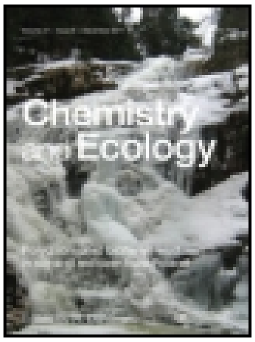

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RESEARCH ARTICLE

Ecotoxicological assessment of sediment from Texcalac River and agricultural soil of riverside area, in Tlaxcala, Mexico

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ABSTRACT

This study assesses the potential risk of Texcalac River and riparian area. The p,p′ DDT and ∑DDTs levels in agricultural soils (3.9–208.0 µg/kg) and in surface sediments (0.6–137 µg/kg) surpassing the guidelines for protection of aquatic life (75% > TEC). The ∑PCBs concentration oscillated from 135 to 93941 µg/kg; the half of sediments exceeded the international guidelines (PEL, PEC), as well as two soils (SQGE = 500 µg/kg). The TEQ concentration of four PCB-DL varied between 0.1 and 24.9 µg TEQ/kg, chiefly affected by PCB 169. Five sediments were lethal for *E. foetida*, two resulted to be cytotoxic and the 58% produced genotoxicity higher than negative control (A = 0.28 ± 0.05; DICA = 72.5 ± 16.9 au). Likewise, 31.6% of samples increased the micronuclei frequency in *V. faba* in comparison with negative control. The analytical data and the bioassays results suggest a significant and immediate risk to exposed biota of this region, highlighting a specific area in Texcalac River and other one in Sambrano Ravine. It is necessity assess the dispersion of pollutants and perform biomonitoring studies that display the exposure levels in biota with the goal to characterise the ecotoxicological risk.

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POPs; surface sediment; agricultural soil; *Eisenia foetida*; *Vicia faba*

1. Introduction

Persistent Organic Pollutants (POPs) are organic compounds that are resistant to environmental degradation, they are capable of bioaccumulate in fatty tissue of organisms and biomagnify in food chains, become environmentally hazardous substances. For 2017, 27 chemicals have been listed in the Stockholm Convention; 18 of these are targeted for elimination, 2 are restricted, and 7 are listed for reduction of unintentional production [1]. In the past, high amounts of DDT were used in agriculture and for malaria control in Mexico [2]. For their part, polychlorinated biphenyls (PCBs) were widely used in the industry, as refrigerants and lubricants in transformers, capacitors and other electrical

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equipment because they are not burn easily and are good insulators [3]. In spite of the restrictions on DDT and prohibition of PCBs, they have been reported in different environmental matrices all over the world; in water and riparian sediments [4–7] and in soils and sediments of wetlands [8]. The presence of organochlorine pollutants in environmental matrices has been associated with industrial activity and agricultural development, where the spraying of pesticides on crops is a common activity (7, 8). Although DDT and PCBs are obsolete pesticides, they have been reported as impurities in herbicides as Dicofol and 2,4 D, respectively, which are still used [9–11].

The sub-basin of Alto Atoyac originates in northern Tlaxcala; the mainstream is the Zahupan River which has an area of 1494 km^2 basin from its source to the confluence with the Atoyac River south of the state, occupying 52% of total surface of Tlaxcala [12]. The preliminary researches have reported significant levels of PCB, DDT, HCB and HCHs in surface sediments from Atoyac River and in the main stream of Zahuapan River [13] and in agricultural soils of the southern region of Tlaxcala [14]. However, tributaries of Zahuapan River have not been studied, Texcalac in one of them where the present study was focused. Texcalac River could be considered a tributary of potential risk by receiving wastewater discharges of two major industrial complexes; Industrial City-Xicohténcatl I (IC-Xicohténcatl) and Industrial Corridor-Xalostoc (IC-Xalostoc), where are placed chemical industries (2,4 D, mancozeb, chlorpyrifos and synthetic dyes), plastic and rubber, metal-mechanics and steel maker manufacturing, whose processes are potential unintentional emitters of polychlorinated biphenyl (PCB) [3,15]. In addition, receive surface runoff and sub-surface flow from the surrounding agricultural fields where organochlorides pesticides have been sprayed, as well as discharges from municipal wastewater.

On the other hand, for assessing the toxicity of complex mixtures of chemical agents present in environmental matrices, several organisms are employed as bioindicators, thereby complementing the analytical methods used to assess the ecotoxicological risk. *Eisenia foetida* (*E. foetida*) earthworm has been used as biological monitor to assess soil and sediment pollution at population level endpoint (survival) and individual level as cellular and molecular damage [16,17] and *Vicia faba* (*V. faba*) has proven to be an excellent test system as pollution bioindicator in water and solid environmental matrices, employing genotoxic biomarkers such as chromosomal aberrations and micronuclei [13,18].

Therefore, the current study aimed to assess the contamination status of DDT and PCBs in surface sediments and agricultural soils riparian of the Texcalac River, by determining analytically p,p′ DDT, p,p′ DDE, and 41 PCBs congeners and using *E. foetida* and *V. faba* as environmental stress bioindicators, providing baseline data for the level of pollutants in the riverine ecosystem that could be significantly valuable in terms of ecological risk.

2. Materials and methods

2.1. Study area

The Texcalac River extends northeast of the state of Tlaxcala, Mexico, between 19° 28′ and 19° 24′ north latitude and 98° 03′ and 98° 07′ west longitude, it originates from the water runoff running down the sides of La Malinche volcano. On its way, it flows through one side of IC-Xicohténcatl I, following a winding course that receives water from the San Cosme and Sambrano ravines; the latter also pass near to IC-Xalostoc. The Texcalac

River receives discharges of urban and industrial wastewater and runoffs from agricultural fields, where the use of diverse agrochemicals has been reported [19].

2.2. Environmental samples collection

Nineteen sampling sites were laid out using the Google Earth 2012 program and then physically located throughout the Texcalac River and tributaries. Twelve sediment samples and seven agricultural soil samples over riverside area were chosen and registered using Global Positioning System equipment (GARMIN 60Cx) (Figure 1).

Six sediment samples were taken in the Texcalac River: SS7, sited after receiving contribution of wastewater coming from IC-Xicothéncatl I; SS6, situated approximately 500 m from IC-Xicothéncatl I passing through a growing area; SS5 and SS4, located before and after the San Cosme Ravine connects to Texcalac River, respectively, this ravine receives urban wastewater discharges from the town of José López Portillo; SS3, placed after Sambrano River which collects the wastewater from IC-Xalostoc; SS1, positioned at the end of the study area, in Atenco River. Three sediment samples (SS9, SS10 and SS11) in Sambrano Ravine which collects the wastewater of IC-Xalostoc and SS8 in San Cosme Ravine that receives urban wastewater discharges from the town of José López Portillo. Two samples were selected as reference sites, both belong to the Alto Atoyac sub-basin where no wastewater discharges were observed: one sediment sample (SS12) was collected in La Mancera River from Emiliano Zapata municipality where the Texcalac River begins and other one (SS2) at El Ojito spring, which is located above the Texcalac River and provides drinking water to Apizaquito town.

