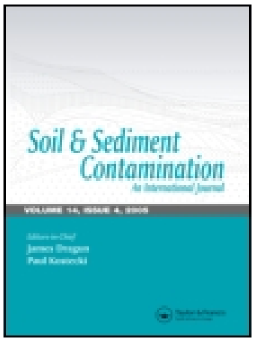

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Assessment of the Genotoxic Potential of Sediments Contaminated with POPs and Agricultural Soils Using *Vicia faba* Micronucleus Assay

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The aim of this study was to assess the levels of some persistent organic pollutants in the surface sediments from the Zahuapan and Atoyac rivers (Tlaxcala, Mexico), as well as to determine the genotoxic potential, by the micronucleus test in Vicia faba*, of the sediments and agricultural soils irrigated with water from these rivers. This document is the first study on the presence of POPs in surface sediments of the above-mentioned rivers; among the compounds analyzed are the HCH isomers, DDT and its metabolite DDE, HCB, mirex, aldrin, and 41 PCB congeners. The concentrations of HCB, ΣDDTs, ΣHCHs, and ΣPCBs ranged from 138–510, 45–450, 3–27, and 59–1876* μ*g kg*$^{-1}$ *dry weight, respectively. The highest levels of HCB, HCH isomers, and PCB congeners were found in the Atoyac River, and these compounds have the potential for causing an environmental impact. On the other hand, biological testing shows that both sediments and agricultural soils possess a genotoxic potential, given that the micronuclei frequency in* V. faba *is increased.*

Keywords Genotoxicity, PCB congeners, pesticides, Zahuapan and Atoyac rivers

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Introduction

The Zahuapan River and the stretch of the Atoyac River that runs within the state of Tlaxcala form part of the Alto Atoyac sub-basin, which has an area of 2031 km^2. The Zahuapan River originates from the Sierra de Tlaxco runoff in the north of the state at a height of 3418 m and 82.75 km long. The Atoyac River arises from the Sierra Nevada de Toluca, at a height of 4000 m from the north slope of the Iztaccíhuatl volcano. It flows into Tlaxcala in the southwest, in the municipality of Tepetitla de Lardizabal, forming a strip that runs almost parallel to the Tlaxcala/Puebla border until its confluence with the Zahuapan River in the municipality of Xicohtzinco (González, 2009).

Discharges of municipal wastewater into both of these rivers have affected them negatively. The environmental problem is aggravated by the liquid wastes discharged by the textile, chemical, and automobile industries located at Puebla and Tlaxcala.

Equally important are the probable pesticide inputs through the runoff from agricultural fields at the municipalities near to the rivers. It is likely that the uncontrolled use of agrochemicals in these zones creates a situation where those residues of pesticides are mixed with the water of both rivers, water which is used for irrigating several crops. In the municipalities of Tepetitla de Lardizabal and Nativitas, located in southern Tlaxcala, 6289 ha of land are used for growing corn, fodder oat, chard, coriander, broad bean, bean, onion, spinach, and pumpkin (COPLADET, 2009). There are almost no studies showing the presence of pollutants in the sediments from these rivers; certain works are focused on assessing the water pollution by the volatile organic compounds (Navarro *et al.*, 2004) and metals (García-Nieto *et al.*, 2011), as well as to evaluate the impact of these contaminants on agriculture soils (Méndez *et al.*, 2000).

Even though the use of persistent organic pollutants (POPs) has been considerably restricted, municipal and industrial effluents, the runoff from contaminated soils, lixiviates from dumpers, and atmospheric pollution continue to be the main sources of contamination for the aquatic environments (Weber *et al.*, 2008; Hong *et al.*, 2010). The fate and behavior of these contaminants in the aquatic systems are determined by the physical, chemical, and biological processes, such as photoxidation, hydrolisis, and breakdown, which transform POPs into their metabolites. These compounds are moved long distances and then accumulate in soils, sediments, and biological tissues (González-Mille *et al.*, 2010; Liu *et al.*, 2011). Sediments are an essential component of the freshwater ecosystems; the studies conducted in several countries, including Mexico, indicate that sediments are contaminated by pesticides and chlorinated biphenyls (PCB) (Echols *et al.*, 2008; González-Mille *et al.*, 2010), polycyclic aromatic hydrocarbon (PAH) (Echols *et al.*, 2008), dioxins, and furans (Wang and Lee, 2010), and almost invariably heavy metals (García-Nieto *et al.*, 2011).

