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CYTOGENETIC EFFECTS IN *VICIA FABA* OF THE POLLUTED WATER FROM RIVERS OF THE TLAXCALA HYDROLOGICAL SYSTEM, MEXICO

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ABSTRACT

In experimental designs several biological systems have shown positive response to specific chemical and physical agents, but in the environment the problem is different because the agents appear as complex mixtures with synergistic, antagonistic or neutral effects. In the water bodies the presence of complex mixtures of substances with genotoxic effects can be detected by mean of a test system quickly and not expensive, based on the exposure to these mixtures of the root tips of *Vicia faba* and the scoring of chromosomal aberrations and centromeric effects induced in the meristematic anaphase cells. In the **Hydrological Atoyac-Zahuapan Rivers System** (Tlaxcala) superficial water and sediments were sampled in nine sites, in which three of the sediments from places named **Afluente de Atenco, Confluencia Apizaquito-Atenco** and **Arroyo Atlixtac** produced chromosomal effects in the significant levels ($P < 0.001$). The plant test used was effective as a monitor of genotoxicants contained in complex mixtures in the water systems.

RESUMEN

En diseños experimentales, varios sistemas biológicos han mostrado respuesta positiva a agentes químicos o físicos específicos, pero en el ambiente el problema es diferente porque se presentan como mezclas complejas con actividades sinérgicas, antagónicas o neutrales. En los cuerpos de agua la presencia de mezclas complejas de sustancias con efectos genotóxicos puede detectarse mediante un sistema de prueba rápido y no costoso que se basa en la exposición a esas mezclas de las raices de *Vicia faba* y en el registro de aberraciones cromosómicas y alteraciones centroméricas inducidas en sus células meristemáticas en anafase. En el sistema **Hidrológico Atoyac-Zahuapan** (Tlaxcala), se colectaron agua superficial y sedimentos en nueve sitios, de los cuales los sedimentos de tres de ellos denominados **Afluente de Atenco, Confluencia Apizaquito-Atenco** y **Arroyo Atlixtac** produjeron efectos cromosómicos a niveles significativos ($P < 0.001$). Se demostró que el ensayo vegetal utilizado fue efectivo para el reconocimiento de agentes genotóxicos contenidos en las mezclas complejas de los cuerpos de agua.

INTRODUCTION

The immoderate increment of wastes from several sources pouring into stream and rivers has produced an increase in pollution and consequently genotoxic effects on plants, animals and human beings. An example of this problem is the **Hydrological System Atoyac-Zahuapan** (Tlaxcala, México) which receives domestic and industrial effluents. In order to detect the effects of low concentrations of pollutants, chromosomes, which are excellent genotoxic indicators have been used as monitors in diverse organisms. A very useful material for these purposes are the meristematic cells of root tips of *Vicia faba,* which offer a wide range of posibilities of cytogenetic analyses, because the chromosomes of this species are large and few in number ($2n = 12$), and thus easy to study

(Villalobos-Pietrini *et al.* 1978). Besides, *Vicia* has its own metabolic activation system (Takehisa *et al.* 1982). The treatments may be applied to the roots directly and the complete experiment is rather inexpensive. This plant has been commonly used to study the effects produced by physical and chemical mutagens, having the frequency of aberrations as an efficient indicator of mutagenic response. In this study, root tips of *Vicia faba* were used as monitor to detect mutagens in the water from the **Hydrological System Atoyac-Zahuapan** of Tlaxcala (México).

MATERIAL AND METHODS

Seeds of *Vicia faba* (var. major) were germinated between two cotton layers. When primary roots reached around 4 cm in length, group of five seedlings were made. The sampled water was stored in the cold (4°C) overnight and used the next day. Each group of seedlings was introduced into the collected water or fresh tap water used as a control, the approach selected to process the complex mixtures for biological analysis is as a single entity and tested in its crude state. Therefore it was avoided artefacts generated by processes like extraction, concentration or fractionation of the samples. After four hours exposure the roots were rinsed and transferred to a bath with fresh tap water for 6 hours with air bubbling constantly for aeration. The root tips were fixed and stained by the acetoorcein technique described by Villalobos-Pietrini (1965) with some modifications. After squash, slides were