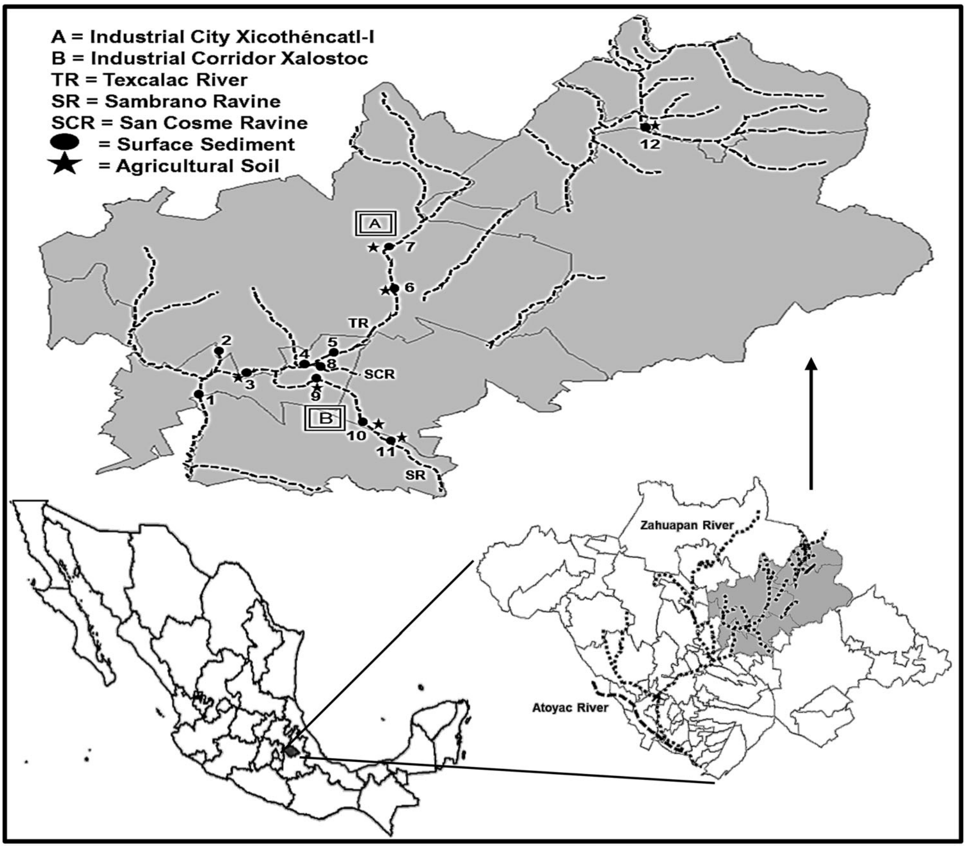
Surface sediment samples (0–10 cm depth) were collected on the river shore from each of the 12 stations. Simple samples were taken directly in amber glass bottle with help of a stainless-steel shovel, previously cleaned with a chromic mixture ($K_2Cr_2O_2 + H_2SO_4 + H_2O$).

With regard to agricultural soils, three samples were collected beside the Texcalac River (AS3, AS6 and AS7); other sample (AS12) was taken in Emiliano Zapata municipality, place considered as reference site, without application of pesticides; and three samples came from the riverside area of the Sambrano Ravine (AS9, AS10 and AS11) near to the chemistry industries of IC-Xalostoc to 1.5, 2.0 and 3.0 km, respectively. For each of seven stations 10 single soil samples (0–10 cm depth) were combined together into a composite sample, using a stainless-steel shovel.

All samples were transported to the laboratory in amber glass containers and oven-dried at 50–60°C. Then were ground in a ceramic mortar and the < 600 µm fraction was separated with a No. 30 sieve (ATSM E11). Finally, the samples were kept under refrigeration at 4°C until analysis.

2.3. Physicochemical properties

The sediments and agricultural soils were characterised according to Mexican standards [20]. Texture was determined using the Bouyoucos, hydrometer method. The pH was assessed in 1N KCl at a soil-to-KCl ratio of 1:2 (w/v) employing a Denver Instrument potentiometer, model 215. Organic matter (OC) was measured through the Walkley and Black method. Electrical conductivity (EC) was assessed in a saturation extract with the help of a multiparameter meter (HANNA instruments®/H1-9828, Woonsocket, RI, USA). Cationic exchange capacity (CEC) was evaluated through the titration method.



Surface sediment	Altitude (masl)	Latitude North	Longitude West	Agricultural soil	Altitude (masl)	Latitude North	Longitude West
SS1	2385	19°24’46.20”	98°07’21.80”	AS3	2406	19°25’05.92”	98°06’24.93’
SS2	2119	19°26’02.73”	98°07’02.68”	AS6	2469	19°27’29.27”	98°02’45.57”
SS3	2406	19°25’07.79”	98°06’23.47”	AS7	2511	19°28’56.10”	98°03’08.80”
SS4	2419	19°25’28.07”	98°05’25.97”	AS9	2433	19°24’47.20”	98°04’47.80”
SS5	2434	19°25’40.18”	98°04’29.80”	AS10	2478	19°23’22.67’	98°03’33.80”
SS6	2469	19°27’29.90”	98°02’43.80”	AS11	2496	19°22’59.80”	98°03’16.27”
SS7	2511	19°28’58.87”	98°03’08.27”	AS12	2517	19°32’28.50”	97°56’052.5”
SS8	2438	19°25’29.54”	98°04’46.17”				
SS9	2431	19°24’50.37”	98°04’49.75”				
SS10	2470	19°23’29.10”	98°03’42.50”				
SS11	2487	19°22’58.47”	98°03’17.68”				
SS12	2517	19°32’30.64”	97°56’51.80”				

Figure 1. Georeferenced samples locations of surface sediment on Texcalac River and agricultural soil on riverside area in Tlaxcala State.