Faced with that lack of information, we try to identify some POPs in surface sediments and characterize their distribution; also, we hope to locate potential point sources of pollution and therefore be able to predict relationships between these pollutants and agricultural soils, and also the consequences for this soil. The *Vicia faba* root tip micronucleus test (MN) is widely used for monitoring studies assessing pollution in soil, water, and sediments (Marcato-Romain *et al.*, 2009; Dong and Zhang, 2010). The use of analytical methods and genotoxicity bioassays will make it possible to determine the genotoxic potential of the surface sediments and agricultural soils in Tlaxcala.

Materials and Methods

Sampling Areas

In order to assess the presence of some POPs in the rainy season of 2008, surface sediment samples (1–5 cm) were taken directly in amber glass bottles [previously cleaned with a solution of $K_2Cr_2O_2 + H_2SO_4 + H_2O$ (chromic mixture)] at 11 sites in the Atoyac and Zahuapan rivers. These sites are Tlaxco (TL), the place where the Zahuapan River originates; it is considered to be a reference site due to the absence of apparent anthropogenic pollution; Atlangatepec (AT), a dam providing water for livestock and carp farming (*Cyprinus carpio*); Apizaco (AP), a tributary of the Zahuapan River, which receives discharges of domestic and industrial wastewaters, especially agrochemical industries. The following four places are located along the Zahuapan River: Atlihuetzia (AL), Apetatitlán (AE), Panotla (PA), and Xicohtzinco Zahuapan (XZ), while the places termed Xicohtzinco Atoyac (XA), Tepetitla de Lardizabal (TE), and Villalta (VI) are situated at the Atoyac River's main course; the last place, Papalotla (PP), is situated 100 m downstream from the point where the Zahuapan and Atoyac rivers join (Figure 1). The soil samples were collected in six agricultural fields impacted by the waters from these rivers. For each site 10 single samples were combined together into a composite sample. All sampling areas were referenced using a Global Positioning System (GPS) (Figure 1).

The soil and sediment samples were oven-dried at 40°C. Then the $<600\ \mu$m fraction was separated with a No. 30 sieve (ATSM E11), and the samples were kept refrigerated at 4°C until analysis.

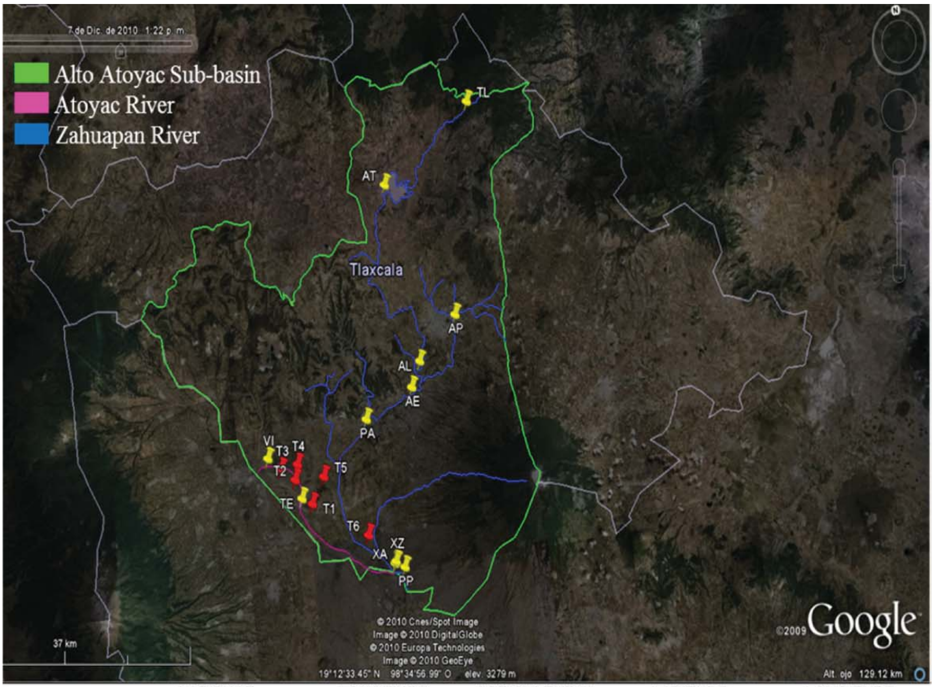
Physicochemical Properties of Soils and Sediments

The measurements of pH, organic matter (OM), total organic carbon (TOC), electric conductivity (EC), and texture were carried out according to the Mexican Official Standard NOM-021-SEMARNAT-2000.

