

PESTICIDE DISTRIBUTION AND ECOTOXICOLOGICAL RISK IN THE AYUQUILA-ARMERÍA RIVER

Distribución de pesticidas y riesgo ecotoxicológico en el río Ayuquila-Armería

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ABSTRACT

Pesticides make an important contribution to increased global agricultural production; however, their intensive use represents a risk to biota in both the short and long terms. The physical and chemical characteristics of these compounds, as well as their versatility in agricultural, domestic, and public health use, have led them to become widely present in the environment. The objectives of this study were to evaluate the spatiotemporal distribution of pesticides in the surface water of the Ayuquila-Armería river and to conduct an ecotoxicological risk assessment in algae, macroinvertebrates, and fish. The presence of 11 analytes was determined, being λ -cyhalothrin, ametrine, and malathion the pesticides with highest concentrations, and carbendazim, malathion, and glyphosate those with the highest frequency of detection (> 70 %). The number of pesticides detected per sample presented no significant temporal differences, and there was no significant effect of the crops or vegetation adjacent to the study sites on the number of pesticides detected per sample. The sampling conducted in November 2019 (wet season) presented the greatest difference in concentrations of most pesticides, compared to the other samplings. The ecotoxicological risk assessment showed that the macroinvertebrates (*Daphnia magna*) are the most vulnerable to the concentrations of pesticides in surface water, followed by fish and algae. Regulation of the importation, trade, and management of malathion and λ -cyhalothrin is crucial in order to reduce the presence of their residues and the risks associated with them.

Palabras clave: contaminación del agua, agroquímicos, agricultura intensiva, monitoreo de plaguicidas, extracción en fase sólida.

RESUMEN

Los plaguicidas contribuyen de manera importante al aumento de la producción agrícola mundial; sin embargo, su uso intensivo representa un riesgo para la biota tanto a corto como a largo plazo. Las características físicas y químicas de estos compuestos, así como su versatilidad en el uso agrícola, doméstico y de salud pública, los han llevado a tener una amplia presencia en el ambiente. Los objetivos de este estudio fueron evaluar la distribución espaciotemporal de plaguicidas en agua superficial del río Ayuquila-Armería, y realizar una evaluación de riesgo ecotoxicológico en algas, macroinvertebrados y peces. Se determinó la presencia de 11 analitos, siendo la λ -cihalotrina, la ametrina y el malatión los plaguicidas con mayores concentraciones, en tanto que el carbendazim, el malatión y el glifosato fueron los de mayor frecuencia de detección (> 70 %). El número de plaguicidas detectados por muestra no presentó diferencias temporales significativas, y no hubo efecto significativo de los cultivos o la vegetación adyacente a los sitios de estudio sobre el número de plaguicidas detectados por muestra. El muestreo realizado en noviembre de 2019 (temporada de lluvias) presentó la mayor diferencia en las concentraciones de la mayoría de los plaguicidas, en comparación con los demás muestreos. La evaluación de riesgos ecotoxicológicos mostró que los macroinvertebrados (*Daphnia magna*) son los más vulnerables a las concentraciones de plaguicidas en el agua superficial, seguidos de los peces y las algas. La regulación de la importación, el comercio y la gestión del malatión y la λ -cihalotrina es fundamental para reducir la presencia de sus residuos y los riesgos asociados a ellos.

INTRODUCTION

The global production of synthetic chemical pesticides is approximately two million tons per year, for use in intensive agriculture for food production (Sharma et al. 2019). However, their intensive use and inefficient application impact the environment and represent a potential risk for living beings (Sumon et al. 2018). Mexico is a producer and exporter of foodstuffs at a global level; however, its production system is based on monocultures and excessive use of pesticides (SAGARPA 2017). The use of synthetic pesticides in Mexico began in the 1940s with the adoption of the technological paradigm for the modernization of agriculture known as the “green revolution” (Bejarano-González 2017).

Aquatic ecosystems are very vulnerable to contamination by pesticides, which can occur via specific or diffuse routes, such as surface runoff, erosion, leaching, and poor waste management (Sumon et al. 2018). Pesticides present high variation in surface waters due to environmental (precipitation, temperature, solar radiation) and anthropic (periods of application, productive activities) variables, which determine the presence and concentration of these chemicals (Belenguer et al. 2014, Vryzas 2018).

Ecotoxicological risk assessment is a useful tool for evaluating potential adverse effects on an ecosystem. Toxicity reference values depend on the response capacity of the species to one or a combination of different stressors, which reflects its sensitivity at a trophic level (Kuzmanović et al. 2016). The potential effect on the biota of stressors can be estimated using two models: Independent Action (IA) or Concentration Addition (CA) (Ccanccapa et al. 2016). Considering that surface water contamination due to the use of pesticides is characterized by the presence of a mixture of compounds, the CA model is considered the first approach to risk assessment (Ginebreda et al. 2014, Castro-Catalá et al. 2016). In this sense, the ecotoxicological risks associated with concentrations of pesticide can be evaluated using Risk Quotients (RQs) and Toxic Units (TUs) (Tsaboula et al. 2016, Carazo-Rojas et al. 2018). RQs are used to evaluate the ecotoxicological risk posed by the concentration of a single pesticide, in terms of chronic exposure, while TUs determine the toxic effects of a combination of pesticides, expressed as a single value per sampling site. These tools are useful for the determination of potential risks to different biotic components of an ecosystem. However, one disadvantage is the current lack of knowledge concerning the antagonistic and

synergistic relationships that occur in a mixture of pesticides (Palma et al. 2014, Kuzmanović et al. 2016).

In Mexico, the Ayuquila-Armería basin is found between the states of Jalisco and Colima and has an area of 9864 km². It has about 450 000 inhabitants and is considered one of the most important basins of western Mexico (Santana et al. 1993). The main economic activities practiced within the basin are intensive and subsistence agriculture, forestry, and fishing, with sugarcane monoculture one of the most economically important productive activities, and one that is dependent on the use of pesticides. The river is recognized as a provider of fishery resources and water at a regional level; however, these have been affected over time by activities that have modified the integrity of the aquatic ecosystem, such as agricultural, domestic, industrial, and livestock production discharges, as well as diversion and retention of water, overexploitation of fishery resources and deforestation of the riparian zones (Meza-Rodríguez 2006, Mercado-Silva et al. 2011).