2.4. POPs analysis

One gram of soil or sediment was placed in Green-Chem LEV glasses and 14 mL of dichloromethane were added. The extraction was performed using a microwave oven (CEM), the extract left to cool at 4°C, was filtered (0.45 µm), diluted with dichloromethane to 20 mL, evaporated to 4 mL, and hexane was added three times (4 mL). In addition, the extract was cleaned with Florisil columns (1000 mg/6 mL, Baker), and the compounds were eluted with 12 mL of 6% diethyl-ether:hexane. Then, was reduced with nitrogen gas flow to 0.5 mL and diluted with hexane to 1 mL. The analysis to separate and characterise 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p′ DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (p,p′ DDE), and 41 PCBs congeners (17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 156, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 201, 205, 206, 208, and 209), was conducted using a Hewlett Packard 6890 gas chromatograph coupled with a Hewlett Packard 5973 mass spectrometer. Analysis of Certified Reference Standards EC-2 'A Lake Ontario Blended Sediment for Toxic Organics' of National Water Research Institute, Canada, was conducted as quality control with recoveries of 78–114% for all tested analytes. The detection limit was 0.3 µg/L approximately [13].

2.5. Toxicity assay using E. foetida

2.5.1. Lethality assay using E. foetida

The assay followed the ASTM E 1676–4 method [21], with slight modifications. Two hundred grams of sediment or agricultural soil were placed into glass recipients and added 40 mL of distilled water. Adult organism of *E. foetida* (n = 10) with clitellum well developed and weighing between 300 and 600 mg, were incorporated in each recipient. The exposure was performed for 15 days under controlled conditions of temperature (22–28°C) and humidity (30–40%). The percentage of lethality was evaluated at the end of exposure, as a lack of response to a gentle mechanical stimulus. A soil with the following properties physicochemical: loam texture (sand 31.4%, silt 42.6%, clay 26%), pH = 7.2, OM = 0.54%, EC = 0.38 dS/m, CEC = 21.8 cmol(+)/kg, was employed as negative control (NC). The same soil contaminated with potassium dichromate at 0.8 mg/kg, was used as positive control for evaluate the *E. foetida* sensibility (lethality 14%, citotoxicity 0.89 ± 0.16 A, and genotoxicity 163.7 ± 20.4 au).

2.5.2. Eisenia foetida *coelomocytes extraction*

Six earthworms were randomly chosen from those that survived in each sample; three were used for each bioassay (cytotoxicity and genotoxicity). The digestive system was purged placing each earthworm on wet filter paper in Petri dishes during 24 h, then were placed in 15 mL test tubes (SARSTEDT 62.554.002, Nümbrecht, Germany) and fully submerged in 3 mL of extrusion solution (4.8% ethanol, 0.85% NaCl, and 0.25% EDTA) and incubated for 15 min at room temperature. Earthworms were removed and the coelomic fluid was diluted with 6 mL of phosphate buffer solution (PBS) at 4° C and centrifuged at 3500 rpm for 5 min, the pellet was resuspended in 100 µl of PBS at 4°C.

2.5.3. Citotoxicity assay using E. foetida

This assay provides an estimation of damage on the coelomocytes-Lysosomal membrane integrity and the flow control of supravital dye neutral red, which accumulates into lysosomal matrix of living cells. One hundred microliters of coelomic suspension were placed in a test tube, then 2 mL of neutral red dye (50 µg/mL) were added, and it was incubated at 22°C for 3 h. The dye was removed by centrifugation at 3500 rpm for 5 min and the pellet was resuspended with 1% acetic acid and 50% ethanol (3 mL). After 15 min at room temperature, the absorbance was determinated using a spectrophotometer (JENWAY 6715 UV/Vis, $\lambda = 540$ nm).

2.5.4. *Genotoxicity by comet assay using* E. foetida

As regards the comet assay, the version utilised was the alkaline method [22], with slight modifications. The coellomic suspension (100 µl) was mixed with an equal volume of low melting point agarose (0.5%). Then, an aliquot of the mixture (75 µl) was spread on microscope slides previously coated with regular agarose and were immersed in lysis solution (NaCl 2.5 N, EDTA 100 mM, Tris-base 10 mM, LSS 1%, DMSO 10% and Tritón X-100 1%, pH 10) during 24 h at 4°C. The microscope slides were placed in an electrophoretic chamber (Thermo EC Midicell EC330) containing alkaline buffer (NaOH 300 mM, EDTA 1 mM, pH > 13, 4°C) for 10 min, then a current electric was applied (12 V, 230 mA) employing a power source (Bio-Rad Model Power Pac Basic) during 10 min, finally they were neutralised (Tris-HCl 0.4 M, pH 7.5). The microscope slides were stained with 30 µl of ethidium bromide (0.02 mg/mL) just before being analysed under an epiflourescence microscope (LEICA DM 2000 Wetzlar, Germany), equipped with a mercury lamp (OSRAM HBO® 100 watts, Augsburg, Germany) and excitation filter (450–490 nm). One hundred DNA molecules per specimen were scored using a scale from zero to three according to the size of the tail or the extent of DNA damage [23]. The comets without head or visible nucleus were classified as ‘clouds’ and were not scored because they are associated with dead cells [24]. The Damage Index Comet Assay (DICA) value was calculated as the sum of the products of the DNA molecules number in each class multiplied by the class value [24]; thus, the DICA oscillated between 0 and 300 arbitrary units (au), according to Eq. 1

2.6. *Micronucleus assay with* V. faba

Seeds of *V. faba* were germinated between two layers of cotton moistened with water at room temperature; when the radicle was 2–3 cm in length, three seeds were placed in Petri dishes contained 50 g of sediment or agricultural soil previously moistened with distilled water (1:2 w/v), the radicle was fully covered into the sample for 6 and 18 h of recovery [13]. The frequency of micronucleus (MN) was determined in 1000 cells in interphase [25]; three experiments were carried independently for each sample. In order to obtain the mitotic index (MI), the same number of cells was evaluated but this time in interphases and mitosis. The MI percentage was calculated dividing the number of cells showing mitosis by the total number of cells and multiplying the result by 100. Distilled water was used as negative control.

2.7. *Hazard quotient*

Risk was characterised by comparing the concentration of each pollutant with the corresponding quality guidelines for protection of the biota. The guidelines applied were the Interim Freshwater Sediment Quality Guidelines (ISQG), Probable Effect Levels (PEL) and Soil Quality Guideline for Environmental Health (SQG_E) of agricultural use recommend by Canadian guidelines (Canadian Sediment Quality Guidelines for the protection of aquatic life [26], or Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health [27]; CSQG) besides the Threshold Effect Concentrations (TEC) and Probable Effect Concentrations (PEC) recommend by Consensus-Based Sediment Quality Guidelines (CBSQG) [28].

2.8. Statistical analysis

The results of the cytotoxicity and genotoxicity experiments with *E. foetida* presented a parametrical distribution (Saphiro-Wilk test, $p = .243$) and homoscedasticity (Equal variance test, $p = .290$). The arithmetic mean and standard deviation from three experiments performed independently for each exposure group were compared with the negative control by one-way analysis of variance (ANOVA) and the Dunnett's test with a level of significance of $p < .05$. The frequency of MN in *V. faba* presented a nonparametric distribution; therefore, statistical analysis was conducted with the median and quartile deviation (QD) as dispersion measure, applying Kruskal–Wallis one-way analysis of variance on ranks and multiple comparison versus negative control (Dunn's method, $p < .05$). All statistical analyses were performed using Sigma Plot version 11 (San Jose, CA, USA).

