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Mutagenic Potential Evaluation of the Water of a River That Recei es Tannery Effluent Using the *Allium cepa* Test System

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Summary Several studies are being conducted to assess the toxicity and cytotoxicity of water bodies receiving industrial and domestic effluents, using the *Allium cepa* test. To assess the toxicity and mutagenicity of water possibly contaminated with chromium, derived from tannery activities, seasonal water samplings were performed in 2001 and 2002 at five different sites along the Sapucaizinho river, Municipality of Patrocínio Paulista, State of São Paulo, Brazil. *A. cepa* seeds were used as the test material and were submitted to germination in waters from the different collection sites, in Milli-Q water (negative control) and in aqueous solution of chromium (positive control). For the determination of cell division rates and mitotic irregularities, slides were prepared with root tip cells according to the standard Feulgen methodology. The results showed that the collection sites most heavily compromised by chromium emission presented low mitotic indices and a higher frequency of mitotic changes such as irregular anaphases (disorganized, multipolar, laggard), cells with chromosomal adherences, cells with micronuclei, and binucleate and/or multinucleate cells.
Key words Chromium, Clastogenicity, Mutagenicity, *Allium cepa*.

The investigation of environmental agents with mutagenic potential has been the target of an increasing number of studies, since the consequences of their actions can result in mutations. The agents to which humans can be exposed are of a physical, chemical or biological nature.

Analysis of the genotoxic potential of a substance through the investigation of the induction of chromosome alterations represents an effective method for biomonitoring studies and for the analysis of the extent of pollution (Harden 2001).

Many plant species have been used as a test organism in environmental monitoring assays. Some plant species such as *Allium cepa*, *Vicia faba* and *Tradescantia poludora* are efficient test organisms for the assessment of mutagenicity and clastogenicity resulting from the contamination with environmental pollutants (Grover and Kaur 1999, Duan *et al.* 1999).

Various studies have determined the toxicity and cytotoxicity of water resources that receive industrial and domestic effluents using the *A. cepa* test system (Smaka-Kincl *et al.* 1997, Matsumoto 2003).

Analysis in water samples from the Paraguai river (Brazil) using the *A. cepa* test system demonstrated the cytotoxic potential of these water samples at collection sites related to the emission of municipal wastewater (Moraes and Jordão 2001). In addition, the authors observed a seasonal influence on the cytotoxicity of the samples analyzed, which was mainly related to seasonal cycles of flooding and drought.

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Hexavalent (VI) chromium is a biologically active compound since the cell membrane is permeable to this metal. Once inside the cell, hexavalent chromium is rapidly reduced to trivalent chromium (O'Brien and Xu 2001, Matsumoto 2003). *In vivo* and *in vitro* studies have shown that hexavalent chromium induces various types of damage to the DNA molecule, including chromosome aberrations, micronucleus formation, sister chromatid exchange, DNA strand breaks, and errors in the replication mechanism (Codd *et al.* 2001).

According Leonard and Lauwerys (1980) and Snow (1992), trivalent (III) chromium in aqueous solution is unable to cross the cell membrane, but can enter the cell through the processes of phagocytosis and endocytosis (Matsumoto 2003). Once inside the cell, trivalent chromium binds to the DNA molecule and damages its structure, a process that characterizes its carcinogenic and mutagenic potential (Sugden *et al.* 1998).

A. cepa roots was used as a test organism to determine the toxicity and genotoxicity of water possibly contaminated with hexavalent chromium in the State of Oressa (India) and observed diverse mitotic irregularities (C-mitosis, anaphase bridges, chromosome stickiness, and chromosome fragmentation and lagging), thus confirming the toxic and genotoxic effects of this metal (Harden 2001, Grover and Kaur 1999). Similar chromosome abnormalities were observed by Liu *et al.* (1982), when submitting *A. cepa* roots to different concentrations of trivalent and hexavalent chromium.

Gomez-Arroyo and Vallalobos-Spietrini (1983) and Smaka-Kincl (1997) reported that the cytotoxic and genotoxic effect of chromium using *A. cepa* roots submitted to different concentrations of potassium dichromate and calcium chromate. The authors observed an increase in the frequency of chromosome irregularities which was directly proportional to the chromium concentration in the solution. The most frequent chromosome abnormalities were anaphase aberrations, isochromosome formation, the presence of micronuclei, and centromere inactivation.

