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Original article

Presence of arsenic and potentially toxic metals (Cd, Cr, Pb) in water and soil of the NE shore of Chapala Lake, Mexico, and its genotoxic effect in the edible chayote fruit (*Sechium edule* (Jacq.) Sw.)

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Summary

The presence of potentially toxic elements in waters used for agricultural irrigation is a latent problem in Lake Chapala, Mexico. Four sites in the polluted area of Chapala lake were studied in this research. Samples of irrigation water and soil were collected from each site in both the dry (DS) and rainy (RS) seasons. Arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) were measured in all the samples. Comet assay was used to measure the migration of DNA from the nucleus in order to detect genotoxicity in fruits. ISSR molecular markers were used to assess the detection power of this marker in correspondence with amounts of metals in the samples. The results show a variation of metal concentrations in the DS and RS soil and water samples. The metalloid As in DS water samples exceeded the permissible limits in three of the sampling areas. Cd and Pb exceeded the prescribed limits from collected water in three of the sampling areas in RS. Cd exceeded the maximum permissible limits in soil collected during both DS and RS in the four sampling sites. Data on the concentration of metals shows significant differences between the sampling areas and the control site. Genetic damage (DNA migration from the nuclei) was found in the chayote fruit and the major migration of DNA was associated principally with the presence of As in DS water samples as well as Pb in soil in RS.

Keywords
comet assay, DNA damage, ISSR marker

Significance of this study

What is already known on this subject?

- Plants absorb elements from soil, some of which are known to be toxic at low concentrations. Arsenic, cadmium, chromium, and lead affect metabolic processes in plants at different levels.

What are the new findings?

- This study shows actual concentrations of As, Cd, Cr and Pb in Lake Chapala and its genotoxic effect in chayote, an important edible plant which is produced on the shore of the lake and distributed all over the country. This work also assesses the concentration of metals in rainy and dry seasons.

What is the expected impact on horticulture?

- The information obtained offers data that can be used in the study of toxic metal accumulation in other vegetables that are produced in the same area, as well as contaminated areas in other lakes. Likewise, it can encourage the planning of studies related to the effect of the intake of these products on the human being.

Introduction

Lake Chapala is by size the third-largest lake in Latin America and the largest in Mexico. The surrounding villages represent more than 16 million people and support an important part of agricultural and industrial activities in Mexico. On the shores of Lake Chapala, vegetables such as chayote (*Sechium edule*) are cultivated and irrigated with water from the lake. Chayote has been cultivated since prehispanic times and its cultivation has spread worldwide from its origin in southern Mexico and Central America. The fruit, when physiologically mature, is the main source of chayote consumption (Ibarra-Manríquez et al., 1998). The presence of diverse metals in the water used to irrigate agricultural crops, as well as their accumulation in soil and crops, can produce a genotoxic affectation that limits adequate development during the plant's vegetative cycle, as well as the yield and nutritional quality of the crop (Maleki et al., 2017). Pollutants predominantly of industrial and agricultural origin can generate cellular stress and cause damage at different levels, including DNA (Moura et al., 2012; Gjorgieva et al., 2013; Siddiqui, 2015). Given that Lake Chapala is endangered (Hansen and Maya, 1997; Rosales-Hoz et al., 2000; Jay and Ford, 2001; Global Nature Fund, 2009; Ontiveros-Cuadras et al., 2014), the potential conflict over regional water resources is high due to overexploitation and contamination. The presence of pollutants such as arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) has been reported in Lake Chapala (Shine et al., 1998; Trujillo-Cárdenas et al., 2010; Badillo-Camacho et al., 2016). Genotoxicity is a term in genetics defined as the destructive effect on the genetic material of a cell (DNA, RNA), thus affecting its integrity (Shah, 2012). Toxicity of such elements in plants is determined by the route, pattern and duration of the exposure. Common routes of exposure involve the consumption of vegetables and fruits grown on contaminated soils, and the consumption of agricultural

products representing a significant cause of the ingestion of pollutants and toxic elements (Nehme et al., 2014; Ajah et al., 2015). Comet assay or single cell gel electrophoresis is one of the methods used to evaluate DNA damage through DNA migration out of the nucleus. During a technique called electrophoresis, damaged DNA migrates away from the nucleus, producing a comet shape. The displacement of DNA from the nucleus can be used as an indicator of damage (Jiang et al., 2014; Carbajal-López et al., 2015). Moreover molecular markers, such as ISSR (Inter Simple Sequence Repeat), have been used for the detection of DNA changes associated with abiotic factors (Bajpai et al., 2015; Kalubi et al., 2015) and genotoxicity (Al-Qurainy, 2010; Poyraz et al., 2018). Due to the importance of this crop for the region around Lake Chapala and the scarce studies for genotoxicity caused by some elements like metals in this crop, the objective of this study was to use comet assay to evaluate genotoxic damage and ISSR for genetic variation, which are caused by the presence of this pollutants in chayote crops cultivated on the NE shores of Lake Chapala.