3. Results

3.1. Physicochemical properties of sediments and agricultural soils

Table 1 exposes the physicochemical characterisation. All samples showed high content of sand (80.4 ± 10.1%), except sediment SS11 (43.5%), the predominant textural class was the loamy sand. The sediments are considered to be neutral (pH = 6.7–7.4), except for the SS11, which along with the agricultural soils, are classified as moderately acids (pH = 5.4–6.6). With regard to the organic matter content, most of the samples presented a low value, but the sediment SS2 (12.9%) and SS6 (8.1%), displayed percentages, which are considered to be high and medium, respectively, for volcanic soils. All the agricultural soils and seven sediments showed negligible salinity values less than one; the rest of the sediments were classified from low to moderately saline. In accordance with the Mexican regulations (MOS, 2000), the sediment samples SS2 and SS11 showed high CEC values (31.4 and 38.6 cmol$^{(+)}$/kg), the remaining samples displayed low values (5.4–13.4 cmol$^{(+)}$/kg) and medium (16.8–20.7 cmol$^{(+)}$/kg).

3.2. Concentration of DDT and PCBs in sediments and agricultural soils

The p,p′ DDT (3.9–208.0 µg/kg) were detected in the seven soils analysed, while p,p′ DDE was found in the AS3, AS9 and AS12 soils, with a maximum value of 3.3 µg/kg and a DDT/DDE ratio of 14.4, 63.0 and 33.3, respectively (Table 2). The AS9 soil showed the greater concentration of $\sum$DDTs of 211.3 µg/kg (Table 2) with an HQ value less than the unity (Table 3).

The p,p′ DDT range in sediments (0.3–137.0 µg/kg) was greater than the p,p′ DDE range, the DDT/DDE ratio was superior to unity with the highest values for SS6 (375.0) and SS12 (109.0) (Table 2).

In the case of p,p′ DDT, the 91.6% of the sediments exceed the limit of 1.19 µg/kg (ISQG) and the 58.3% was above the PEL value of 4.77 µg/kg (Table 2), both limits established

Table 1. Physicochemical properties of surface sediment and agricultural soil from Texcalac River and riverside area.

SITE	Sand (%)	Silt (%)	Clay (%)	pH	OM (%)	EC (dS/m)	CEC (cmol$^{(+)}$/kg)
SS1	93.6	1.1	5.3	6.7	5.7	0.87	17.3
SS2	91.6	1.1	7.3	7.2	12.9	0.28	31.4
SS3	84.2	1.1	14.7	6.8	5.4	2.78	17.4
SS4	90.6	1.1	8.3	6.7	2.6	1.70	12.2
SS5	87.4	5.1	7.5	7.1	3.4	1.11	16.8
SS6	85.8	3.9	10.3	6.8	8.1	0.78	18.3
SS7	84.3	3.2	12.5	6.9	4.8	0.90	20.7
SS8	94.9	1.1	4.0	6.8	2.2	0.48	9.3
SS9	87.0	8.5	4.5	7.4	0.8	0.05	17.2
SS10	84.2	7.9	7.9	7.0	4.8	1.65	17.0
SS11	43.5	27.9	28.6	5.4	3.8	0.47	38.6
SS12	96.3	3.2	0.5	6.9	4.1	1.34	20.4
AS3	89.6	6.2	4.2	5.8	1.4	0.04	10.9
AS6	91.4	5.3	3.3	6.6	0.9	0.07	5.4
AS7	83.6	11.7	4.7	6.1	1.9	0.14	10.9
AS9	65.6	23.6	10.8	6.5	5.5	0.27	13.4
AS10	71.1	18.6	10.3	6.1	1.7	0.04	8.6
AS11	85.2	10.5	4.3	5.8	1.4	0.03	7.9
AS12	65.6	21.7	12.7	6.4	3.9	0.09	19.5

Note: Surface sediment (SS); agricultural soil (AS); organic matter (OM); electrical conductivity (EC); cationic exchange capacity (CEC).

by the Canadian SQG for the protection of aquatic life for total DDT [25]; when the concentrations are compared with the consensus based SQG [27] the 58.3% and 25% of the sediments are higher than TEC (4.16 μg/kg) and PEC (62.9 μg/kg). Meanwhile the p,p′ DDE concentrations in the 25.0% of the sediments are above of 1.42 and 3.16 μg/kg,

Table 2. Persistent organic pollutants levels in surface sediment of Texcalac River and agricultural soil of riverside area in Tlaxcala State.

SITE	Concentration (μg/kg)				
	p,p′ DDT	p,p′ DDE	DDT/DDE	∑DDTs	∑PCBs
SS1	8.5	nd	–	8.5	1412.0
SS2	1.4	5.4	0.3	6.8	201.8
SS3	1.7	0.5	3.4	2.2	4764.0
SS4	8.2	3.1	2.6	11.3	1660.0
SS5	1.4	4.7	0.3	6.1	325.6
SS6	112.5	0.3	375.0	112.8	32546.6
SS7	137.0	nd	–	137.0	241.5
SS8	9.3	0.3	31.0	9.6	24458.0
SS9	0.3	0.3	1.0	0.6	93941.3
SS10	2.2	nd	–	2.2	135.2
SS11	23.0	0.6	38.3	23.6	185.9
SS12	76.3	0.7	109.0	78.0	247.3
AS3	11.5	0.8	14.4	12.3	191.8
AS6	36.2	nd	–	36.2	313.0
AS7	3.9	nd	–	3.9	202.4
AS9	208.0	3.3	63.0	211.3	4077.0
AS10	11.8	nd	–	11.8	88022.1
AS11	6.5	nd	–	6.5	399.8
AS12	10.0	0.3	33.3	10.3	0.0

Notes: The consensus-based sediment quality guidelines: TEC, threshold effect concentrations (DDT = 4.16, DDE = 3.16, ∑DDTs = 5.28 and ∑PCBs = 59.8 μg/kg); PEC, probable effect concentrations (DDT = 62.9, DDE = 31.3, ∑DDTs = 572 and ∑PCBs = 676 μg/kg). The Canadian guidelines: ISQG, interim freshwater sediment quality guidelines (DDT = 1.19, DDE = 1.42 and ∑PCBs = 31.1 μg/kg); PEL, probable effect levels (DDT = 4.77, DDE = 6.75 and ∑PCBs = 277 μg/kg); SQG$_E$, soil quality guideline for environmental health of agricultural use (∑DDTs = 700 and ∑PCBs = 500 μg/kg).