The Municipality of Patrocínio Paulista, São Paulo, is a region characterized by intense industrial activity related to leather processing (tanning). Various toxic substances including chromium, which are considered to be harmful to the environment, are used for the tanning of cow leather. In this region, all water used during tanning is submitted to treatment before being dumped into the river in an attempt to reduce the impact of pollution. The objective of the present study is to investigate the possible persistence of contaminating agents with mutagenic potential which were released during the tanning process into the Sapucaizinho River, Municipality of Patrocínio Paulista, by means of mutagenenicity assays using *A. cepa* as the test organism.

Materials and methods

Seasonal collections were performed in 2001 and 2002 at five distinct sites along the Sapucaizinho River, Municipality of Patrocínio Paulista, State of São Paulo, Brazil. The collection sites were standardized as follows: A – river water: 1 km before the disposal site of the tannery effluent (upstream of the river – PA), about 3 m before the disposal site (PB-A), at the disposal site (PB-L), and 1 km after the disposal site (downstream of the river – PC); B – previously treated tannery effluent water before disposal into the river (PB-T) (Fig. 1).

The analyses were based on the Standard Methods for the Examination of Water and Wastewater (Franson 1995).

For cation determination, the samples were first acidified with HNO_3, pH 1, and the cations were analyzed sequentially by inductively coupled plasma atomic emission spectrometry (ICP-AES) with ultrasonic nebulization. The following elements were determined: calcium, magnesium, strontium, silicon, iron, manganese, aluminum, zinc, chromium, cobalt, nickel, lead, cadmium, phosphorus, copper, and barium.

The standard solutions used to construct the calibration curves for the elements analyzed in

0.1% HNO_3 medium were obtained by appropriate dilution of the standard solution of 1000 ppm Titrisol (Merck).

A. cepa seeds were used as test material. Seeds were submitted to germination in water obtained from the different collection sites. Seeds submitted to germination in Milli-Q water only were used as negative control. The aqueous solution of trivalent chromium (0.089 mg/l) was used as positive control. The assays were performed with seeds from a single *A. cepa* variety to avoid variations in the responses to the different steps of the procedure.

The seeds were submitted to two types of treatment: A) continuous treatment: soaking and germination of the seeds in the water derived from the collection sites; B) discontinuous treatment: the seeds were initially submitted to germination in Milli-Q water until reaching a length of 2 cm and then transferred to a Gerbox containing water from the collection sites and incubated for 20 h (acute treatment). After this period, some roots were randomly selected, while the remaining roots were maintained under the same conditions as described above until completing 72 h of treatment (chronic treatment) and then collected. For the assays performed with water samples collected in 2002, some roots were transferred to plates containing Milli-Q water after chronic treatment and incubated for a further 48 h (recovery treatment) before collection.

All root tips were fixed in Carnoy's solution diluted 3 : 1 (3 parts ethanol and 1 part acetic acid) for 24 h.

For cytological analysis, samples on a slides were obtained by the common method of gentle squashing. Root tips were submitted to acid hydrolysis in 1 N HCl at 60°C for 8 min, followed by washing in distilled water. The samples were stained with Schiff's staining for 2 h in the dark. For each treatment about 5000 cells were analyzed. All cells showing any type of alteration were recorded and the most significant cells were photographed for documentation of the material.

For the determination of the rate of cell division, slides containing root tip cells from both treatments were mounted as described above. Analysis was done taking into account the percentage of cells of each phase of cell division.

For assessment of the mutagenic potential, root tip cells showing irregular division such as irregular anaphases (disorganized, multipolar, delayed, *etc*.), cells with chromosome adhesions, cells with micronuclei, and binucleate and/or multinucleate cells were analyzed.

The results were analyzed statistically by the Kruskal-Wallis test.

Results and discussion

In the present study, *A. cepa* roots treated with water samples collected during the 2001 and 2002 rainy periods did not show significant differences in the mitotic index compared to the negative control for all treatments performed (Table 1).