POPs Analysis in Sediments

The sediment samples (1 g) were microwave-extracted in 14 ml of dichloromethane and 1ml of the internal standard (α-HCH-C^{13}, Endrin-C^{13} y PCB-141-C^{13}). After the extraction, the samples were evaporated to 0.2 ml with a gentle flow of nitrogen to 37°C, which in turn was re-suspended to 2 ml with hexane. Then, the extract was cleaned in a Florisil column (1000 mg/6 ml Phenomenex) and Silica:H_2SO_4 1:2 elusion was carried out utilizing 12 ml of 6% diethyl ether in hexane and concentrated to 1 ml by nitrogen current.

The analysis for separating and characterizing 41 PCB congeners: 18, 17, 31, 28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 151, 82, 149, 118, 153, 132, 105, 138, 158, 187, 183, 128, 177, 171, 156, 180, 191, 169, 170, 201, 208, 195, 194, 205, 206 and 209, as well as alpha (α-HCH), beta (β-HCH), gamma hexachlorocyclohexane (γ-HCH), hexachlorobenzene (HCB), mirex, aldrin 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), was carried out in a Hewlett Packard 6890 Series gas chromatograph (Hewlett Packard, Atlanta, GA equipped with an HP 6890 automatic injector and a Hewlett Packard 5973 mass spectrometer; helium was utilized as a carrier gas. All injections (2 μL) were made in split pulse mode onto a HP5-MS column, 60 m × 0.25 mm ID, 0.25-μm film thickness (J&W Scientific, Bellefonte, PA, USA). The injector temperature was set at 250°C. The GC temperature program was as follows: 100°C



SEDIMENT	LATITUDE NORTH	LONGITUDE WEST	AGRICULTURAL SOIL	LATITUDE NORTH	LONGITUDE WEST
TL	19° 37' 49.7"	98° 04' 35.3"	T1	19° 14' 00.4"	98° 20' 49.0"
AT	19° 33' 23.5"	98° 12' 08.2"	T2	19° 15' 41.0"	98° 22' 02.0"
AP	19° 24' 28.0"	98° 07' 25.4"	T3	19° 16' 30.0"	98° 23' 06.0"
AL	19° 21' 53.3"	98° 10' 45.0"	T4	19° 16' 38.0"	98° 21' 43.0"
AE	19° 20' 19.3"	98° 11' 37.9"	T5	19° 15' 36.0"	98° 19' 39.0"
PA	19° 18' 45.6"	98° 15' 40.0"	T6	19° 11' 34.4"	98° 17' 23.4"
XZ	19° 09' 33.5"	98° 14' 24.9"			
PP	19° 09' 7.2"	98° 13' 44.5"			
XA	19° 09' 27.1"	98° 14' 30.7"			
TE	19° 14' 26.3"	98° 21' 37.7"			
VI	19° 17'16.7"	98° 24' 06.7"			

Figure 1. GPS location of sampling sites (Color figure available online).

for 2 min, ramp 20°C/min to 200°C, hold for 0 min, then ramp 15°C/min to 310°C, hold for 5 min.

We used individual compounds (Ultra Scientific Analytical Solutions, North Kingstown, Rhode Island, USA) as quality control. The standards consisted of a 41 PCB mixture and 14 organochlorinated compounds. The mass spectrum was detected for the 55 individual compounds using SCAN mode for finding ions and retention time. Samples were analyzed using selective ion monitoring mode (SIM). The calibration curve was 2 to 100 ppb; in blank samples 50 ppb of the 55 compounds was added and we obtained recoveries of 82% to 115%; we used Certified Reference Standards EC-2 "A Lake Ontario Blended Sediment for Toxic Organics" of National Water Research Institute, Canada, obtaining an accuracy of 78% to 114% for 24 PCBs and 2 organochlorinated compounds.

Soils and Sediments Genotoxicity

The broad bean seeds (*V. faba*) were germinated between two layers of cotton moistened with water at room temperature; when the radicle was 2–3 cm long, two seeds were exposed to sediments or agricultural soil previously moistened with distilled water (1:2 w/v) for 6 and 24 h. Distilled water was used as negative control (NC). The staining was made according to Gómez-Arroyo and Villalobos-Pietrini (1995). The frequency of MN was determined in 1000 cells in interphase following the criteria described in Zalacain *et al.* (2005); three experiments were conducted independently for each exposure time. In order to obtain the mitotic index (MI), the same number of cells was evaluated but in interphases and mitosis. The percentage was calculated by dividing the number of cells in mitosis by the total number and multiplying the result by 100.