Table 3. Hazard quotient of surface sediment and agricultural soil from Texcalac River and riverside area.

	p,p′ DDT				p,p′ DDE				$\sum$DDTs			$\sum$PCBs				
	HQ[a]		HQ[b]		HQ[a]		HQ[b]		HQ[a]		HQ[b]	HQ[a]		HQ[b]		
	TEC	PEC	ISQG	PEL	TEC	PEC	ISQG	PEL	TEC	PEC	SQG_E	TEC	PEC	ISQG	PEL	SQG_E
SS1	2	–	7	2	–	–	–	–	2	–		24	2	41	5	
SS2	–	–	1	–	2	–	4	–	1	–		3	–	6	–	
SS3	–	–	1	–	–	–	–	–	–	–		80	7	140	17	
SS4	2	–	7	2	1	–	2	–	2	–		28	3	49	6	
SS5	–	–	1	–	2	–	3	–	1	–		5	–	10	1	
SS6	27	2	95	24	–	–	–	–	21	–		544	48	954	118	
SS7	33	2	115	29	–	–	–	–	26	–		4	–	7	–	
SS8	2	–	8	2	–	–	–	–	2	–		409	36	717	88	
SS9	–	–	–	–	–	–	–	–	–	–		1571	139	2755	339	
SS10	–	–	2	–	–	–	–	–	–	–		2	–	4	–	
SS11	6	–	19	5	–	–	–	–	5	–		3	–	6	–	
SS12	18	1	64	16	–	–	–	–	15	–		4	–	7	–	
AS3											–					–
AS6											–					–
AS7											–					–
AS9											–					8
AS10											–					176
AS11											–					–
AS12											–					–

[a] Hazard quotient on the consensus-based sediment quality guidelines: TEC, threshold effect concentrations (DDT = 4.16, DDE = 3.16, $\sum$DDTs = 5.28 and $\sum$PCBs = 59.8 µg/kg); PEC, probable effect concentrations (DDT = 62.9, DDE = 31.3, $\sum$DDTs = 572 and $\sum$PCBs = 676 µg/kg).

[b] Hazard quotient based on the Canadian guidelines: ISQG, interim freshwater sediment quality guidelines (DDT = 1.19, DDE = 1.42 and $\sum$PCBs = 31.1 µg/kg); PEL, probable effect levels (DDT = 4.77, DDE = 6.75 and $\sum$PCBs = 277 µg/kg); SQG_E, soil quality guideline for environmental health of agricultural use ($\sum$DDTs = 700 and $\sum$PCBs = 500 µg/kg).

corresponding to the ISQG and TEC, respectively (Table 2). With respect to ∑DDTs concentrations, 75% of the sediments exceed the 5.28 µg/kg TEC limit (Table 2). In general, the HQ values for p,p′ DDT are higher than p,p′ DDE, 11 sediment samples show HQ values for p,p′ DDT ranged from 1 to 115 compared with the ISQG and only three samples exhibit HQ values slightly above the unity when they were compared with the PEC (Table 3).

With regard to PCBs, the AS12 soil were not detected levels of ∑PCBs, while in the SS12 sediment the concentration was 247.3 µg/kg, they were considered as reference samples for the Texcalac River area. The ∑PCBs levels in the samples AS11 and SS11, chosen as reference samples for the Sambrano Ravine, were 399.8 and 185.9 µg/kg, respectively (Table 2). The samples SS9 (93941.3 µg/kg), AS10 (88022.1 µg/kg), SS6 (32547.6 µg/kg) and SS8 (24458.0 µg/kg) presented the highest ∑PCBs levels (Figure 1 and Table 2), with HQ values based on the Canadian guidelines of 339, 176, 118 and 88, respectively (Table 3).

3.3. Profile distribution of 45 PCBs congeners in 4 selected samples

Figure 2 shows the PCBs congener profiles of the samples with the highest concentration. In Texcalac River was observed greater variability than in Sambrano Ravine, were recorded 36 of 45 congeners analysed. The homologs more abundant were the penta-PCBs and hexa-PCBs with concentrations up to 15804.0 and 13242.0 µg/kg in the SS6 sediment, in the case of the 82, 87, 95, 99, 101, 118, 128, 132, 138, and 151 congeners, were recorded concentrations above 1000 µg/kg (Figure 2(a)). In Sambrano Ravine, the concentration of penta-PCBs and hexa-PCBs homologs reached levels of 38047.0 µg/kg in SS9 and 52667.0 µg/kg in AS10. The congeners 44, 138 and 153 in the AS10 soil, and the congeners 99, 132 and 180 in the SS9 sediment, showed concentrations above 10000 µg/kg (Figure 2(b)).

The dioxin-like PCBs (PCBs-DL) have a spatial structure that is similar to dioxins, which is related with their toxicity by binding to aryl hydrocarbon receptor. Indeed, 12 PCB congeners (77, 81, 105, 114, 118,123, 126, 156, 157, 167, 169, and 189) are defined as such [3]. The congeners 105, 118, 156, and 169 were analysed and detected in 5 agricultural soils and 10 surface sediments samples with the higher concentrations in the SS6, SS8 and AS10 samples. In the Texcalac River sediments SS6 and SS8, the ∑PCBs-DL values correspond to 8.7% (2824.0 µg/kg) and 14.3% (3499.0 µg/kg) of total ∑PCBs, respectively (Figure 2 (a)), while the agricultural soil AS10 of Sambrano Ravine, the ∑PCBs-DL value (1949.0 µg/kg) correspond only to 2.2% of total ∑PCBs (Figure 2(b)).

3.4. Toxicity of sediments and agricultural soils in E. foetida

The sediments SS2, SS3, SS6, SS9 and SS10 showed a 100% of lethality followed by the SS4 sediment (80%) and the AS9 soil (40%), in the remaining samples did not occur lethality; therefore, the cytotoxicity (Figure 3(a)) and genotoxicity (Figure 3(b)) assays were conducted.