The Ayuquila-Armería river is one of the 15 most important rivers among the 100 found on the Pacific slope of the country and presents important biodiversity in the two states it occupies, with endemic and threatened species according to Mexican Official Standard NOM-059-SEMARNAT-2010 (CONABIO 2020). In the basin, at least 143 active ingredients are available for sale, along with an even higher number of commercial products. Commonly used pesticides in agricultural activities within the basin include glyphosate, carbofuran, malathion, ametrine, paraquat, cypermethrin, and atrazine (Rodríguez-Aguilar et al. 2019). The aims of this study were (1) to evaluate the spatiotemporal distribution of the pesticides in the surface water of the Ayuquila-Armería river, and (2) to conduct an ecotoxicological risk assessment in algae, macroinvertebrates, and fish, based on pesticide residues in the surface water samples and adopting the RQ and TU methods.

METHODS

Sampling sites

Twenty-four sites were chosen based on the main sources of contamination by domestic, industrial, and agricultural discharges within the Ayuquila-Armería basin. These included the Melchor Ocampo sugar mill; the agave production area of Tuxcacuesco, Tonaya, Tolimán and San Gabriel Jalisco; the greenhouse zone of San Gabriel, Jalisco, and residual discharges from the cities of Autlán, El Grullo and

Colima (**Fig. 1**). Three reference sites were established: one in the state of Colima (Zacualpan creek) and two in the state of Jalisco (La Taza spring and Manantlán creek). These reference sites were selected because they are close to or within the polygon of the Sierra de Manantlán Biosphere Reserve (SMBR) and present null or very low agricultural activity.

The sites are found in a surrounding manner, with different land uses associated with various productive activities, including irrigated and seasonal agriculture and the sugarcane and agave distillate production industries. The exceptions to this were the reference sites (8, 10, and 18), and those found mainly in zones with canyons (1, 9, 17, and 19), which generally present tropical low deciduous forest vegetation (**Table I**). The choice of pesticides was based on their sale, the extent, and frequency of application to crops. These pesticides comprise a mixture of active ingredients that control different types of organisms, such as fungicides, insecticides, and herbicides (Rodríguez-Aguilar et al. 2019).

Sample collection

Four water samples were taken at each site over the course of a year. Two were taken during the dry season (May 2018 and February 2019) and two during the rainy season (September and November 2018). Water samples were taken with 1 L jars, previously washed with methanol and ultrapure water prior to being used, in zones with a flowing current, from the middle of the water column and in the center of the river flow, based on the protocol published in NMX-AA-003-1980 (SECOFI 1980). During transport and fieldwork, the samples were placed at 4 °C in order to increase the time of preservation and reduce microbial activity. Once in the laboratory, the samples were stored at -20 °C until analysis.

MATERIALS AND EQUIPMENT

The pesticides under study were 2,4-D, acetochlor, ametrine, atrazine, carbendazim, carbofuran, diazinon, dimethoate, emamectin, glyphosate, imazalil, λ -cyhalothrin, malathion, methomyl, metoxuron, molinate, parathion, picloram, pyraclostrobin, and thiabendazole. Formic acid, acetonitrile, sodium chloride, ammonium formate, and methanol (MeOH), all analytical grade and sourced from Sigma-Aldrich, were used in the extraction of pesticides and preparation of the mobile phase of the chromatograph. Solid-phase extraction (SPE) cartridges Supel-select HLB 500 mg/12 mL (Supelco) were used.