Materials and methods

Sampling location

Four sampling sites were selected from the polluted area of the NE shore of Lake Chapala: PC (20°20'4.9"N, 102°47'18.8"W), FA (20°19'3"N, 102°47'48.9"W), NH38 (20°18'39.8"N, 102°48'24.2"W), NH40 (20°18'42.3"N, 102°48'19.5"W) (Figure 1). Two controls (vi and H) were used 76 km from the shore of Chapala lake, vi at the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) located at Rancho Las Agujas, Zapopan, Mexico, in the central part of the state of Jalisco at 1,548 m above sea level (20°25'30"N, 103°19'30"W) and H (20°45'55.98"N, 103°31'42.72"W). Irrigation water and soil samples were collected from each site during the dry (DS) and rainy (RS) seasons. Fresh young leaves and chayote fruit growing at the sites were collected from ten individuals and kept frozen until processed.

Treatment of water and soil samples

The irrigation water samples were collected during DS and RS in 1-L HDPE bottles, according to the technical guidelines established by the Official Mexican Standard and international similar analyses (NOM-AA-14-1980; Estefan et al., 2013). For the soil analysis, a composite sample was taken at a depth of 30–50 cm, with the samples then being stored in plastic bags until analyses could be conducted. The heavy metals Cd, Cr and Pb were determined via the atomic absorption method with equipment detection values for As of 0.001 mg L^{-1}, Cd 0.01 mg L^{-1}, Cr 0.01 mg L^{-1} and Pb 0.05 mg L^{-1} according to the technical guidelines stipulated in NOM-021-RECNAT-2000.

Comet assay

The alkaline comet assay procedure was performed using the method described by Koppen and Verschaeve (1996), with some modifications. The fruit was cut into small pieces