Statistical Analysis

The results of the genotoxicity experiments were explored for normality (Saphiro-Wilk) and homoscedasticity (Levene). The arithmetic mean from three experiments conducted independently for each exposure time was compared by a variance analysis (ANOVA) and the Dunnett's test with a level of significance of $p < 0.05$. Correlation analysis was done using a bivariate Pearson correlation coefficient to determine the relationships between the MN frequency and the levels of POPs in sediments. All statistical analyses were performed using SPSS software (version 8.0).

Results and Discussion

Physicochemical Properties of the Soil and Sediments

The values of the physicochemical properties measured in the sediments and agricultural soils (pH, OM, TOC, and EC) are shown in Table 1. According to the Mexican Official Standards on soils, the sediments AP, PA, and XZ, as well as the agricultural soils T5 and T6, are classified as neutral; the remainder fall within the moderately acid range. The organic matter content is very low in both matrices (in the case of volcanic soils, content lower than 4% is regarded as very low), which facilitates the POPs' biodegradation (ATSDR, 2004). As far as the electrical conductivity is concerned, the salinity effect of all the samples is considered to be negligible ($EC < 100\ dS\ cm^{-1}$), although agricultural soils show a value 10 times greater than that of the sediments.

Figure 2 shows the percentages of sand, silt, and clay in sediments and agricultural soils. The high-energy hydrodynamics of the Zahuapan and Atoyac environments during the rainy season contribute to the sandy texture of the sediments studied. Surface sediments showed a sand content ranging between 80% and 96.7%, except for sediment VI, which showed a 60.1 sand percentage. In general, the sand fraction of the sediments is higher than that of agricultural soils, which ranges between 16.6% and 78.6%. The texture class of the sediments and agricultural soils varies from sand to sandy loam, except for soils T1 and T4, which fall into the loam and clay loam classes, respectively, and T6, which falls into the silt clay loam.

The texture properties seem to have a direct effect on the POPs' adsorption capacity. The fine fractions of the sediments are prone to the retention/adsorption of the chemicals

Table 1
Physicochemical properties of sediments and agricultural soils

SITE	pH	OM (%)	TOC (%)	EC (dS cm^{-1})
TL	6.25	3.77	2.18	8.8
AT	6.87	1.61	0.94	3.1
AP	7.32	1.88	1.09	3.3
AL	6.55	1.61	0.94	2.7
AE	6.81	2.15	1.25	3.4
PA	7.24	5.38	3.12	4.2
XZ	7.23	1.61	0.94	2.6
PP	6.82	1.88	1.09	3.7
XA	6.62	1.34	0.78	2.6
TE	6.92	1.35	0.78	4.5
VI	6.86	2.96	1.72	2.4
T1	5.22	1.75	1.01	15.4
T2	5.44	1.35	0.78	23.1
T3	5.96	2.55	1.48	58.4
T4	6.18	1.61	0.94	29.5
T5	7.12	0.40	0.23	47.2
T6	7.20	2.02	1.17	30.8

Superficial sediment samples of Zahuapan and Atoyac rivers: Tlaxco (TL); Atlangatepec (AT); Apizaco (AP); Atlihuetzia (AL); Apetatitlán (AE); Panotla (PA); Xicohtzinco Zahuapan (XZ); Papalotla (PP); Xicohtzinco Atoyac (XA); Tepetitla de Lardizabal (TE); Villalta (VI). Agricultural soils composite samples of Tepetitla de Lardizabal: T1-T6. Organic matter (MO); total organic carbon (TOC); electric conductivity (EC).

from anthropogenic sources, and diminish the POPs breakdown (ATSDR, 2004). In sediments, clay and slit were found in low percentages since values are within the ranges 1.8–12% and 0.4–16.6%, respectively; however, POPs' concentrations in this matrix are substantial.