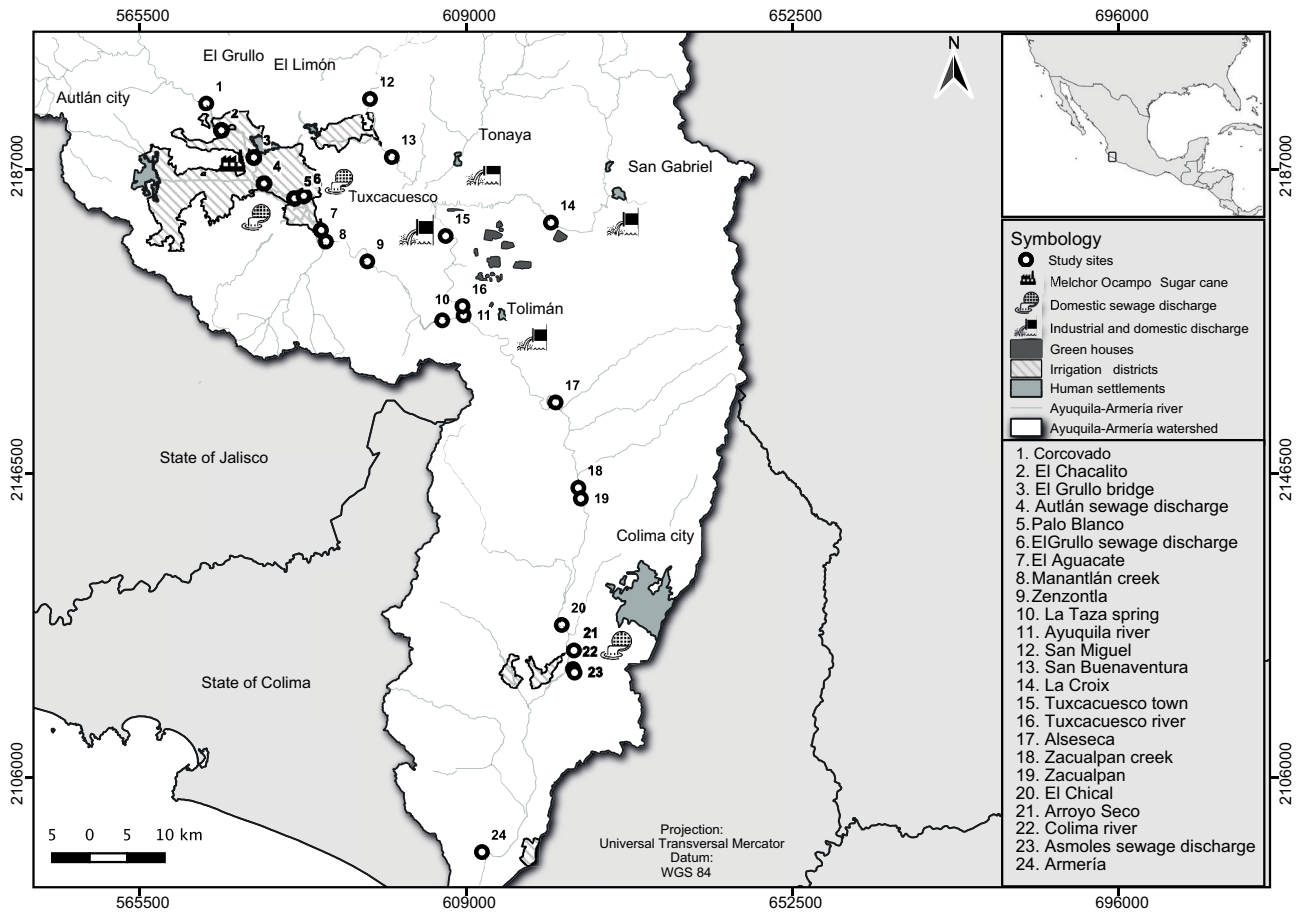


Fig. 1. Sampling sites and main sources of contamination in the Ayuquila-Armería basin in western Mexico.

TABLE I. PHYSICAL MORPHOLOGY AND LAND USE AT THE SURFACE WATER SAMPLING SITES IN THE AYUQUILA-ARMERÍA RIVER BASIN.

Study sites	Land use	Geomorphic type
Corcovado; Manantlán creek and Zenzontla in Jalisco; Alseseca Zacualpan creek and Zacualpan in Colima	Deciduous tropical dry forest	Canyon
El Chacalito; El Grullo bridge; Autlán and El Grullo sewage discharges; Palo Blanco and El Aguacate in Jalisco; San Miguel; San Buenaventura; El Chical; Arroyo Seco; Colima river; Asmoles sewage discharge and Armería in Colima	Sugarcane	Valley
La Taza spring in Jalisco	Deciduous tropical dry forest	Valley
Ayuquila river; Tuxcacuesco town; Tuxcacuesco river	Agave and greenhouses	Valley
La Croix in Jalisco	Agave and greenhouses	Canyon

A range of equipment was used during the process of extraction of pesticides from the water samples, including Milli-Q water purification system of Millipore, Vacuum pump EV-40 of EVAR, 24-port SPE Manifold system of Phenomenex, Orion 3 Star pH meter, and Cimarec stirring hotplate, both of Thermo Scientific. For quantification of the pesticides, a liquid chromatograph model 1200 was used, coupled to a mass spectrometry detector model 6430B, of Agilent Technologies. The software packages used for data acquisition were MassHunter Workstation Acquisition Software v. B.02.01 and MassHunter Workstation Quantitative Analysis Software v. B.03.02. A chromatographic column Zorbax Eclipse XDB-C18 Rapid Resolution 2.1 mm in diameter \times 50 mm in length and particle size 3.5 μm of Agilent Technologies was used.

Extraction and analysis of pesticides

Water samples were filtered in a vacuum system with filter paper of pore size 0.45 μm . A volume of 400 mL of each sample was used for the extraction of the pesticides. Prior to extraction, 5 g of sodium chloride was added per 100 mL of sample. The SPE cartridges were then conditioned with 5 mL of MeOH and 10 mL of ultrapure water. Samples were passed through the cartridge at a flow rate of 2 mL/min. The cartridges were washed with 10 mL of ultrapure water followed by 5 min of vacuum drying. Elution of the analytes was conducted with 6 mL of MeOH. The samples were evaporated at 1 mL at ambient temperature, according to the procedure published by El-Osmani et al. (2014). The final extract was placed in 2 mL vials for injection into the liquid chromatography. The samples were analyzed based on Sierra-Díaz et al. (2019).

Risk quotient and toxic units for water

The toxicity values of effective concentration (EC_{50}), lethal concentration (LC_{50}), and no observable effect concentration (NOEC) are used in ecotoxicological risk assessments as points of comparison for the concentrations of pesticides in water and to establish their possible effect at a trophic level in an ecosystem (Carazo-Rojas et al. 2018). Ecotoxicological risk assessment was conducted using the approaches of RQs and TUs (Palma et al. 2014, Castro-Català et al. 2016) at three trophic levels of the aquatic ecosystem: (a) *Raphidocelis subcapitata*, *Pseudokirchneriella subcapitata*, and *Scenedesmus subspicatus* (algae), (b) *Daphnia magna* (aquatic invertebrates), and (c) *Oncorhynchus mykiss*, *Lepomis macrochirus*, and *Pimephales promelas* (fishes).

The toxicity reference values EC_{50} and CL_{50} were used to evaluate acute effects (Mu et al. 2016, Yuniari et al. 2016), while NOEC was used for the analysis of chronic effects (Warne and van Dam 2008). To evaluate the acute effects, acute toxicological data EC_{50} of 72 h were used for algae; EC_{50} of 48 h for the aquatic invertebrates, and LC_{50} of 96 h for the fishes. For the chronic effects, of each pesticide, data of 96 h NOEC were used for algae, 21 days NOEC for aquatic invertebrates, and 21 days NOEC for the fishes. This information was obtained from the Pesticide Properties Database developed by the Agriculture and Environment Research Unit (AERA) of the University of Hertfordshire (Lewis et al. 2016).

$$TU_i = C_i/EC_{50} \text{ or } LC_{50} \quad (1)$$

where TU_i is the toxic unit of the pesticide i ; C_i is the quantified concentration of a pesticide ($\mu\text{g/L}$) in the water samples, and EC_{50} or LC_{50} ($\mu\text{g/L}$) are the effective and lethal concentrations that affect 50 % of the individuals when exposed to a given pesticide (Cancapá et al. 2016). The TU_i was obtained for each of the pesticides in a sample and later summed to obtain the ecotoxicological risk level per site. Sites that present a value of 0.1-1 suggest moderate acute effects, and ≥ 1 suggest a high acute risk (Carazo-Rojas et al. 2018).