A high frequency of aberrant cells was observed in *A. cepa* roots treated with water samples that receive tannery effluents (Table 1). For samples collected in 2001, the frequency of aberrant cells in relation to the total number of dividing cells was higher at the PB-T site (water resulting from tannery effluent treatment) during the dry period for the continuous (6.06) and acute discontinuous −20 h (11.78) treatments (Table 1). As mentioned earlier, during the rainy period, the frequency of aberrant cells was low at all sites and for all treatments, with no significant differences compared to the negative control (Table 1). The most frequent aberrations identified in *A. cepa* root tip cells for the dry and rainy periods were cells with micronuclei and irregular anaphases (anaphase bridges and multipolar anaphases), as shown in Table 1 and Fig. 2.

Analysis of *A. cepa* roots treated with water samples collected during the 2002 dry and rainy periods revealed a higher frequency of aberrations for the chronic discontinuous treatment (72 h). During the dry period, the highest frequency was observed at the PC site (downstream of the effluent), with a mean value of 9.62%, followed by the PB-T site (water resulting from tannery effluent

Table 1. Cell changes observed in *Allium* cepa root tip systems submitted to the action of waters contaminated with chromium residues derived from leather tanning industries, collected from the Sapucaizinho River in 2001, 2002

(%)

Period	Treatment	A		B		C		D		E		F		G		H	
		'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02
Rainy																	
	Continuous	—															
	NC	—	2	—	—	—	—	—	—	—	0.9	—	1.0	—	—	0.0 a	0.18 d
	PC	0.3	1.8	1.2	1.6	0.7	2.0	0.2	—	0.4	2.6	2.6	5.4	—	—	10.95 b	15.81 ab
	PA	1	0.3	0.9	0.4	0.9	0.2	—	0.2	—	0.5	—	1.2	0.8	0.2	1.75 a	3.66 c
	PB-A	1.1	0.6	0.9	0.4	1	0.5	—	—	—	1.2	0.3	1.6	2.2	0.4	0.82 a	5.30 bc
	PB-T	1	0.5	1	0.7	1	0.2	—	0.1	—	1.7	0.3	3.2	1.3	0.1	1.29a	9.33 ab
	PB-L	1.5	0.4	0.9	0.6	0.5	0.1	—	0.3	—	0.7	—	1.8	2.1	0.1	1.25 a	4.68 bc
	PC	1	0.1	1.1	—	0.6	—	—	—	0.1	0.6	—	1.3	1.8	—	1.45 a	2.89 c
	20 h																
	NC	—	2	—	—	—	—	—	—	—	0.9	—	0.1	—	—	0.0 a	0.18 c
	PC	0.3	1.8	1.2	1.6	0.7	2.0	0.2	—	0.6	2.6	2.8	5.4	—	—	10.95 b	15.81 a
	PA	0.6	0.5	0.4	0.4	0.4	0.6	—	0.1	0.1	0.4	0.7	1.5	0.2	—	2.72 a	4.85 b
	PB-A	2.1	0.4	0.25	2.5	0.1	0.9	—	—	—	0.4	0.1	1.8	0.3	0.1	2.20 a	5.88 b
	PB-T	0.7	0.8	0.2	—	0.1	—	0.1	—	0.1	—	—	0.3	1	0.9	1.96 a	2.57 bc
	PB-L	1.7	0.2	0.2	0.8	0.2	0.3	0.1	0.1	0.1	0.7	0.2	2.0	0.8	0.1	1.35 a	5.83 b
	PC	1	0.2	0.5	0.4	0.4	0.2	—	—	0.2	0.1	—	1.0	1.1	0.3	2.22 a	3.00 bc
	72 h																
	NC	—	2	—	—	—	—	—	—	—	0.9	—	0.1	—	—	0.0 a	0.18 d
	PC	0.3	1.8	1.2	1.6	0.7	2.0	0.2	—	0.6	2.6	2.8	5.4	—	—	10.95 b	15.81 a
	PA	0.3	0.4	0.3	0.3	0.2	—	—	—	—	0.9	—	2.6	0.5	0.2	0.55 a	6.49 bc
	PB-A	0.4	0.4	0.4	0.3	0.2	0.1	—	0.1	—	0.5	0.1	1.1	0.7	0.6	0.71 a	2.65 cd
	PB-T	1	2.0	0.2	0.6	0.1	0.7	—	0.1	—	0.6	—	2.8	0.3	0.3	0.50 a	9.64 ab
	PB-L	0.9	0.7	0.3	1.0	0.2	0.6	0.4	0.1	—	0.9	0.1	1.9	1	0.1	1.60 a	8.51 ab
	PC	0.2	0.6	0.1	0.2	—	0.2	—	—	—	0.1	—	1.2	0.1	—	0.47 a	3.67 bc
	Recovery																
	NC		2		—		—		—		0.9		0.1		—		0.18 c
	PC		1.8		1.6		2.0		—		2.6		5.4		—		15.81 a
	PA		0.5		—		—		—		0.7		2.1		—		6.84 b
	PB-A		0.8		0.1		0.3		0.2		1.1		0.6		—		4.92 b
	PB-T		2.0		0.4		0.2		0.2		0.9		1.0		—		5.08 b
	PB-L		—		0.1		—		—		0.2		0.2		—		4.02 bc
	PC		0.9		0.5		0.4		0.1		0.3		1.9		0.3		8.23 ab