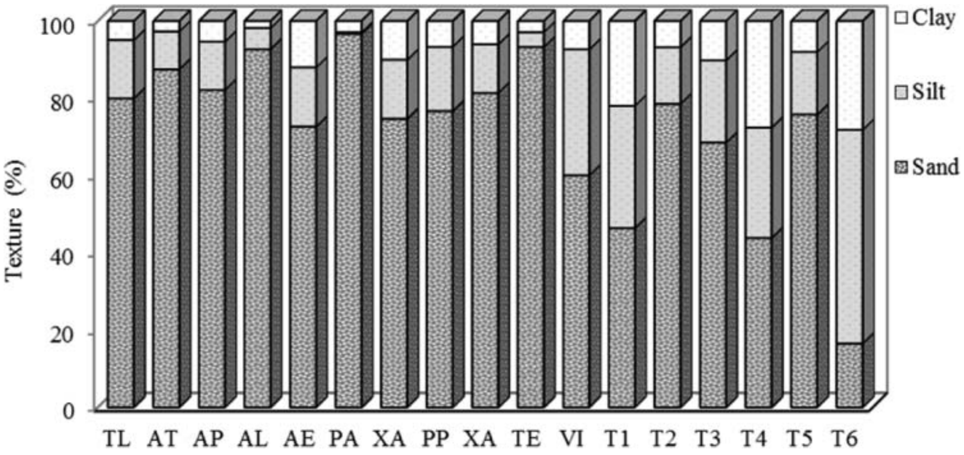


Figure 2. Percentage of texture of sediments and agricultural soils.

Table 2

Levels of POPs (μg/kg) in sediments of Zahuapan and Atoyac rivers

SITE	HCB	DDT	DDE	ΣDDT	αHCH	βHCH	γHCH	ΣHCH	ΣPCB
TL	165	18	4	22	155	15	50	220	284
AT	175	nd	3	3	214	31	91	336	80
AP	87	nd	4.5	4.5	315	nd	110	425	167
AL	153	nd	4	4	300	60	36	396	403
AE	115	19	8	27	150	nd	122	272	124
PA	355	nd	10	10	115	30	46	191	59
XZ	91	nd	5	5	180	24	16	220	64
PP	450	nd	nd	nd	410	nd	100	510	648
XA	353	nd	nd	nd	251	nd	22	273	1876
TE	45	nd	3	3	98	nd	40	138	89
VI	45	nd	6	6	170	38	51	259	82
Mean	184.9	18.5	5.3	7.7	214.4	33.0	62.2	294.5	352.4
SD	138.4	0.7	2.4	8.8	95.6	15.3	36.9	111.4	537.4
[a]LEL	20	8	5	7	6	5	3	—	70
[b]ISQG	—	1.19	1.42	—	—	—	0.94	—	34.1

Tlaxco (TL); Atlangatepec (AT); Apizaco (AP); Atlihuetzia (AL); Apetatitlán (AE); Panotla (PA); Xicohtzinco Zahuapan (XZ); Papalotla (PP); Xicohtzinco Atoyac (XA); Tepetitla de Lardizabal (TE); Villalta (VI). [a]Lowest Effects Level (Guidance for Sediment Quality Evaluations, New Jersey Department of Environmental Protection, 1998). [b]Interim Sediment Quality Guidelines (Canadian Sediment Quality Guidelines for the Protection of Aquatic Life, 2002). Detection limit = ranged from 0.6 to 2 μg/kg; nd = not detectable. SD = standard deviation.

POPs Analysis in Sediments

The concentrations of individually resolved peaks were summed to obtain total concentrations of PCB congeners (ΣPCBs), *p,p’* DDT and *p,p’* DDE (ΣDDTs), and HCH isomers (ΣHCHs), and thus compare the results with the sediment quality guidelines (Table 2).

The distribution of HCB, ΣDDTs, ΣHCHs, and ΣPCBs in sediment samples shows a wide variation among sites (Table 2), while aldrin and mirex were not detected in either of the assessed sites. The levels of the POPs oscillated between 45 to 450 μg/kg of HCB, not detectable (nd) to 27 μg/kg of ΣDDTs, from 138 to 510 μg/kg of ΣHCHs and from 59 to 1876 μg/kg of ΣPCBs, indicating an extreme heterogeneity in the distribution pattern.

The HCB was introduced in 1945 as a fungicide for cereal seeds treatment; currently, it is the main byproduct from the production of a great number of chlorine compounds (ATSDR, 2002). The presence of HCB in the area studied is perhaps caused by the use of pesticides and industrial activity. The total of the analyzed samples showed HCB levels higher than 20 μg/kg (ISQG, 2002); higher levels were found in the sites XA, PA, and PP. These values are similar to those in other Mexican rivers considered highly contaminated (González-Mille *et al.*, 2010).