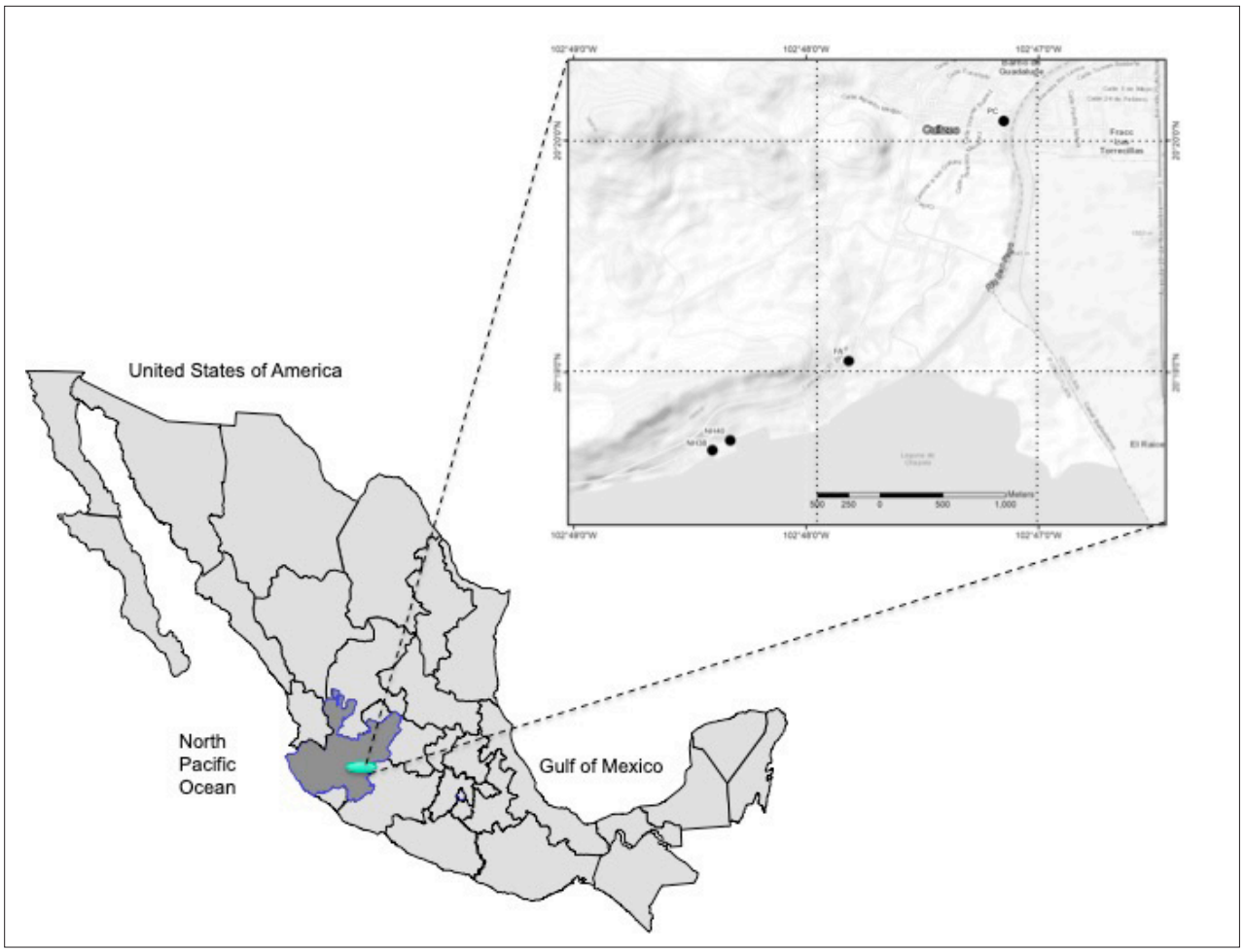


Figure 1. Location of sampling sites for chayote plants, water, and soil at Lake Chapala.

and placed in cold mortar with a phosphate buffer (160 mM NaCl, 8 mM Na_2HPO_4, 50 mM EDTA, pH 7). The nuclei were obtained from juice that was filtered using 20–25 µm Whatman 4 paper and centrifuged for 10 min at 3,000 rpm in a refrigerated centrifuge (4°C). The migration of the nuclei was obtained on slides containing one layer of 1% Normal Melting Point agarose and a second layer of 0.6% Low Melting Point (LMP) agarose, after which 0.5% LMP agarose was mixed with 5 µL of the nuclear suspension and placed on the first layer. Finally, a third layer of 100 µL 0.5% LMP agarose was added to cover the second layer. The sheets containing the kernels of the chayote were immersed in a lysis solution (2.5 mM NaCl, 10 mM Na_2EDTA, 10 mM Tris-HCl, 1% lauryl sarcosinate, 1% Triton X-100 and 10% DMSO, pH 10) for 24 h at 4°C. After lysis, the slides were placed in a horizontal electrophoresis system with an alkaline pH buffer (30 mM NaOH, 1 mM Na_2EDTA, pH 13) for 60 min at 4°C to start the run under the following conditions: 200 mA and 9 Volts by 10 min. The slides were stained with Ethidium Bromide (2 mg L^{-1}) for observation under a fluorescence microscope (Axioskop 40). A 515–560 nm excitation filter was then performed using Comet Assay System II software. Testing was conducted on ten fruit per sample site and 100 nuclei per fruit.

DNA extraction and molecular marker analysis

DNA was isolated from the fresh leaves using the Nucleon PhytoPure Kit (GE Healthcare Life Sciences®). The yield and quality of DNA was tested, using electrophoretic and spectrophotometric methods, and stored at -20°C until processing.

Amplification of DNA with ISSR molecular marker

Ten ISSR primers from the University of British Columbia, published at NAPS Unit list of standard primers, were tested. The four that presented the best amplification standards were selected. Table 1 shows the sequences of the selected primers. The PCR reaction was carried out in a final volume of 20 µL containing: PCR buffer 1X, $MgCl_2$ 2.5 mM, primer 0.8 µM, dNTPs 0.25 mM, 0.05 U taq DNA pol and 5 ng of target DNA. A thermocycler (Techne Flexigene® 96-Wel) was used for amplification under the following conditions: initial denaturation at 95°C for 3 min; 40 cycles of 30 s at 94°C for denaturation; 1 min at 53°C for primer annealing; 2 min at 72°C for extension; and a final extension of 7 min at 72°C. The PCR products were separated in 1.2% w/v agarose HR gel and stained with EtBr for its visualization.