Table 1. Continued

(%)

Period	Treatment	A		B		C		D		E		F		G		H	
		'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02
Dry																	
	Continuous																
	NC	0.6	2.2	0.1	1.6	—	—	—	—	0.7	—	—	—	1.2	—	0.52 c	2.04 c
	PC	1.2	4.4	1.8	2.2	2.2	0.8	0.1	0.9	3	1	7.9	5.1	—	0.1	18.07 a	14.42 a
	PA	0.7	2.0	0.2	1.0	0.3	0.3	—	0.4	0.4	0.7	—	2.4	0.9	0.1	1.61 bc	7.37 bc
	PB-A	0.7	1.1	0.4	0.3	0.3	0.2	0.4	0.1	0.3	1.5	0.1	1.5	1/5	0.1	3/16 b	5.17 b
	PB-T	0.6	1.1	0.2	0.6	0.4	1.0	1.1	0.3	0	2.1	0.2	1.4	2.3	0.1	6.06 b	5.65 b
	PB-L	3.1	1.3	0.7	1.5	0.6	1.1	1.8	0.2	0.3	1.2	1	0.8	4.8	0.3	3.85 b	5.62 b
	PC	0.9	1.4	0.5	0.4	0.31	0.2	0.5	0.1	0.7	1.0	—	1.0	2.3	0.3	3.03 b	3.94 b
	20 h																
	NC	0.6	2.2	0.1	1.6	—	—	—	—	0.7	—	—	—	1.2	—	0.52 c	2.04 c
	PC	1.2	4.4	1.8	2.2	2.2	0.8	0.1	0.9	3	1.0	7.9	5.1	—	0.1	18.07	14.42 a
	PA	1.5	1.2	0.2	0.5	0.3	0.2	0.7	—	0.6	1.6	0.1	2.1	1.9	0.2	2.06 bc	5.85 bc
	PB-A	1.4	0.2	0.2	1.1	0.1	0.8	0.8	—	0.2	2.8	—	3.8	1.4	—	1.52 bc	6.32 bc
	PB-T	7.6	0.6	0.1	0.4	4.1	0.4	—	—	—	0.1	1.2	2.3	5.4	0.4	11.78 bc	4.32 bc
	PB-L	1.6	0.4	0.5	1.9	0.7	0.9	1.7	—	1.3	0.8	—	2.1	4.2	—	4.84 b	5.22 b
	PC	0.8	1.0	0.3	1.1	0.4	0.8	1.7	—	0.7	1.5	—	2.7	2.1	0.2	2.99 b	5.61 b
	72 h																
	NC	0.6	2.2	0.1	1.6	—	—	—	—	0.7	—	—	—	1.2	—	0.52 a	2.04 b
	PC	1.2	4.4	1.8	2.2	2.2	0.8	0.1	0.9	3	1.0	7.9	5.1	—	0.1	18.07 b	14.42 a
	PA	—	1.1	—	0.5	—	1.2	—	0.2	—	0.3	—	3.4	—	0.1	0.0 a	5.52 b
	PB-A	1.6	1.2	0.2	1.0	0.1	0.2	—	—	0.2	1.4	—	3.7	0.5	0.1	0.70 a	5.65 b
	PB-T	2.3	3.6	0.4	1.2	0.6	0.6	—	—	—	1.2	0.1	1.5	1.1	0.3	1.93 a	9.23 b
	PB-L	1.2	1.3	0.2	2.3	0.32	0.9	0.1	—	0.4	3.1	—	3.3	1	—	1.56 a	8.42 b
	PC	1.5	1.2	0.1	0.3	0.3	0.6	0.7	0.4	0.6	2.1	0.1	6.1	1.8	—	2.58 a	9.62 b
	Recovery																
	NC		2.2		1.6		—		—		—		—		—		2.04 c
	PC		4.4		2.2		0.8		0.9		1.0		5.1		0.1		14.42 a
	PA		1.8		0.5		0.3		0.1		1.9		1.6		0.6		4.82 c
	PB-A		0.9		—		0.2		0.2		1.4		0.3		—		3.57 c
	PB-T		2.1		1.1		0.6		1.4		2.1		4.3		0.3		11.69 ab
	PB-L		1.5		1.6		1.4		0.4		4.4		2.9		0.2		11.72 ab
	PC		1.8		1.0		0.7		0.2		2.9		2.9		—		8.38 bc

