD = limit of detection. (4) Limit of quantification.

Table A5. Pesticide threshold values established by national and international agencies for the protection of aquatic life.

Nr	Pesticide	LFD (µg/L) [1]	U.S. EPA (µg/L) [2]				CWQG (µg/L) [3]		ANZECC (µg/L) [4]	TC_{Avg} (µg/L)
		Protection of Freshwater Organisms	Freshwater Vertebrate		Freshwater Invertebrate		Protection of Freshwater Organisms		Protection of Freshwater Organisms	Average Toxicity Values
			Acute	Chronic	Acute	Chronic	Acute	Chronic		
1	α-BHC	1.000	ND	ND	ND	ND	ND	ND	ND	1.0000
2	β-BHC	1.000	ND	ND	ND	ND	ND	ND	ND	1.0000
3	γ-BHC (Lindane)	2.000	0.8500	2.9000	0.5000	54.0000	ND	0.0100	0.2000	8.6371
4	Heptachlor	0.500	0.5200	0.0038	0.3000	0.0100	ND	0.0100	0.0100	0.1934
5	Aldrin	0.300	3.0000	ND	ND	ND	ND	ND	0.001	1.1003
6	α-Endosulfan	0.200	0.0500	0.0230	0.3000	0.0100	0.0600	0.0030	0.0002	0.0923
7	Chlorpyrifos-methyl	ND	7.0000	ND	0.0850	ND	ND	ND	ND	3.5425
8	Dicofol	ND	26.5000	4.4000	70.0000	19.0000	ND	ND	0.500	24.0800

ND = no data available. [1] LFD = Federal Law of Rights [53]. [2] USEPA = U.S. Environmental Protection Agency [45,46]. [3] CWQG = Canadian Water Quality Guidelines [54]. [4] ANZECC = Australian and New Zealand Environment and Conservation Council [55].

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