Statistical analysis

For the comet assay, a variance analysis (one-way ANOVA) was used to detect the statistical significance of the migration of nuclei among treatments. The Dunnett test for multiple comparisons of means was performed ($P < 0.05$). Pearson correlation was performed to determine the degree of the relationship between metal concentrations and DNA migration, using the MINITAB 17 statistical program. For the molecular marker, the ISSR bands for each gel were scored as either present (1) or absent (0). Jaccard's similarity coefficient was calculated for use in a clustering analysis via the Unweighted Pair-group Method with Arithmetic Average (UPGMA). The results are presented in a dendrogram. NTSYS software v. 2.21 was used to perform the analysis (Rohlf, 2009).

Table 1. Primers for the ISSR molecular marker and sequences.

Primer	Sequence
844	5'-CTCTCTCTCTCTCTCTRC-3'
901	5'-GTGTGTGTGTGTYR-3'
812	5'-GAGAGAGAGAGAGAGAA-3'
16	5'-YRGACAGACAGACA-3'

R = A or G; Y = C or T.

Results and discussion

The average values for element concentrations in the water and soil during both DS and RS are presented in Table 2. All concentrations obtained were compared with Official Mexican Standards, Environmental Protection Agency (EPA, 1995) and World Health Organization (OMS, 2011). NOM-001-SEMARNAT-1996 establishes the maximum permissible limits of pollutants in wastewater discharges NOM-147-SEMARNAT/SSA1-2004 standard which establishes criteria to determine the concentrations of remediation of soils contaminated by Arsenic, Barium, Beryllium, Cadmium, Hexavalent chromium, Mercury, Nickel, Silver, Lead, Selenium, Thallium and Vanadium and NOM-021-RECNAT-2000 which sets the specifications of fertility, salinity and soil classification studies, sampling and analysis, and using data reported by Trujillo-Cárdenas et al. (2010) for metal content in basaltic rock, because this is the major kind of rock in this lake. According to these standards, the metalloid As in DS water exceeded the permissible limits in three of the sampling areas (FA, NH38 and NH40). Cd and Pb exceeded the prescribed limits from collected water in three of the sampling areas (PC, FA and NH38) in RS. Cd exceeded the maximum permissible limits in soil collected during both DS and RS in the four sampling sites. Present data suggests that control sites vi and H did not present dangerous levels in water for Cr in DS. Historically, pollutant content has been found at Chapala Lake since 1990, due to the establishment of industries in the Lerma-Chapala basin (Hansen et al., 1995; Shine et al., 1998). The level of As detected in those years was 51.6 µg L^{-1} (0.0516 ppm) during the DS, which contrasts with the results of the present study (0.176 mg kg^{-1} in soil). Regarding the other metal content, the Cd concentration was higher than the monthly and daily average stipulated in Mexican regulations (NOM-001-ECOL-1996) for irrigation water and was present in all water and soil sampling sites in both seasons. The presence of Cd in water during RS was greater than DS, which is generally associated with water levels in the lake and suspended sediments (Asati et al., 2016) as a result of decreasing water levels and the action of waves, Cd may be more susceptible to desorption in contaminated sediments and the increase of various ions (Hansen and Van Afferden, 2004). It was possible to observe the presence of lead in the soil during both DS and RS. According to Vázquez-Alarcón et al. (2005), the presence of Pb can be explained by the possible deposition of this metal via products used on the crops. According to reports by several authors (Shine et al., 1998; Trujillo-Cárdenas et al., 2010), pollutants such as Cr, found in the water of Lake Chapala, are anthropogenic in origin, introduced into the lake by discharge from industrial and municipal sources via the Lerma river. Batayneh (2012) carried out a study comparing the concentrations of Cr in DS and RS, finding a slight increase in DS, which concurs with the results of this study. A study conducted in Algeria found also that the highest concentrations were observed during the rainy season and exceeded the limits permissible under the country's regulations (Khérici-Bousnoubra et al., 2009).

Table 2. Concentration of heavy metals in the water and soil during the dry (DS) and rainy (RS) seasons at sample sites on the shore of Lake Chapala.