A. micronucleus; B. anaphase with a bridge; C. multipolar anaphase; D. delayed anaphase; E. sum of chromosome breaks and losses; F. sum of C-metaphase and adherence; G. binucleate cell; H. Frequency of aberrant cells in relation to dividing cells. Means followed by the same letters did not at the 5% level of siginificance (mean rak Kruskal-Wallis test). PA. 1 km before the disposal site of the tannery effluent; PB-A. About 3 m before the disposal site; PB-T. Previously treated tannery effluent water before disposal into the river; PB-L. At the disposal site; PC. 1 km after the disposal site.

Table 2. Determination of elements (ppm) by ICP-AES in water samples collected at three different points in the four seasons of the year in 2001, 2002

		Chemical elements																			
		Pb		Fe		Cd		Cr		P		Al		Zn		Cu		Ba		Co		Ni	
		'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02	'01	'02
Summer																							
	PA	<0.025	<0.025	1.2	1.1	<0.005	<0.005	<0.01	<0.01	0.1	<0.1	0.06	0.12	0.02	<0.01	0.01	<0.01	0.03	0.02	<0.01	<0.01	<0.01	<0.01
	PB-A	<0.025	<0.025	2.0	1.1	<0.005	<0.005	<0.01	<0.01	<0.1	<0.1	0.10	0.09	0.03	<0.01	<0.01	<0.01	0.03	0.03	<0.01	<0.01	<0.01	<0.01
	PB-T	<0.025	<0.025	0.2	0.13	<0.005	<0.005	0.37	0.2	0.5	0.51	0.7	5.6	0.06	0.03	0.01	<0.01	0.005	0.05	<0.01	<0.01	0.01	0.01
	PB-L	<0.025	<0.025	1.8	1.2	<0.005	<0.005	<0.01	0.01	0.1	<0.1	0.17	0.2	0.05	<0.01	0.01	<0.01	0.03	0.03	<0.01	<0.01	<0.01	<0.01
	PC	<0.025	<0.025	0.7	1.1	<0.005	<0.005	<0.01	<0.01	2.5	<0.1	0.30	0.1	0.05	<0.01	<0.01	<0.01	0.05	0.02	<0.01	<0.01	<0.01	<0.01
Autumn																							
	PA	<0.025	<0.012	1.3	0.96	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	0.05	<0.01	<0.005	<0.01	<0.005	0.02	0.02	<0.01	<0.005	<0.01	<0.005
	PB-A	<0.025	<0.012	1.0	0.92	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	0.06	0.06	0.01	<0.005	<0.01	<0.005	0.02	0.03	<0.01	<0.005	<0.01	<0.005
	PB-T	<0.025	<0.012	0.2	0.12	<0.005	<0.003	0.04	0.2	0.15	0.26	0.35	0.35	0.01	0.01	<0.01	<0.005	0.01	0.01	<0.01	0.01	<0.01	0.005
	PB-L	<0.025	<0.012	1.1	0.81	<0.005	<0.003	<0.01	0.005	<0.1	<0.1	0.10	0.1	0.09	<0.005	<0.01	<0.005	0.02	0.03	<0.01	<0.005	<0.01	<0.005