As seen in Figure 3(a), the AS6 and AS10 soils proved to be cytotoxic and statistically significant ($p < .05$); since they had an absorbance 5.6 times less than the NC (0.28 ± 0.05). With respect to the comet assay, the samples SS5, SS8, SS11, SS12, AS6, AS10, and

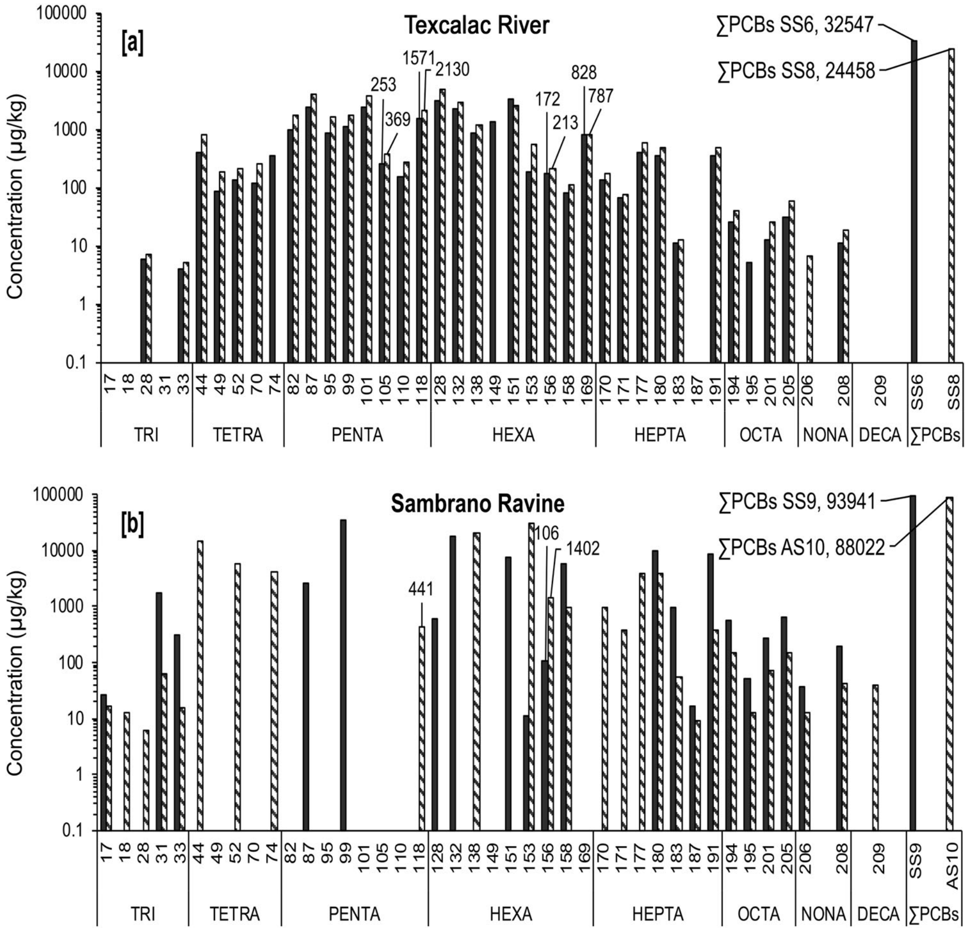


Figure 2. Polychlorinated biphenyls profile congeners of the samples with higher ∑PCBs levels from Texcalac River (a) and Sambrano Ravine (b). The consensus-based sediment quality guidelines.

Note: TEC, threshold effect concentrations (∑PCBs = 59.8 µg/kg); PEC, probable effect concentrations (∑PCBs = 676 µg/kg). The Canadian guidelines: ISQG, interim freshwater sediment quality guidelines (∑PCBs = 31.1 µg/kg and PCDD/PCDF = 0.85 ng TEQ/kg); PEL, probable effect levels (∑PCBs = 277 µg/kg and PCDD/PCDF = 21.5 ng TEQ/kg); SQGE, soil quality guideline for environmental health of agricultural use (∑PCBs = 500 µg/kg and PCDD/PCDF = 4 ng TEQ/kg).

AS11 proved to be genotoxic, with statistically significant differences regarding to the NC (69.9 ± 19.8 au), outstanding among these samples is the SS12 soil, with a DICA value of 142.9 ± 24.9 au (Figure 3(b)).

3.5. Genotoxicity of sediments and agricultural soils in **V. faba**

The MN assay results are shown in Figure 4. The MN frequency in *V. faba* exposed to sediments ranged between 9.0 ± 4.0 MN/1000 (SS7) and 41.5 ± 10.8 MN/1000 (SS10), while the agricultural soils ranged from 11.5 ± 2.5 MN/1000 (AS11) to 21.5 ± 6.5 MN/1000 (AS3). The sediments SS2, SS5, SS9, SS10, SS11 and the AS3 soil had a significant increase ($p < .05$) in the MN frequency with respect to NC (10.0 ± 1.6 MN/1000), no significant changes in mitotic index when compared to NC (9.7 ± 2.6%).

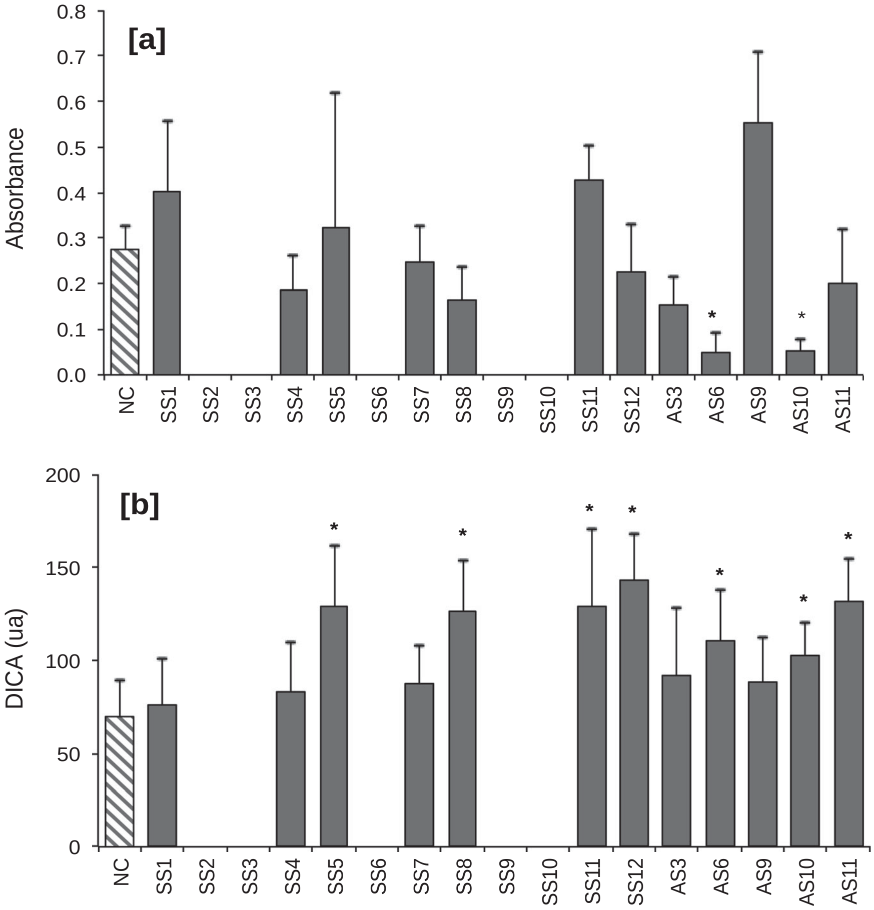


Figure 3. (a) Citotoxicity (absorbance mean ± SD) and (b) DNA damage (DICA mean ± SD) in coelomocytes of *E. foetida* following 15-day exposures to surface sediment (SS) and agricultural soil (AS) from Texcalac River and the riverside area in Tlaxcala State.