Sample site	Heavy metals in water (mg L^{-1})								Heavy metals in soil (mg kg^{-1})							
	DS				RS				DS				RS			
	Cr	As	Cd	Pb	Cr	As	Cd	Pb	Cr	As	Cd	Pb	Cr	As	Cd	Pb
PC	0.084	0.179	0.047	0.274	ND	0.003	0.424	ND	0.086	0.016	0.136	0.782	ND	0.020	0.126	0.416
FA	0.083	0.265	0.054	0.417	ND	0.004	0.816	0.971	ND	0.016	0.123	0.140	ND	0.016	0.087	ND
NH38	0.067	0.247	0.065	0.385	ND	0.003	0.230	ND	ND	0.008	0.128	1.825	ND	0.010	0.119	0.233
NH40	0.054	0.294	0.071	0.455	ND	0.004	0.192	ND	ND	0.004	0.084	ND	ND	0.005	0.076	0.667
vi	0.036	0.331	0.065	0.548	ND	0.001	0.191	ND	ND	0.002	0.083	ND	0.041	0.001	0.055	ND
H	ND	ND	ND	ND	ND	0.001	0.190	ND	ND	ND	0.082	ND	0.039	ND	0.050	ND

ND = not detected.

The presence of Pb was observed in water at all the sampling points during DS, whereas, during RS, it was only quantified at site FA. According to reports of Maya and Hansen (1995) the presence of Pb during DS may be due to a higher concentration of dissolved solids and the chemical characteristics of water, such as the pH level. Despite the entry of anthropogenic Pb into bodies of water during RS, the Pb adsorbed into sediments may become desorbed due to the presence of certain ions in the dissolved solids. The results indicate that although there was no Pb accumulation found at the sampling points during RS, (except in FA), the Pb retained in sediments may undergo a desorption process due to its displacement from the absorption sites to the soil of the lakeshore through irrigation. According to Vázquez-Alarcón et al. (2005), the presence of Pb during both DS and RS can be also explained by the possible deposition of this metal by agrochemical products applications. Batayneh (2012) carried out a study comparing the concentrations of Cr during DS and RS and found a slight increase during DS, which coincides with the results of the present study. In another study conducted in Algeria, Khérici-Bousnoubra et al. (2009) found the highest concentrations during RS, which exceeded the permissible limits under the country's regulations. It is reported that As becomes highly soluble at alkaline pH and in soils with low clay content (Akter et al., 2005), which is the case in the study area in the present research. This situation can cause the metal to be present in the crop, thus causing genotoxic affectations. Although the concentrations of As only increase during DS, its effects can occur in low concentrations and can cause damage to the mitochondrial and nuclear DNA, as well as the inactivation of enzymes through the generation of ROS (Reactive Oxygen Species) (Norton et al., 2012; Pozo-Molina et al., 2015; Asati et al., 2016).

This phenomenon, observed on the shores of Chapala Lake, could occur due to the possible dragging of pollutants during RS and their deposition in the water and soil, which concurs with Reynoso et al. (2014), who found an increase in pollutants during RS. At general, levels of potentially toxic elements in Chapala Lake are higher than those reported by Shine et al. (1998) and Trujillo-Cárdenas et al. (2010). Vargas et al. (2001) suggested a relationship between metals in sediments and genotoxicity in a river contaminated by industrial waste in Brazil, while Barbosa et al. (2010) have consistently observed that Cd, Pb and Cr found in bodies of water are agents capable of inducing genotoxic damage. Hansen et al. (1995) reported the movement of suspended sediments in the Lerma-Chapala basin, showing that this represents the main transport of metals and that their deposition in the soil can occur during irrigation.

Comet assay

The results obtained show significant differences between the sampling areas and the control site vi ($P < 0.05$). Table 3 shows the distance (µm) of DNA migration in the nuclei of all fruit tested and the statistical significance of the Dunnett test. These results are evidence of the genetic damage found in chayote fruit grown in the presence of pollutants, and the genotoxicity this causes. Figure 2 shows the migration of DNA compared with a control sample.

The highest values for DNA damage were found in FA in RS, in accordance with DNA migration. The correlation analysis shown in Table 4 shows the strength and direction of the relationship between the metal content in the water and soil and the migration of DNA from the nuclei in the

Table 3. Average distance of DNA migration from the nuclei for each sample site and mean comparison (The Dunnett test).