The toxic units per site (TU_{site}) were subsequently determined using a cumulative approach, conducted by summing the toxic units (TU_i) of each pesticide quantified in a sample (Sánchez-Bayo et al. 2002). The TU_{site} was obtained for each of the samples collected during the different samplings.

$$\text{Sum } TU_{\text{site}} = \sum_{i=1}^n TU_i \quad (2)$$

The RQ values were calculated according to the following equation:

$$RQ = EC/PNEC \quad (3)$$

where EC is the mean and maximum quantified concentration of each pesticide in the water samples, with the aim of evaluating the general and worst-case scenarios, respectively (Palma et al. 2014). The value of the predicted no-effect concentration (PNEC) was estimated through the NOEC. Where the NOEC value was unavailable in the database, EC_{50} was used. The PNEC values were then divided by 1000 as an

assessment factor (AF) when at least a short-term assay was available (LC_{50} or EC_{50}) at a trophic level. These were divided by 100 when data were available from long-term assays (EC_{10} or NOEC) with fish or aquatic invertebrates, and by 50 and 10 when two or three long-term assays (EC_{10} or NOEC) were available, respectively (EC 2011, Tsaboula et al. 2016). AF is an arbitrary value that considers the inherent uncertainty of the acquisition of toxicological laboratory data, according to the Technical Guidance for Deriving Environmental Quality Standards of the European Commission (EC 2011). Finally, if the RQ value was >1 , it was considered that chronic adverse effects could be expected, if the value was 0.1-1, it was considered an intermediate risk and if it was <0.1 , the risk was considered low.

Statistical analysis

To evaluate the temporal distribution of the number of pesticides detected per sample, a statistical analysis was conducted using a generalized linear model (GLM) of Poisson type, in order to determine the significant differences between the number of pesticides detected per sample and the samplings conducted; likewise, to determine differences between the number of pesticides detected per sample and the land use adjacent to each study site.

To evaluate the spatiotemporal distribution of each pesticide, statistical analyses were performed in order to determine significant differences per season and per sampling. The statistical tests for each pesticide were conducted through left-censored data analysis, with the non-parametric Kaplan-Meier test and a parametric test with maximum likelihood estimation, using R project v. 3.6.2 and the packages Survival v. 3.1-8 and NADA v. 1.6-1. This type of analysis allows working with data found below the limit of quantification and conferring greater robustness to the results obtained, in place of truncating or substituting the results below the limit, whether for a zero or a fraction of the limit, causing bias in the results and increasing the probability of committing errors in the conclusions and subsequent decision-making (Helsel 2012). According to the theory of censored data analysis, it is not possible to conduct analysis with this statistical test for analytes that present censorship, or more than 80 % of results below the quantification limit, in the total number of samples (Hewett and Ganser 2007, Fox 2015). For this reason, the analytes acetochlor, dimethoate, emamectin, metoxuron, parathion, pyraclostrobin, thiabendazole, carbofuran, imazalil, and methomyl were excluded from the statistical analyses.

Finally, a temporal analysis was conducted of the TUs results produced by the ecotoxicological risk assessment for each level (algae, aquatic invertebrates, and fish). An analysis was conducted to determine differences among the results of the TUs of each sampling site per season, using the Wilcoxon Rank Sum test. A further analysis was conducted to determine differences among the results of the TUs of each sampling site per sample, using the Kruskal-Wallis test. The use of non-parametric tests was necessary since none of the results behaved normally according to the Shapiro-Wilk test.

RESULTS AND DISCUSSION

Occurrence and concentration of pesticides in surface water

The concentrations of 20 pesticides were analyzed in the samples (Fig. 2). Of these, six pesticides were invariably found below the limit of quantification ($0.025 \mu\text{g/L}$) (Table II). The maximum individual

Sites	AME	ATZ	CBZ	CBF	DZN	IMZ	λ CY	MAL	MTM	MLT	PIC	GPS	24D
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
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23													
24													

Fig. 2. Pesticides detected by each site during the four samplings in the surface water of the Ayuquila-Armería river. The gray color in the box represents the pesticide positive detection. AME: ametrine, ATZ: atrazine, CBZ: carben-dazim, CBF: carbofuran, DZN: diazinon, IMZ: imazalil, λ CY: λ -cyhalothrin, MAL: malathion, MTM: methomyl, MLT: molinate, PIC: picloram, GPS: glyphosate, and 24D: 2,4-D. Thiabendazole was only detected at site 17. Acetochlor, dimethoate, emamectin, metoxuron, parathion, and pyraclostrobin were not detected.

TABLE II. MEAN AND MAXIMUM CONCENTRATION, AND STANDARD DEVIATION OF ALL DETECTED CONCENTRATIONS OF PESTICIDES IN SURFACE WATER SAMPLES FROM THE AYUQUILA-ARMERÍA RIVER.

Pesticide*	Mean concentration (µg/L)	Maximum concentration (µg/L)	SD	Pesticide	Mean (µg/L)	Maximum concentration (µg/L)	SD
2,4-D	0.14	0.81	0.19	Imazalil	0.19	0.93	0.25
Ametrine	0.58	18.12	2.36	l-cyhalothrin	1.75	50.92	9.13
Atrazine	0.47	2.59	0.46	Malathion	3.73	16.82	3.28
Carbendazim	0.26	1.86	0.37	Methomyl	0.09	0.12	0.01
Carbofuran	0.12	0.25	0.08	Molinate	0.18	0.40	0.09
Diazinon	0.73	10.08	2.06	Picloram	0.71	2.02	0.39
Glyphosate	0.73	3.34	0.64	Thiabendazole	0.15	0.24	**

SD: standard deviation.