TABLE I. PHYSICO-CHEMICAL CHARACTERISTICS OF THE WATER SAMPLES OF THE SEDIMENTS THAT PRODUCED CHROMOSOMAL EFFECTS

Characteristic		Sampled sites number		
		3	4	5
ph		7.42	7.00	7.70
Temperature (°C)		21		22
Conductivity (Mhos/cm)		699	470	772
Settleable solids (ml/L)		26.0	0.70	0.01
Flow (l/seg)			484.4	1660.0
Alkalinity (mg/L)		276.65**	231.0**	231.0**
Hardeness (mg/L)	Calcium	166.94	74.77	85.47
	Magnesium	99.13	60.87	119.32
	Total	266.07	135.64	204.79
Chlorides (mg/L)		3.97	12.84	30.02
Sulphates (mg/L)		53.30	16.82	91.51
Phosphates (mg/L)	Orto	0.52	0.57	3.51
	Total	1.01	1.67	4.22
Total fixed solids (mg/L)		484	292	160
Total volatile solids (mg/L)		436	148	448
Total solids (mg/L)		920*	440	608
Suspended fixed solids (mg/L)		105*	33	58
Suspended volatile solids (mg/L)		310*	22	27
Total suspended solids (mg/L)		415*	55	85
Dissolved fixed solids (mg/L)			259	102
Dissolved volatile solids (mg/l)			126	421
Total dissolved solids (mg/L)			385	523
Dissolved oxygen (mg/L)		0.56	3.64	0
Biochemical oxygen demand (mg/L)		142.0	31.08	20.20
Chemical oxygen demand (mg/L)		432.23**	79.55	252.0
Nitrogen (mg/L)	Ammonia	0	0	19.93
	Organic	6.00	0.44	9.63
	Total	6.00	0.44	29.56
Greases and oil (mg/L)		21.7	41.73	
Detergents (mg/L)		0.62	0.24	3.62

weak
medium*
hard**

TABLE II. ABNORMAL ANAPHASES, CHROMOSOMAL ABERRATIONS AND CENTROMERIC ALTERATIONS PRODUCED IN *VICIA FABA* ROOT TIP CELLS BY WATER OF THE RIVERS OF TLAXCALA, MEXICO

Site	Abnormal Anaphases (%)	Chromosomal Aberrations (%)	Centromeric Alterations (%)
1	1.27 NS	0.93 NS	0.34 NS
2s	1.38 NS	1.32 NS	0.07 NS
3s	3.50 NS	2.51 NS	1.49 NS
3	12.54*	8.76*	10.12 *
4	5.37 *	5.04 *	1.25 NS
5	6.41 *	4.15 *	3.40 *
6	1.50 NS	1.34 NS	0.29 NS
7	3.99 NS	3.43 NS	1.25 NS
8	2.52 NS	2.00 NS	1.28 NS
9	3.15 NS	3.07 NS	1.79 NS
Control A	1.98	1.31	1.27
Control B	1.71	0.84	1.18
Control C	1.03	1.17	0.11

* Significant, $P < 0.001$
NS = Not significant

made permanent with dry ice, two changes in butanol and mounted in Canada balsam.

By microscopic examination, the chromosomal alterations in anaphase cells as fragments, bridges, isochromosomes and chromosomes with inactivated centromeres were scored. In order to compare the frequencies obtained by each treatment with those scored in their own controls **(Table II),** the difference in the proportion test was used (Spiegel 1970).

In the **Hydrological Atoyac-Zahuapan System** the following places were sampled **(Fig. 1): Parque Ecológico Tizatlán** (1s), **Zahuapan Puente Trébol** (2s), **Afluente de Atenco** (3 and 3s), **Confluencia Apizaquito-Atenco** (4), **Arroyo Atlixtac** (5), **Atoyac Villalta** (6), **Atoyac Xochiteca-**