Because of its climatic conditions, the state of Tlaxcala was not considered as a malaria zone, and consequently DDT was not used in as copious quantities as in other regions of Mexico (Díaz-Barriga *et al.*, 2002; Yáñez *et al.*, 2002). However, some farmers mention its use in the past for fighting agricultural pests. Considering that in this case its metabolite, DDE, showed a larger distribution, it is likely that the DDT found within the study area is

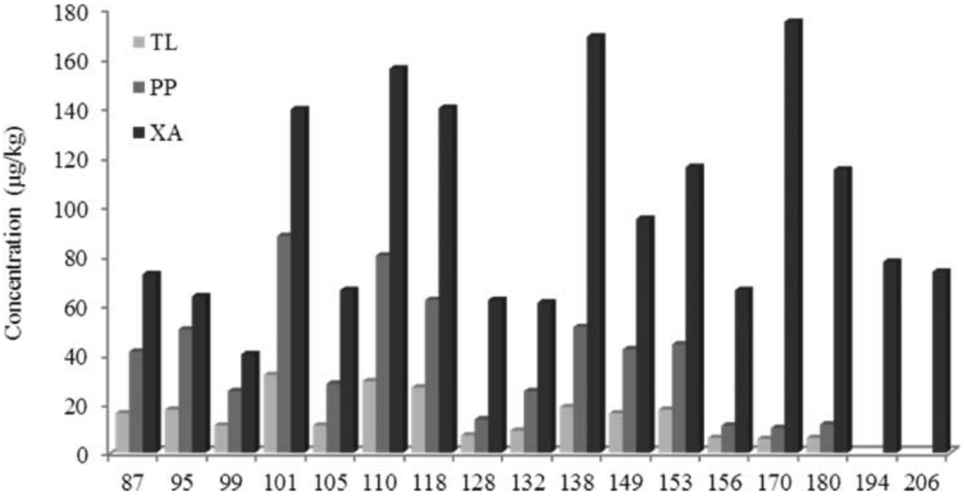


Figure 3. Congener profiles of polychlorinated biphenyls in sediments.

residual. In nine out of the 11 sites, DDE was identified (mean = 5.4 μg/kg), but DDT was only detected in two (18 and 19 μg/kg). These values are threefold lower than those found in sediments from a malaria-endemic zone; despite this, the levels exceed the Canadian standards for the protection of aquatic life (ISQG = 1.19 μg/kg).

The average concentrations of the αHCH, βHCH, and γHCH (lindane) displayed in Table 2 are 69.3%, 10.7% and 20.0%, which is the formulation of the technical-grade HCH [$\alpha HCH_{(53–70\%)}$, $\beta HCH_{(3–14\%)}$, $\gamma HCH_{(11–18\%)}$, $\delta HCH_{(6–10\%)}$ and $\varepsilon HCH_{(3–5\%)}$] (INE, 2004). The ratio αHCH/γHCH for the technical-grade HCH was 5, and in this work it ranged from 1 to 11 with a mean of 4.8. In environmental conditions, under ultraviolet light, lindane is converted by bioisomerization into the more stable isomer αHCH (Steinwandter, 1976). Both the high level of αHCH in the technical-grade HCH and the environmental transformation of γHCH into αHCH might be the reasons for its abundance in the sediments (Senoo and Wada, 1989; Liu *et al.*, 2011). Lindane has been widely utilized for controlling pests in crops and livestock, treating seeds and soils, foliar applications in fruit crops, and wood protection (USEPA, 2000). For this reason, it is possible that runoff from agricultural lands might contribute to the HCH build-up in sediments.

The sediments from sites XA and PP showed the higher PCB levels (Table 2). Their concentrations are up to nine times higher than those encountered in surface sediments from the Missouri River (Echols *et al.*, 2008), Baiyangdian Lake (Hu *et al.*, 2010), and Haihe River (Zhao *et al.*, 2010), but lower than the values reported in the Coatzacoalcos River by González-Mille *et al.* (2010). Thirty-seven of the 41 congeners analyzed were found in the sediments from the sites studied, and only four (PCB 17, PCB 169, PCB 208, and PCB 209) were not found in such sites. The congeners predominating in the two most contaminated sites and reference site (Figure 3) belong to the Penta-PCB (87, 95, 99, 101, 105, 110, 118), Hexa-PCB (128, 132, 138, 149, 153, 156), and Hepta-PCB (170, 180) groups. The Octa-PCB 194 and 206 were identified only in sediment XA. The profile of PCB congeners is similar to those found in the Coatzacoalcos River (González-Mille *et al.,* 2010). The penta-, hexa-, and hepta-, PCB are both bioavailable and resistant to degradation and these PCB homologs bioaccumulate in the organism to the greatest extent (ATSDR, 2004).