*Acetochlor, dimethoate, emamectin, metoxuron, parathion and pyraclostrobin were below the limit of quantification in all samples;

**pesticide quantification on only two occasions.

concentration detected was for λ -cyhalothrin at 50.92 µg/L in the site El Grullo bridge in the sample from September (wet season), followed by ametrine at 18.12 µg/L in the site El Grullo sewage discharge in the sample from May (dry season) and by malathion at 16.82 µg/L in the site La Croix in the sample from September.

The pesticides with the greatest frequency of detection in the total number of samples were carbendazim, malathion, and glyphosate, being found in more than 70 % of the total number of samples analyzed. Their high frequencies of detection are associated with their wide sale and use in different crops in the study area, causing their residues to be distributed across most of the basin (Rodríguez-Aguilar et al. 2019).

The broad spectrum of use of malathion, probably with different periods of application in agriculture, has led to its permanence in the environment and thus its continuous detection (Deknock et al. 2019). However, the frequent detection of this pesticide isn't only related to agricultural practices but also to its use in urban areas (Masiá et al. 2013); it isn't only used to control insects in different crops, but also to control vectors of diseases and for domestic use. This leads to its permanent presence through continuous use and incorporation in the environment, which coincides with the definition of "pseudo-persistent" pollutants provided by Daughton (2003).

All the samples presented at least one pesticide. For this reason, the reference sites also presented concentrations of mixed pesticides in all their samples, apart from the sample taken from the Manantlán creek in September 2018 (wet season), in which only one analyte was detected. However, the Zacualpan

creek presented a mean of five pesticides during the four samplings, the Manantlán creek a mean of 3.2 and La Taza spring presented a mean of 2.5, with the latter presenting the lowest mean relative to all the study sites.

According to various authors (Bailly-Comte et al. 2008, Guo et al. 2010), karst zones are vulnerable systems that are prone to contamination by pesticides, since their hydrogeological condition favors the movement of pollutants found on the surface towards the subterranean water. These zones present a minimum natural autodepuration of pollutants that, added to the rapid infiltration of precipitation, short residence times of the water, and diminution of photolysis and thermal decomposition of the pesticides, contribute to maintaining these pollutants in a stable condition and distributing them widely within these systems (Alam et al. 2014). The SMBR comprises Sierra Manantlán, Cerro de Enmedio, and Cerro Grande, the latter two of which are karstic mountainous massifs. The reference sites La Taza spring and Zacualpan creek are located on the slopes of Cerro de Enmedio and Cerro Grande, respectively, for which reason their hydrogeological situation favors the transfer of pesticides even though Cerro de Enmedio presents no agricultural activities, while in Cerro Grande the main activities are forestry and agriculture.

This indicates that some pesticides could already be contaminating subterranean waters used to supply the human population (Polanco-Rodríguez et al. 2018). The Zacualpan creek, one of the reference sites, supplies water to the conurbation of the municipalities of Colima and Villa de Álvarez and is considered the main source of potable water for both municipalities.

Some triazines and organophosphates have the capacity to move throughout the system of caverns and represent a risk of contamination for the aquifers (Lorenzo-Flores et al. 2017, Modrá et al. 2017). The results obtained not only revealed the presence of pesticides belonging to the groups of the triazines and organophosphates, but also pyrethroids, carbamates, thiocarbamates, benzimidazole, pyridine, and phosphoglycine, which not only increases the risk of the effects that these concentrations can have on the biota but also has the adverse effects on the other uses of the water from the springs, such as is human consumption.

Temporal and spatial distribution of pesticides in surface water

The analytes with the greatest frequency of detection during the dry season were malathion, glyphosate, and atrazine while, in the wet season, these were malathion, carbendazim, and glyphosate. Mean concentrations of ametrine, atrazine, carbofuran, malathion, picloram, and glyphosate were higher during the samplings conducted in the dry season compared to those of the wet season; however, carbendazim, diazinon, imazalil, λ -cyhalothrin, methomyl, molinate, 2,4-D, and thiabendazole presented the highest mean concentrations during the wet season. The highest concentration detected of ametrine during the dry

season was 18.115 $\mu\text{g/L}$, while in the wet season this was 50.917 $\mu\text{g/L}$ (Table III).

There are no temporal differences between the number of pesticides found in the study sites and the samplings ($P = 0.371$). These results differ from those presented by Masiá et al. (2013) since, in the case of the Ayuquila-Armería basin, the number of pesticides detected per sample over the entire period presented no temporal variation. The May 2018 sampling coincided with the period of least rainfall, which facilitated the process of pesticide concentration due to evaporation of the water and diminution of river flows. However, this same condition of low river flow facilitates greater contact between sunlight and the pesticides, causing even greater photolysis in the intertropical zones where the solar incidence angle is almost perpendicular, which could explain why the mean value for May was the lowest (Narváez-Valderrama et al. 2012).

The results of this study don't coincide with that indicated by Belenguer et al. (2014) and Deknock et al. (2019), since there were no differences between the number of pesticides detected in the samples with respect to the crops and vegetation types adjacent to each sampling site ($P = 0.271$). Nevertheless, the results coincide with those of Carazo-Rojas et al. (2018) in that the frequency and concentration of the pesticides are not a function of the areas of intensive

TABLE III. RANGE, MEAN AND DETECTION FREQUENCY OF PESTICIDES QUANTIFIED DURING THE DRY AND WET SEASONS IN THE SURFACE WATER OF THE AYUQUILA-ARMERÍA RIVER.