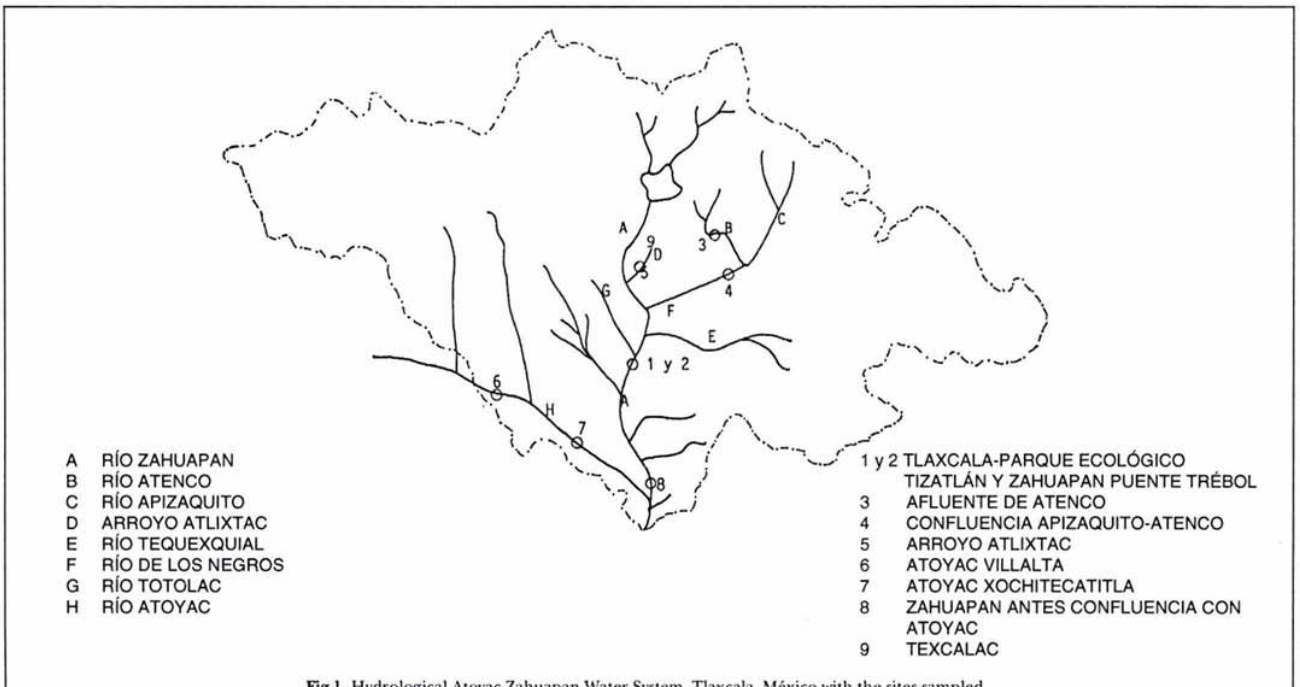

Fig 1. Hydrological Atoyac-Zahuapan Water System, Tlaxcala, México with the sites sampled

titla (7), **Zahuapan** before **Confluencia** with **Atoyac** (8) and **Texcalac** (9). Samples 1s, 2s and 3s were taken from surface water, while the others were obtained from sediments. In the sites in which the genetic damage was observed, the assay was confirmed once again.

The Zahuapan river has its origen from drains going down the south watershed of the Puebla mountains. Its initial course was from the southwest up to Atlangatepec, where a store vessel was built. In the state of Tlaxcala, the direction of the Zahuapan river is irregular up to the confluence with the Atoyac River, 10 km north of Puebla (INEGI 1986).

The Delegación Estatal in Tlaxcala of SEDESOL (Ministry of Social Development, before SEDUE) determined the physico-chemical characteristics of the samples **(Table I).**

RESULTS AND DISCUSSION

From the total samples taken those from the following sites were chosen for analysis: **Afluente de Atenco** (3), **Confluencia Apizaquito-Atenco** (4) and **Arroyo Atlixtac** (5), all three sites which induced damage to the chromosomes; other samples taken from the surface water and another sediments did not produce significant effects different from those of the controls **(Table II).**

The scoring of chromosomal alterations in anaphase cells supply adequate data to assess damage at the genetic level that is produced by environmental pollutants (Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1985, Grant *et al.* 1992). In this study fragments, bridges as well as chromosomes with inactivated centromeres were detected similar to those observed in *Allium cepa* and *Ornithogalum virens* of waste waters from textil industries (Ravindran and Ravindran 1978), industrial effluents in *Allium cepa* (Santhamurthy and Rangaswamy 1979) and dye-related industries in *Allium cepa* and *Chlorophytum amaniense* (Somashekar *et al.* 1985, 1987).

The waste discharges accumulated in the sediments where the positive response were obtained, came from different sources. In **Afluente de Atenco**, the industries has been involved in the recycling of paper and their main wastes were cellulosae, minerals, greases, oils and dyes. In **Confluencia Apizaquito-Atenco**, the main industrial wastes were dyes, sodium hydroxide solids, greases and oils. In **Arroyo Atlixtac**, the industries related discharged cellulosae and chemical products like salts originated from dyes and sulfonic acids.