*Statistical differences with respect to negative control (NC), ANOVA and Dunnett's test $p < .05$.

4. Discussion

4.1. Relationship between the physicochemical properties and POPs environmental behavior

The results of the physicochemical characterisation of sediments and agricultural soils suggest a poor reservoir of nutrients, and a moderate degree of weathering. The low organic matter content and the loamy sand texture reduce the capacity of soils and sediments to adsorb POPs, thus favouring the mobility of these compounds to other environmental matrices and their bioavailable [3].

4.2. POPs environmental levels and ecotoxicological risk assessment

Due to its climatic conditions, the Tlaxcala State is not considered a malarial zone, for that reason the use of DDT was not as intense as in other regions of Mexico [29]. DDT degrades

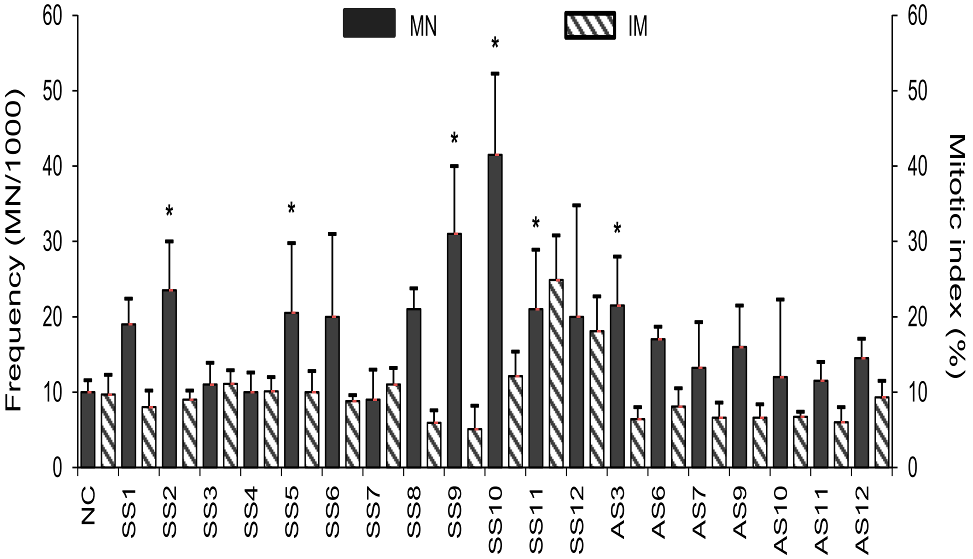


Figure 4. Frequency of micronucleus (MN) and mitotic index (MI) in *V. faba* following 6-h exposure to surface sediment (SS) and agricultural soil (AS) from Texcalac River and the riverside area in Tlaxcala State.

*Statistical differences with respect to negative control (NC), Kruskal-Wallis and Dunn's method, $p < .05$.

primarily to DDE under aerobic conditions and to DDD under anaerobic condition, DDE is more stable metabolite, therefore the DDT/DDE ratio can be used as a rough estimate of aged and degradation of DDT [30–32]. In the present study, the dominance of DDT in agricultural soils and sediments with a DDT/DDE ratio >1, may be attributed to recent input of DDT in the environment rather than historical usage, in addition to a slow degradation of DDT given the tepid weather in Tlaxcala [31,32].

The AS9 soil situated in the Sambrano Ravine showed the greater concentration of ∑DDTs; nevertheless, it does not exceed the limits set of 700 µg/kg recommended by the Canadian SQG for environmental health of agricultural use [27]. The ∑DDTs values (3.9–211.3 µg/kg; 41.8 ± 75.5 µg/kg) are similar to the reported in surface soils of malaria endemic areas in some states of Mexico; among others are Veracruz [33], Tabasco [34] and Chihuahua [35] with values ranged from nd to 49.9, 2.0–123.0 and 2.0–1417.0 µg/kg for total DDT, respectively. Likewise, the ∑DDTs levels are equivalent to those found in wetland surface soils of Pearl River Estuary from China (10.0–650.0 µg/kg) [36], but less than those reported for Chiapas ranged from nd to 26980.0 µg/kg [29], and Valle de Mayo in Sonora ranged from nd to 24100.0 µg/kg [37].

In the case of p,p′ DDT in surface sediment, special attention should be paid to the samples SS6, SS7 and SS12 (reference sediment) localised on the Texcalac River, with HQ values greater than the unity when are compared with the PEC, predicting sediment toxicity.

The ∑DDTs levels on sediment samples (0.6–137.0 µg/kg; 33.2 ± 47.9 µg/kg) are in accordance or slightly lower than the findings in the Coatzacoalcos River in the state of Veracruz, Mexico (73.3 ± 38.5 µg/kg) [4], in the Jiaomen River and Shawan River located in the Pearl River Delta, China (4.1–136.2 µg/kg) [38] and in the Chenab River (40.3 ±

26.2 μg/kg) [39] and River Ravi (76.9 ± 306.5 μg/kg), Pakistan [40]. The ∑DDTs concentrations are higher than found in the lagoon system Navachiste-Macapule, in Sinaloa, Mexico (2.9 ± 1.1 μg/kg) [41], in the Yellow River, China (0.05–5.03 μg/kg) [42] and in the Tiber River, Italy (0.28–3.86 μg/kg) [7]. However, those findings are lower than reported in sediments of Aiba Reservoir, Nigeria (460–7810.00 μg/kg) [31].

The ∑PCBs levels in the samples reference (nd–399.8 μg/kg) are similar to those found in soil at a mining site where the ∑PCBs drums were stored (nd–190.0 μg/kg), located in Zacatecas, Mexico [43] and in surface sediment/soil of three types of wetlands of the Pearl River Estuary (17.7–169.2.0 μg/kg) [8]. The ∑PCBs concentrations are between the ISQG and the PEL or consensus based SQG (TEC-PEC), they are considered to represent potential risks to exposed organisms and to predict toxicity occasionally.

All sediments exceeded the limits of Canadian SQG (ISQG = 34.1 μg/kg) and consensus based SQG (TEC = 59.8 μg/kg) for the protection of aquatic life and two agricultural soils are above the Canadian SQG for the environmental health (SQG_E = 500 μg/kg). The half of the sediment samples showed HQ values above the unity when were compared with the PEL (5–339) and PEC (2–139), they are considered to represent significant and immediate risks to exposed biota of this region.