Sample site	Migration average of tail length (μm)		The Dunnett test (P<0.05)
	DS	RS	*
PC	3.71 ± 1.39	5.91 ± 1.99	A
FA	3.85 ± 1.68	6.51 ± 1.64	B
NH_{38}	5.13 ± 1.40	5.61 ± 1.81	B
NH_{40}	4.01 ± 1.95	5.61 ± 2.04	B
vi	1.57 ± 0.76	1.57 ± 0.76	C

* Treatments with the same letter have no statistical differences.

fruit collected. A positive coefficient (r>0) indicates that an increase in the metal content corresponds to an increase in the genetic damage. According to results, the higher level of genetic damage in the fruit collected during RS was caused by Cr, As and Pb in the water and also in the soil.

A higher correlation was found between the migration of DNA from the nuclei and the presence of metals in water. Even when the localities were not far from the lakeside area, the migration values of the DNA in the fruits sampled in NH38 and NH40 were lower.

While the genotoxicity that was evaluated via comet assay shows damage in fruit collected at all the sampling sites, the control site (vi) presented lower levels of migration. The migration during DS may be attributable to other contaminants, such as the pesticides Heptachlor, Aldrin and Dieldrin used in this crop, as reported by Reynoso et al. (2014).

ISSR molecular marker

The results obtained by ISSR marker, clustering the individuals according to their sampling sites, which corresponds to levels of pollutants. This molecular marker was used to detect genotoxicity in *Eruca sativa* (Al-Qurainy, 2010), in which its efficiency was proven to detect treatments with different concentrations of Zn, Pb and Cd. In *Allium cepa*, ISSR techniques offered insight into detecting genotoxicity caused by Gentiana extracts (Poyraz et al., 2018). In the present study, the clustering corresponds to differences among sample sites and the detection of different concentrations of metals (Figure 3). Individuals from organic crop (H1 and H2) were clustering totally out from the other clusters.

Conclusions

The presence of As, Cr, Cd and Pb in the water and soil on the shore of Lake Chapala was verified in the present study. During the DS in water, the concentration of As can be considered hazardous; this could promote the higher migration of DNA from the nuclei of fruit cells collected during DS and RS. Cd and Pb exceeded the permissible limits in three of the sampling areas. Cd exceeded the maximum permissible limits in soil collected during both DS and RS in the four sampling sites. DNA migration was higher in those fruits collected during RS which shows a strong correlation with the As content in the water. Results obtained reveal that As and Cd were the metals that exceeded the concentration levels stipulated by the NOM and international standards used to compare. The genotoxic affectation associated with chemical agents used for producing the chayote crop supposes a possible transfer of contaminants into the food