Analyte	Dry season			Wet season		
	Range ($\mu\text{g/L}$)	Mean ($\mu\text{g/L}$)	D.F. (%)	Range ($\mu\text{g/L}$)	Mean ($\mu\text{g/L}$)	D.F. (%)
2,4-D	LQ-0.771	0.12	31	LQ-0.806	0.16	40
Ametrine	LQ-18.115	0.96	60	LQ-0.708	0.20	62
Atrazine	LQ-2.587	0.60	67	LQ-0.904	0.31	55
Carbendazim	LQ-0.464	0.11	60	LQ-1.857	0.37	81
Carbofuran	LQ-0.248	0.15	6	LQ-0.120	0.08	6
Diazinon	LQ-4.560	0.47	29	LQ-10.076	1.00	28
Glyphosate	LQ-3.340	0.85	87	LQ-1.730	0.59	77
Imazalil	LQ-0.236	0.16	10	LQ-0.928	0.21	15
λ -cyhalothrin	LQ-0.336	0.10	31	LQ-50.917	3.30	34
Malathion	LQ-12.140	4.38	94	LQ-16.819	2.97	81
Methomyl	LQ-0.093	0.09	21	LQ-0.120	0.09	13
Molinate	LQ-0.397	0.16	42	LQ-0.369	0.21	28
Picloram	LQ-1.585	0.76	44	LQ-2.017	0.68	68
Thiabendazole	LQ-0.06	*	2	LQ-0.241	*	2

DF: detection frequency; LQ: 0.025 $\mu\text{g/L}$.

*Only one quantification.

agriculture, since the reference sites of the Manantlán creek and the La Taza and Zacualpan creek, where agricultural activity is low or null, presented concentrations and presence of pesticides that were similar to other sites located in the agricultural valleys. The Zacualpan creek presented a mean of five different pesticides during the sampling period, which is equal or greater than the number detected in the sites San Miguel, Tuxcacuesco, El Chical, Arroyo Seco, and Armería, which are all located in agricultural zones where pesticides are intensively applied.

The wide spatial distribution of pesticides in the study area is due to a lack of training in their application related to the dosage and the pesticide mixing, the use of equipment that is obsolete or in poor condition, and the absence of regulation of the sale and adequate management of the containers of these agrochemicals (Rodríguez-Aguilar et al. 2019). In addition, the highly diffuse characteristics of certain pesticides also favor their dispersion across most of the surface water of the Ayuquila-Armería River (Rasmussen et al. 2016).

Of the 10 pesticides included in the statistical analysis with the censored data in order to evaluate their temporal distribution, only atrazine, carbendazim, malathion and glyphosate presented significant differences per season (**Table IV**). These pesticides present different intrinsic characteristics that range from high to low water solubility and from a moderate to low octanol-water partition coefficient (K_{ow}). For these reasons, their residuality in the surface water must respond to other intrinsic aspects of the

pesticides, to the quantity or frequency of use, or to environmental factors within the study area.

Except for carbendazim, the pesticides malathion, atrazine, and glyphosate presented greater concentrations during the dry season, coinciding with the findings of Palma et al. (2014) and Papadakis et al. (2015) who indicated that periods of drought promote the concentration of pesticides due to reduced river flow. However, the dry season also coincides with the period of pesticide application in the sugarcane crop.

The results showed that the concentrations of carbendazim, diazinon, λ -cyhalothrin, malathion, molinate, picloram, and glyphosate in the surface water presented significant differences per sample (**Table IV**). The analytes that presented no differences per sample suggest that their wide temporal distribution could be the result of their frequent use in concentrations greater than those recommended, leading to their constant presence in the surface water, which coincides with that published by Carazo-Rojas et al. (2018). However, the results do not coincide with the findings of Belenguer et al. (2014), who indicate that the compounds that present the greatest persistence were those with relatively more stable values.

The analysis per sample provided more detail regarding the temporal behavior of the pesticides in the surface water of the Ayuquila-Armería river, compared to the analysis per season. The reduced concentrations of glyphosate, molinate, malathion, and diazinon during November could be a function of the increased frequency and intensity of hydrometeorological events due to the rainy season,

TABLE IV. RESULTS OF THE STATISTICAL TEST BY LEFT-CENSORED DATA ANALYSIS OF PESTICIDE CONCENTRATIONS PER SEASON AND SAMPLING IN THE SURFACE WATER OF THE AYUQUILA-ARMERÍA RIVER.

Analyte	Statistical test	Censorship percentage (%)	Season (p value)	Sampling (p value)
2,4-D	MLE	64.2	0.170	0.190
Ametrine	K-M	38.9	0.600	0.100
Atrazine	K-M	38.9	0.030*	0.060
Carbendazim	K-M	29.5	5×10^{-5} *	1×10^{-4} *
Diazinon	MLE	71.6	0.930	0.009*
Glyphosate	K-M	17.9	0.040*	$4e-10$ *
λ -cyhalothrin	MLE	67.4	0.42	3.8×10^{-8} *
Malathion	K-M	12.6	0.001*	2×10^{-14} *
Molinate	MLE	65.3	0.240	0.041*
Picloram	K-M	44.2	0.090	9×10^{-5} *

K-M: Kaplan-Meier, MLE: maximum likelihood estimation.

*Statistically significant.

facilitating the dilution of the analytes in the surface water, thus reducing the capacity for quantification (Carazo-Rojas et al. 2018).

Ecotoxicological risk assessment of pesticides in surface water

The results of the TU_{site} values evidenced that pesticide concentrations can be associated with acute toxic effects for *D. magna* in all the study sites. However, this does not mean that the sites present no live invertebrates, since the effect generated by a pesticide in live organisms is a function of the concentration to which they are exposed, the toxicity of the pesticide, and the species involved, which results in different responses to the presence of pesticides. This situation is even more complex when pesticides are found in combination due to the antagonistic, synergistic, and cumulative relationships involved in their toxicity (Hoffman 2002, Rand 2008).

According to the results, the sites El Grullo bridge, San Miguel, La Croix, and Tuxcacuesco presented

the highest TU_{site} values for *D. magna*, standing out for its high values in the three trophic levels of El Grullo bridge. These results don't coincide with those obtained by Ginebreda et al. (2014), Ccancapa et al. (2016), and Carazo-Rojas et al. (2018), since they show that the risk associated with the combination of pesticides is distributed in neither a diffuse nor specific manner, nor does it respond to the loss of ecological quality. This study shows that the processes of pesticide dispersion have led to the presence of these chemicals across most of the basin, regardless of the degree of conservation of the sites or their surrounding productive activities (**Table V**).