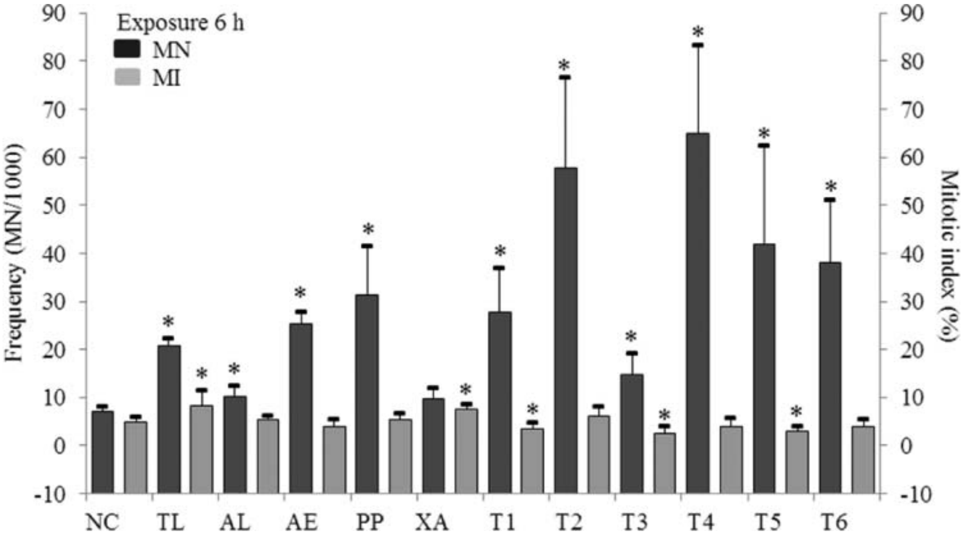


Figure 4. Micronucleus frequency and percentage of mitotic index in *Vicia faba* roots tips after 6 h of exposure to sediments and agricultural soils.

What is surprising are the POPs levels found in the site TL, especially PCB congeners (Figure 3), within which it is not possible to identify point sources of pollution, and which at the start of the work was considered a reference site. Perhaps this presence is the result of the long-distance atmospheric transport from other parts of Mexico through Eolic and wet deposition.

In the state of Tlaxcala, agriculture and industry are the major threats to the contamination of the Zahuapan and Atoyac rivers. Studies have been carried out reporting the presence of toluene, chloroform, methylene chloride, heavy metal traces, and pesticides. Our study showed that the levels of POPs exceed the international standards on sediment quality for aquatic life protection.

The sub-basin receives directly or through its tributaries urban and industrial wastewater running over agricultural areas. Also, in the vicinity of the sub-basin there are lands cultivated with vegetable crops, such as chard, cauliflower, lettuce, tubers, radish, and broad bean, irrigated with river water. Food such as crop vegetables, tubers, and fresh fruits contain a larger amount of pesticide residues (Schinas *et al.*, 2000; Fenske *et al.*, 2002; Moysich *et al.*, 2002; Gebara *et al.*, 2011; Kobayashi *et al.*, 2011).

Soils and Sediments Genotoxicity

Results of the MN test reflect the genotoxic capacity of the sediments and agricultural soils. All of the samples showed an increase in the frequency of MN by comparison with the negative control, both at 6 and 24 h exposures (Figures 4 and 5), except for the XA sediment. The MN assay is an efficient technique for evaluating damage caused by some genotoxic agents to DNA. For this reason, it can be considered a good effect biomarker in biomonitoring studies for detecting exposure to clastogenic agents (Marcato-Romain *et al.*, 2009). These agents induce DNA strand breakages, which are transformed in mutations, and for acting before or after the synthesis phase of the cell cycle are classified as S-dependent or S-independent (Loza *et al.*, 2005).