The genotoxic effects of the effluents of pulp and paper mill have been observed in *Salmonella* by base-pair substitution (Douglas *et al.* 1980, Lee *et al.* 1981), mutations, chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells (Douglas *et al.* 1980, 1983, Lee *et al.* 1981, Rannung *et al.* 1981, Langi and Priha 1988), gene conversion and mitotic recombination in yeast (Douglas *et al.* 1983), micronuclei in fish erythocytes (Das and Nandas 1986) and chromosomal aberrations in onion mitotic cells (Shanthamurthy and Ranganswamy 1979, Mohapatra *et al.* 1985).

Fern (*Osmunda regalis*) populations living in places near discharges of pulp and paper mill wastes have shown higher frequencies of mutations (Klekowski and Levin 1979). When extracts of tissues of fishes living in the effluent of pulp and paper mills were used to treat *Salmonella*, mutagenicity also increased significantly (Blevins 1991). Seedlings of *Vicia faba* and cuttings of *Tradescantia* were exposed *in situ* in a creek containing raw effluent from a pulp and paper mill which resulted in the production of micronuclei in tetrads of *Tradescantia* and chromosomal aberrations in the root tips of *Vicia faba* (Grant *et al.* 1992). In these processes involving the production of paper from wood, lignin was removed by digestion at very high temperature and in order to facilitate the fiber separation sulfate (Kraft process) and sulfite were used, but in spite of that still some residues and other organic constituents must be taken out by means of bleaching, obtained by different reactions of chlorination, alkaline extraction, hypoclorite and chlorine dioxide and only the chlorination stage effluent has exhibited significant mutagenic activity in a crude state (Houk 1992). The discharge of the factories involved in this study were not from processes of digestion and bleaching because the procedure started from commercial cellulosae fibers (virgen or recycled), therefore the genotoxic effects were not depending upon the mentioned compounds.

On the other hand, in relation to the waste water from the dye manufacturing industries, which represent one of the major sources of water pollution, many toxic compounds including heavy metals, sometimes in high concentrations are found in the waste waters. In root tips of *Allium cepa* this waste waters decreased the mitotic index and produced abnormalities such as chromosomal breaks, C-mitosis, binuclear cells as well as tri- and tetrapolar cells (Somashekar *et al.* 1985). Such waste waters also induced chromosomal damage in meiotic cells of *Chlorophytum amaniense* (Somashekar *et al.* 1987). In the case of the dye industry, the authors considered heavy metals as the potential mutagens.

The values of the physico-chemical characteristics of the samples are given in **Table I** and they indicate low pollution due to the inorganic components being higher for the organic compounds, but in general the pollution in the sites samples is not critical. The pH values are almost neutral and quite similar to water recommended for irrigation, the same happend for the alkalinity, chlorides and sulphates. The total hardness as well as calcium and magnesium had also low values. The results of electric conductivity are low, being the lowest for sample 4, possibly due to the dilution of the waste waters. The lower limit of the dissolved oxygen (DO) depends upon the water temperature. For biota of temperate water the limit of DO should not be less than 5 mg/L and for cold water, not less than 6 mg/L (Stocker and Seager 1981). As shown in **Table I,** the level of DO in the three positive response samples is below 5 mg/L, possibly due to the fact that the industrial wastes are mainly organic compounds

with very high demands of oxygen, since its degradation depends upon aerobial bacteria (Stocker and Seager 1981). The chemical oxygen demand (COD) that determines the amount of oxygen required to oxydate organic compounds in sample 3, showed high concentration whereas sample from site 5 is medium and that from site 4 is low **(Table I),** but they are not so high to be considered as heavy contamination. These data agree in general with the scarcity of DO. It was found a large variation of settleable solids in the three samples that are in agreement with the BOD_5 values. The concentrations of ammonia, organic and total nitrogen and also total phosphates and ortophosphates are low and it should not cause any problem to plants or animals using these waters.

As a conclusion, it is possible to consider that the first approach to the pollution problems in the waters bodies is the response of the plant assay and in this manner to detect the presence of genotoxicants in the complex mixtures, step that is inexpensive. The next steps require, if it is desirable to go deep into the analysis, additional experimental designs, more sophisticated equipment and higher expenses and thus to point out some specific substance that generates the genotoxic activity of this complex mixtures. In addition, it was been shown that the *Vicia faba* plant assay used in this study is sensitive enough to become an effective monitor of genotoxicants in water bodies.

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