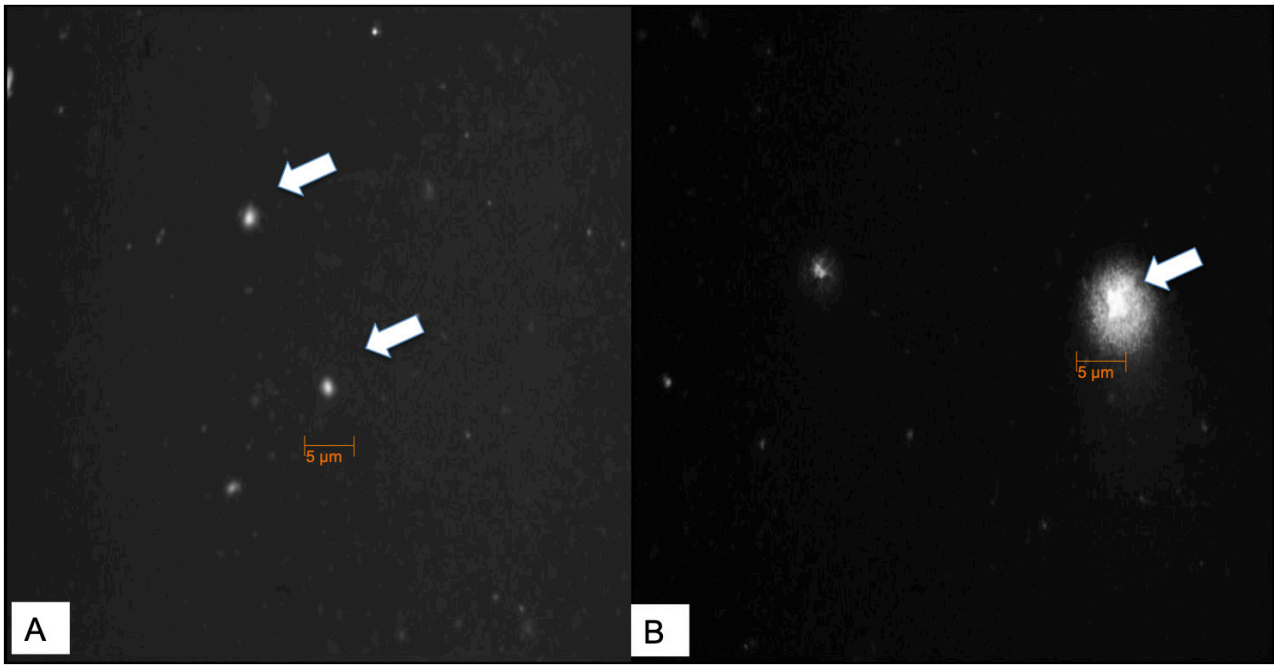


Figure 2. Migration of DNA from nucleus, detected with comet assay. (A) Control samples. (B) Fruit cultivated in the presence of heavy metals.

Table 4. Pearson correlation among the migration of DNA from the nucleus in fruit collected during DS and RS and the heavy metals detected in the water and soil samples.

Heavy metal	DNA migration (µm)	
	Samples	
	DS	RS
Cr		
Water DS	-0.862	0.931
RS	ND	ND
Soil DS	-0.767	0.842
RS	-0.037	0.083
As		
Water DS	0.639	-0.691
RS	-0.721	0.817
Soil DS	0.051	-0.085
RS	-0.553	0.652
Cd		
Water DS	0.236	-0.183
RS	-0.514	0.379
Soil DS	0.174	-0.142
RS	-0.298	0.446
Pb		
Water DS	0.651	-0.709
RS	-0.694	0.611
Soil DS	-0.778	0.863
RS	-0.772	0.858

ND = not detected.

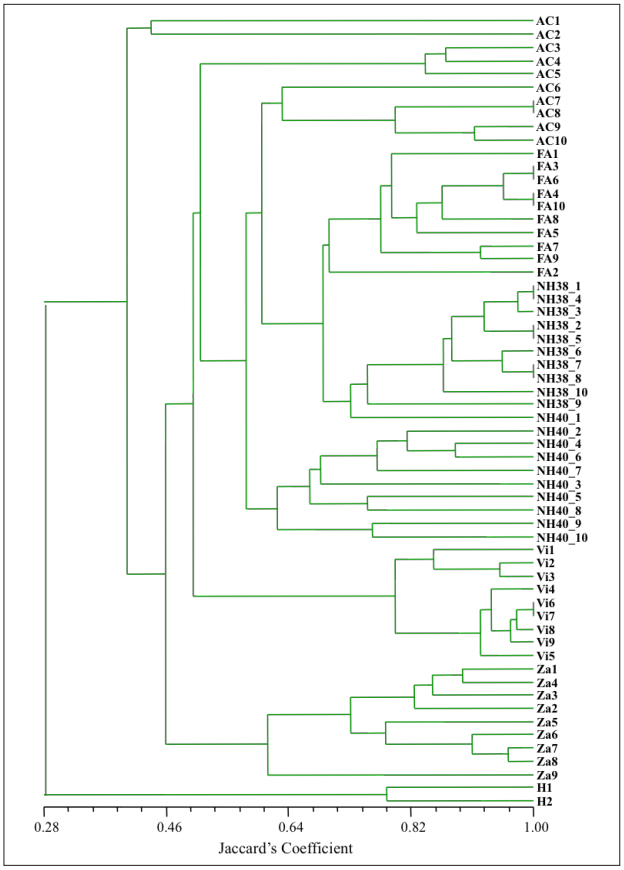


Figure 3. Dendrogram produced by ISSR data for chayote individuals collected from sites with different heavy metal concentrations.

chain. The status of the soil and water found on the shore of Lake Chapala and its environs has been heavily compromised due to the indiscriminate disposal of untreated waste. This work assesses the effect in chayote crop as a clue of possible future negative effects in other crops and in human beings. Unfortunately, this lake is the principal source of water for the nearest city, Guadalajara, and for the production of crops that are consumed throughout the region. Elements found in water and soil in the study area such as As, Cd and Pb have toxicological importance due to their possible bioavailability towards established crops in the area and their potential entry into the trophic chain involving effects on the public health of the region.

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