Samples with ∑PCBs levels above 88000.0 μg/kg were collected from the Sambrano Ravine after passing by IC-Xalostoc, and the two remaining samples with the highest ∑PCBs levels between 24458.0 and 32547.6 μg/kg were obtained in the Texcalac River after passing close to IC-Xicothéncatl I and the San Cosme Ravine. Along the river course, the concentration gradually decreases; however, the SS1 sediment still displays high concentrations (1412.0 μg/kg). The results of current study are higher than the previously reported data on sediments samples of downstream of Zahuapan River (59.0–648.0 μg/kg) and in the stretch of the Atoyac River at south of the Tlaxcala State (82.0–1876.0 μg/kg) [13], the concentrations in the agricultural soils are comparable to that reported in Tepetitla, Tlaxcala (118.3–26982.9 μg/kg) [14].

To understand the magnitude of ∑PCBs pollution, the concentrations found in agricultural soils and sediments from the Texcalac River (135.2–93941.3 μg/kg) were compared with those from other sites in the world. The ∑PCBs concentrations were far higher than those presented in Chenab River, Pakistan (12.5–144.2 μg/kg) [44], in Tiber River, Italy (3.7–79.3 μg/kg) [7], in soil (78.6–1272.6 μg/kg) [33] and sediment (nd–12702.6 μg/kg) [4], both from the industrial zone of Coatzacoalcos and the river with the same name, where the main activity is the chemical and petrochemical industry. However, they were close to those reported in an urban tidal estuary from New Bedford Harbor, USA (2800.0–109 000.0 μg/kg) [45] and in soils nearby to a manufacturing industry of electrical capacitors in Alpuyeca, Morelos, Mexico (6.2–108460.6 μg/kg) [46].

The PCBs-DL are considered to have potentially negative effects in biota and human health (3). The ∑PCBs-DL concentrations in sediments (5.8–3499.0 μg/kg) and in agricultural soils (nd–1843.0 μg/kg), were similar to those measured in soils of Alpuyeca, Morelos, Mexico (0.5–4992.0 μg/kg) [46]. The toxic equivalents (TEQ) concept is widely used to assess the overall risk of a dioxin-like chemical mixture. The TEQ values were calculated with the four PCB-DL congeners, measured in this study, by using the WHO_{05}-TEQ [47]. The PCB 169 contributed significantly to the total calculated TEQ. The sites from Texcalac River provide a toxicity corresponding to 23.7 (SS6) and 24.9 μg TEQ/kg (SS8), while in Sambrano Ravine the toxicity was 0.1 μg TEQ/kg (AS10). The magnitude of the TEQs of four PCB-DL analysed was higher than found in wetlands sediment/soils of Pearl River Estuary, China (0.04×10^{-3}–8.23×10^{-1} µg TEQ/kg), where 11 PCB-DL were detected [8].

The three samples were exceeding the Canadian guideline for sediment (ISQG = 0.85 ng TEQ/kg, PEL = 21.5 ng TEQ/kg) [26] for the protection of aquatic life and the Canadian guideline for agricultural soil (SQG = 4 ng TEQ/kg) [27].

4.3. Potential risk of sediments and agricultural soils

Usually, in scenarios where multiple sources of pollution are identified, exists a complex mixture of chemical agents which interact with each other and with environmental particles; so that, the specific characteristics of each site influence the bioavailability of chemical agents and toxicity over ecological receptors at individual, cellular and molecular level [48].

Therefore, to predict the ecological risk of environmental matrices by using only the analytical techniques is a difficult task. The bioassays employing wildlife organisms or cultured in laboratory, are a valuable complement to gathering information about bioavailable and toxicity of the chemical agents mixture present in the environment.

At individual level, the assay with *E. foetida* revealed lethality in the 29.4% of samples; sediments were more lethal than agricultural soils. At cellular level, the 11.8% of the remaining samples were cytotoxic; the AS6 and AS10 agricultural soils located near to Texcalac River and Sambrano Ravine. At molecular level, the 41.2% of the remaining samples proved to be genotoxic; the comet assay with earthworms is a useful method to detect genotoxic agents [49]. Similar data were found in soils of Coatzacoalcos, Veracruz, Mexico, contaminated with a mixture of HCBs (520 µg/kg), HCHs (370 µg/kg) and $\sum$PCBs (600 µg/kg), the exposed group presented a greater DNA fragmentation (tail length = 9.7 ± 0.2 µm) in coelomocytes of *E. foetida* than control group (6.6 ± 0.1 µm) [16].

4.4. Potential genotoxic risk of sediments and agricultural soils

The MN assay is considered a good biomarker to identify clastogenic agents and variations in the mitotic index, which can be considered as indicator of cytotoxic damage [50,51]. The MN assay results with *V. faba* suggest the presence of clastogenic agents non-cytotoxic in sediments and agricultural soils.

The genotoxicity of agricultural soils was lower (from 11.5 ± 2.5–21.5 ± 6.5 MN/1000) than soils from the Tlaxcala State south area (from 14.8 ± 4.3–65 ± 18.2 MN/1000), but the DNA damage of *V. faba* exposed to the sediments (from 9.0 ± 4.0–41.5 ± 10.8 MN/1000) was similar to that reported in Zahuapan River and Atoyac River, in Tlaxcala (from 9.7 ± 2.1–31.3 ± 10 MN/1000) [13].

5. Conclusions

The results of this research have helped to identify two potential risk areas taking into consideration high PCBs levels and the bioassay results; the first, in Texcalac River (SS6 and SS8), and the second in Sambrano Ravine (SS9 and AS10). These sites could be acting as point sources of contamination for other environmental media assuming the slow degradation rates of these compounds in sediment. The high $\sum$PCBs levels in SS3, SS6 and SS9 sediments, match with the 100% lethality in *E. foetida*. The bioassays using *E. foetida* and *V. faba* were capable of identifying a latent risk in 50 and 70% of the samples analysed, respectively. The 25 and 58% of the sediment samples with concentrations of p,p′ DDT and ∑PCBs equal to or greater than PEC values, highlight potential risk to aquatic life.

It is stalwartly recommended that the study area should be asserted hazardous for activities such as livestock grazing, cultivation and irrigation with river water, until remediation measures are taken to safeguard ecological integrity.

Furthermore, areas with higher PCBs levels highlight the necessity of assess the dispersion of pollutants and perform biomonitoring studies that display the exposure levels in biota living in the study area with the goal to characterise the ecotoxicological risk.

Therefore, the data obtained in this study can be used by ecological decision makers in Tlaxcala to prepare intervention programs that diminish the risk in Texcalac River.

Disclosure statement

The authors declare that they have no conflict of interest .

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