It is worth noting that most of the risk is caused by concentrations of malathion and λ -cyhalothrin. Excepting the concentrations of malathion and λ -cyhalothrin, only three sites could present a high potential risk (**Table V**). Of the three sites, Manantlán creek is the only one found in the SMBR and presents little agricultural activity; the high TU_{site} value is due to the concentration of diazinon, from the group of

TABLE V. TOXIC UNITS (TU) FOR SITES IN ALGAE, AQUATIC INVERTEBRATES AND FISHES BASED ON THE CONCENTRATIONS OF ALL PESTICIDES DETECTED (EXCLUDING THOSE OF MALATHION AND λ -CYHALOTHRIN) IN EACH OF THE SAMPLING SITES OF THE AYUQUILA-ARMERÍA RIVER.

Sites	All pesticides			Excluding malathion and λ -cyhalothrin		
	Algae	Aquatic invertebrates	Fishes	Algae	Aquatic invertebrates	Fishes
Corcovado	0.05	7.72	0.64	0.03	0.13	< 0.01
El Chacalito	0.1	4.55	0.79	0.07	0.65	< 0.01
El Grullo bridge	10.35	232.05	242.88	0.17	0.1	< 0.01
Autlán sewage discharge	0.27	15.16	0.59	0.27	0.14	< 0.01
Palo Blanco	0.24	8.71	0.67	0.22	0.15	< 0.01
El Grullo sewage discharge	5.11	7.29	0.92	5.08	0.63	0.01
El Aguacate	0.35	9.73	1.14	0.31	0.26	< 0.01
Manantlán creek	0.11	16.97	1.11	0.08	4.56	< 0.01
Zenzontla	0.31	6.46	0.25	0.31	0.12	< 0.01
La Taza spring	0.02	8.99	0.77	< 0.01	0.01	< 0.01
Ayuquila river	0.35	6.61	0.85	0.32	0.01	< 0.01
San Miguel	0.02	17.58	0.3	0.02	10.08	0.01
San Buenaventura	0.08	10.14	0.39	0.08	0.1	< 0.01
La Croix	0.1	24.63	1.24	0.09	0.33	0.01
Tuxcacuesco town	0.14	5.11	0.89	0.11	0.15	< 0.01
Tuxcacuesco river	0.08	17.5	2	0.02	< 0.01	< 0.01
Alse seca	0.22	11.68	0.87	0.2	0.26	< 0.01
Zacualpan creek	0.06	15.95	1.26	0.03	0.01	< 0.01
Zacualpan	0.07	10.73	0.82	0.05	0.2	0.01
El Chical	0.1	6.81	0.6	0.09	0.1	< 0.01
Arroyo Seco	0.3	10.85	0.82	0.28	0.77	0.01
Colima river	0.07	14.22	1.2	0.05	0.05	< 0.01
Asmoles sewage discharge	0.28	15.55	1.27	0.25	0.11	< 0.01
Armería	0.03	10.27	0.9	0.01	0.25	< 0.01

Numbers in bold denote high risk of acute effects.

the insecticides. This pesticide is highly toxic for aquatic invertebrates and could be used in different settings, such as agricultural, livestock production, and urban activities (EPA 2004). For this reason, its presence could be related to activities ongoing in the communities upstream of the sampling site, or to atmospheric transport and deposition with subsequent entry into the aquatic medium.

Malathion and λ -cyhalothrin have the characteristics of insecticides, which are authorized and recommended by the Mexican National Center of Preventive Programs and Disease Control (CENAPRECE, by its Spanish acronym) for the control of insect vectors of disease (CENAPRECE 2019). The dengue virus is a public health problem for the populations found within the Ayuquila-Armería basin, for which reason campaigns of control of the mosquito *Aedes aegypti* are common practice and are conducted through the spraying of insecticides in the urban zones (Anguiano-Moreno et al. 2011). The use of these chemicals in the control of vectors is a situation that contributes to the number of residues dispersed in the environment.

In most of the samples that presented high toxicity, this was due to the concentration of one or more compounds that contribute in greatest measure to the risk, and not to the set of pesticides in combination, which coincides with Stenström (2013). Regarding the TU_{site} results for algae, El Grullo bridge and El Grullo sewage discharge were the sites that presented a potentially high risk. In El Grullo bridge, the concentration of λ -cyhalothrin presented the greatest effect on the growth of algae *P. subcapitata*, while the herbicide ametrine had the highest impact on the growth of the algae *R. subcapitata* in El Grullo sewage discharge. El Grullo bridge, El Aguacate, Manantlán creek, La Croix, Tuxcacuesco, Zacualpan creek, Colima river, and the Asmoles sewage discharge were the study sites that presented the greatest potential acute risk in fish (*P. promelas* and *O. mykiss*), along with the concentrations of malathion and λ -cyhalothrin, making the greatest contribution to this risk.

The result of the statistical analysis to evaluate the temporal variation of the TU_{site} results per season through the Wilcoxon test showed that aquatic invertebrates are the only biotic component that presented differences ($P = 0.005$). The temporal analysis of the TU_{site} values per sampling, through the Kruskal-Wallis test, showed that all the biotic components (algae, $P = 0.015$; aquatic invertebrates, $P = 3.72 \times 10^{-8}$; fishes, $P = 0.002$) presented differences. These differences in the three components are due to the diminution of the risk values obtained during the samplings conducted in the wet season (September and November 2018).

The analytes ametrine, carbendazim, and λ -cyhalothrin are associated with a chronic risk ($RQ > 1$) in algae (*R. subcapitata*, *S. subspicatus*, and *P. subcapitata*, respectively), at both their maximum and mean concentrations. Carbofuran, diazinon, λ -cyhalothrin, malathion, and methomyl also represent a danger for *D. magna* in both their maximum and mean concentrations. Finally, the concentrations of carbendazim, carbofuran, imazalil, l-cyhalothrin, malathion, and thiabendazole presented RQ values of moderate to high risk in both their maximum and mean concentrations (Table VI). The similar concentrations of a wide variety of pesticides represent a high risk, which leads to a high probability of potential affectation in the biotic communities of the aquatic ecosystem, mainly due to concentrations of ametrine, carbendazim, carbofuran, l-cyhalothrin, and malathion (Ccanccapa et al. 2016, Carazo-Rojas et al. 2018).