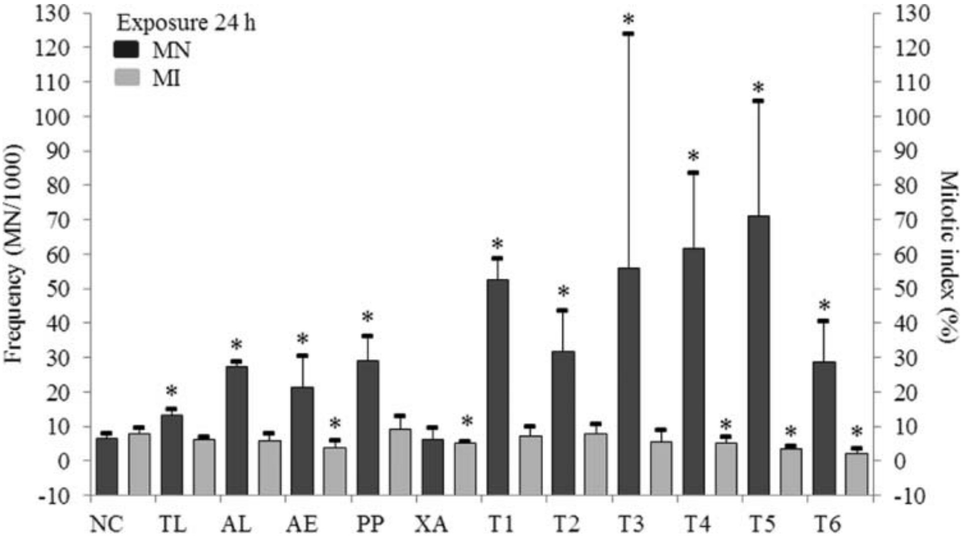


Figure 5. Micronucleus frequency and percentage of mitotic index in *Vicia faba* roots tips after 24 h of exposure to sediments and agricultural soils.

Even though the results show that the soils and sediments are genotoxic, the simple correlations of Pearson did not show correlation of the MN frequency in sediments with any of the analyzed contaminants. Studies under laboratory-controlled conditions for assessing the genotoxic capacity of single POPs have showed undoubtedly that DDT (Ennaceur *et al.*, 2008; Canales-Aguirre *et al.*, 2011) and the HCH isomers (Mattioli *et al.*,1996; Anguiano *et al.*, 2007) possess this capacity, while the HCB is considered to be a weak genotoxic (Canonero *et al.*, 1997), or non-genotoxic (Brusick 1986; Siekel *et al.*, 1991; Ennaceur *et al.*, 2008). In the case of PCB toxicity, mechanisms depend on the congener; some are regarded as non-genotoxic carcinogens (Knerr and Schrenk, 2006) and others as genotoxics (Jacobus *et al.*, 2010) with an S-dependent mechanism (Nagayama *et al.*, 1999). In environmental samples, contaminant mixtures make it difficult to establish a dose-response relationship. Agricultural soils contaminated with PAH, PCB, and metals showed an increase in the frequency of MN, but there was no correlation between the effect and concentration of the analyzed contaminants (Song *et al.*, 2006). In evaluating the genotoxicity of soils exposed to DDT, Loza *et al.* (2005) found only slightly significant results.

In this test system, an increase in the MN frequency reveals the genotoxic potential of both the soils and sediments, and in turn this increase assumes the presence of chemicals harmful to DNA, which are mutagens and potential carcinogens (Loza *et al.*, 2005; Song *et al.*, 2006; Dong and Zhang, 2010). Moreover, both samples TL, XA, T1, T3, and T5 with an exposure for 6 h (Figure 4) and samples AE, XA, T4, T5, and T6 with 24 h of exposure (Figure 5) showed a significant difference in the% MI with regard to the negative control. Some reports indicate that the chromosomal disorders cause direct cell death (Leme and Marin-Morales, 2009; Kasurka *et al.*, 2011); because of this, changes in the mitotic index may be regarded as indicators of cytotoxic damage.

Conclusions

POPs found in sediments constitute a potential risk to human health and the environment. Their concentrations exceed the international guidelines for the protection of aquatic life.

The low OM content and sandy texture prevailing in these samples favor POPs mobilization to other environments. In the vicinity of the rivers studied, it is common to see cattle and goats grazing; similarly, there are areas where fish farming is practiced, as well as irrigation canals conveying water from this hydrologic system; consequently meat and vegetables produced in the zone could be an important exposure route to POPs in humans.

Apparently, the magnitude and distribution of the POPs along the hydrological system are evidence of the incorporation of PCB and HCB from point sources as a result of anthropogenic activities, especially in the case of Atoyac River. However, as far as the rest of POPs analyzed are concerned, anthropogenic inputs show a diffuse pattern related to a generalized environmental pollution, perhaps as a consequence of the great scale atmospheric transport.

Given that they are complex mixtures, it is difficult to prove that there is a relationship between the contaminants found in an environmental sample and damage to an organism. Nevertheless, the MN assay in *V. faba* has demonstrated that agricultural soils have a great genotoxic capacity. Even though initially we thought it was quite possible that water in the hydrological system utilized for irrigating crops (whose sediments have high levels of POPs) increased the genotoxicity of the agricultural soils, from our results the opposite turns out to be true. The pesticide residues found in the agricultural areas and attaining the rivers washed away by surface runoff are likely to be the contributors to the genotoxic capacity of sediments.

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