Pesticide combination is common in the environment worldwide; however, the relationships that occur among them when in combination are generally unknown (Xie et al. 2019, De Souza et al. 2020). Organophosphate and carbamate concentrations can be managed using an additive approach since both are recognized as inhibitors of acetylcholinesterase; however, the effect of the mixture with other pesticide families remains largely unknown (Laetz et al. 2009).

Risk assessments of combinations of pesticides through an additive model of concentration would allow to determine the most problematic sites and evaluate the influence of concentrations of certain pesticides on the risk level, with the aim of directing actions for the regulation of the sale and use of these pesticides (Zhang et al. 2008). The results of the ecotoxicological risk assessment associated with concentrations of pesticides in the surface water of the Ayuquila-Armería basin should not be ignored, since the concentrations present are associated with both acute and chronic effects, mainly in the sites located within the valley of Autlán-El Grullo, in which there is a high potential of acute affectation at different trophic levels. Insecticides were found in greater measure with RQ values > 1 , compared to the values obtained for herbicides and fungicides, which coincides with the findings of Palma et al. (2014).

CONCLUSIONS

Pesticides in the water samples from the Ayuquila-Armería river are current-use, their intensive application, in both quantity and frequency, leads to

TABLE VI. RESULTS OF THE RISK ASSESSMENT BY RISK QUOTIENT (RQ) IN ALGAE, AQUATIC INVERTEBRATES AND FISHES.*

Pesticides	Chronic 96 h NOEC and 72 h EC ₅₀ in algae			Chronic 21 days NOEC and 48 h EC ₅₀ in aquatic invertebrates			Chronic 21 days NOEC in fishes		
	PNEC (µg/L)	RQ mean	RQ max	PNEC (µg/L)	RQ mean	RQ max	PNEC (µg/L)	RQ mean	RQ max
Ametrine	0.072	8.0	252	6.4	<0.1	2.8	14	<0.1	1.3
Atrazine	10	<0.1	0.3	25	<0.1	0.1	200	<0.1	<0.1
Carbendazim	0.003	85.3	619	154	<0.1	<0.1	0.064	4.0	29.0
Carbofuran	320	<0.1	<0.1	0.8	0.1	0.3	0.22	0.5	1.1
Diazinon	1000	<0.1	1.0	0.056	13.0	179.9	70	<0.1	0.1
Imazalil	8.7	<0.1	0.1	35	<0.1	<0.1	0.43	0.4	2.2
λ-cyhalothrin	0.5	3.5	102	0.0002	7964	231441	0.003	565.2	16424.8
Malathion	260	<0.1	<0.1	0.0012	3112	14016	1.82	2.1	9.2
Methomyl	2000	<0.1	<0.1	0.032	2.8	3.8	1.52	<0.1	<0.1
Picloram	1204	<0.1	<0.1	135.8	<0.1	<0.1	11	<0.1	0.2
Thiabendazole	320	<0.1	<0.1	4.2	<0.1	<0.1	1.2	0.1	0.2

*The mean and maximum RQ for each pesticide represent the general and the worst-case scenarios, respectively.

NOEC: non-observable effect concentration, EC: effective concentration, PNEC: predicted no-effect concentration.

Numbers in bold denote high potential risk of chronic effects; 2,4-D, glyphosate and molinate presented RQ values lower than 0.1 in the three trophic levels.

the presence of these compounds in combination, regardless of the season. However, concentrations of certain pesticides decreased during the November sampling in response to the general increase of the flow in the stretches of the river under study, with the consequent dilution effect.

Attention to the use and management of malathion, carbendazim, and glyphosate is important, given that they presented a frequency of detection of more than 70 % in the total samples. This suggests that their use is common and excessive in the basin, leading to the presence of their residues in most of the sites sampled.

This study used the model of concentration addition for the risk assessment per site. This procedure does not consider the antagonistic or synergistic relationships that can occur with a mixture of pesticides, but for practical terms, would allow determining the sites under greatest ecological risk. This is crucial in order to focus efforts on the regulation of certain pesticides and management measures for the protection of species associated with the aquatic ecosystem.

The results with the TU_{site} for the determination of potential acute risk showed a high risk for aquatic invertebrates (*D. magna*) in all the study sites, being the most sensitive group. Followed by the group of fish, the sites that presented a high risk were El Grullo bridge (*P. promelas*), El Aguacate, Manantlán creek, La Croix, Tuxcacuesco, Zacualpan creek, Colima

river and Asmoles sewage discharge (*P. promelas* and *O. mykiss*, both species in the previous sites). And finally, the least sensitive group was algae, being the sites El Grullo bridge (*P. Subcapitata*) and El Grullo sewage discharge (*R. subcapitata*) the ones that presented a high risk. The concentrations of malathion and λ-cyhalothrin influenced to the greatest extent the TU_{site} values for most of the study sites.

The use of RQ as a tool to determine chronic risk showed that all three trophic levels are potentially influenced by chronic effects, mainly due to the concentrations of ametrine, carbendazim, carbofuran, λ-cyhalothrin, and malathion. The use of TU_{site} and RQ to determine acute and chronic risk, respectively, are viable tools to evaluate the risk in different trophic levels, with the aim of protecting the integrity of an ecosystem and generating suitable information for natural resources managers and for the development of public policies.

Regulation of the importation, sale, and management of synthetic pesticides has become a substantial aspect in reducing the concentration of their residues in surface waters of the country's rivers. Special attention should be paid to malathion and λ-cyhalothrin, which generate the greatest influence on the associated risks in this study; however, it is possible that the reduction of these two pesticides alone will not resolve the situation of pesticides in the basin, because more active ingredients may be present, which generate greater impacts. For this

reason, it is recommended to generate a long-term monitoring plan for the surface waters, considering a greater number of pesticides that are currently used within the basin and were not considered in this study.

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