	PC	<0.025	<0.012	0.8	0.85	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	0.07	<0.01	<0.005	<0.01	<0.005	0.02	0.02	<0.01	<0.005	<0.01	<0.005
Winter																							
	PA	<0.025	<0.02	0.27	0.57	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	0.1	<0.01	<0.005	<0.01	<0.005	0.02	0.02	<0.01	<0.005	<0.01	<0.005
	PB-A	<0.025	<0.02	0.07	0.35	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	0.05	<0.01	<0.005	<0.01	<0.005	0.30	0.02	<0.01	<0.005	<0.01	<0.005
	PB-T	<0.025	<0.02	1.4	0.29	<0.005	<0.003	0.21	<0.005	0.19	0.43	0.4	0.03	0.03	0.01	0.03	<0.005	0.20	0.02	<0.01	0.005	0.01	0.005
	PB-L	<0.025	<0.02	0.08	0.13	<0.005	<0.003	<0.01	0.17	<0.1	0.48	<0.05	0.35	<0.01	0.01	<0.01	<0.005	0.02	0.01	<0.01	<0.005	<0.01	<0.005
	PC	<0.025	<0.02	0.10	0.43	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	0.08	<0.01	<0.005	<0.01	<0.005	0.02	0.02	<0.01	<0.005	<0.01	<0.005
Spring																							
	PA	<0.025	<0.02	0.61	2.6	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	4.17	<0.01	0.01	<0.01	0.02	0.02	0.02	<0.01	<0.005	<0.01	<0.005
	PB-A	<0.025	<0.02	0.61	2.77	<0.005	<0.003	<0.01	<0.005	<0.1	<0.1	<0.05	4.33	<0.01	0.01	<0.01	0.01	0.02	0.01	<0.01	<0.005	<0.01	<0.005
	PB-T	<0.025	<0.02	0.09	0.16	<0.005	<0.003	0.45	0.39	0.10	0.49	0.24	1.04	0.02	0.03	<0.01	0.01	0.01	0.02	<0.01	0.005	0.01	0.01
	PB-L	<0.025	<0.02	0.42	3.15	<0.005	<0.003	0.01	<0.005	<0.1	0.11	<0.05	5.27	<0.01	<0.005	<0.01	0.02	<0.01	<0.01		<0.005	<0.01	<0.005
	PC	<0.025	<0.02	0.37	3.24	<0.005	<0.003	<0.01	<0.005	<0.1	0.11	<0.05	5.23	<0.01	<0.005	<0.01	0.01	0.02	0.01	<0.01	<0.005	<0.01	<0.005

PA. 1 km before the disposal site of the tannery effluent; PB-A. About 3 m before the disposal site; PB-T. Previously treated tannery effluent water before disposal into the river; PB-L. At the disposal site; PC. 1 km after the disposal site.

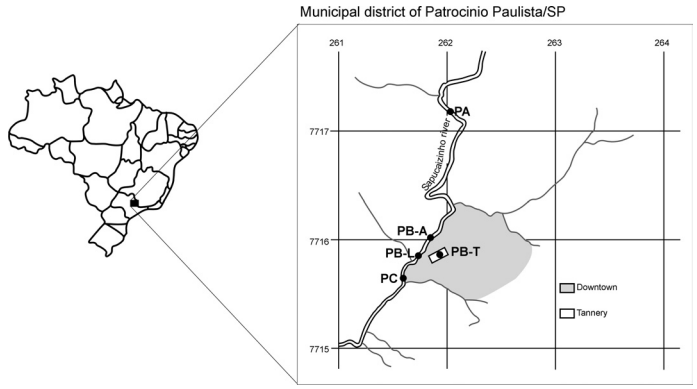

Fig. 1. Geographical localization of the collected site along the Sapucaizinho River. PA. 1 km before the disposal site of the tannery effluent; PB-A. About 3 m before the disposal site; PB-T. Previously treated tannery effluent water before disposal into the river; PB-L. At the disposal site; PC. 1 km after the disposal site.

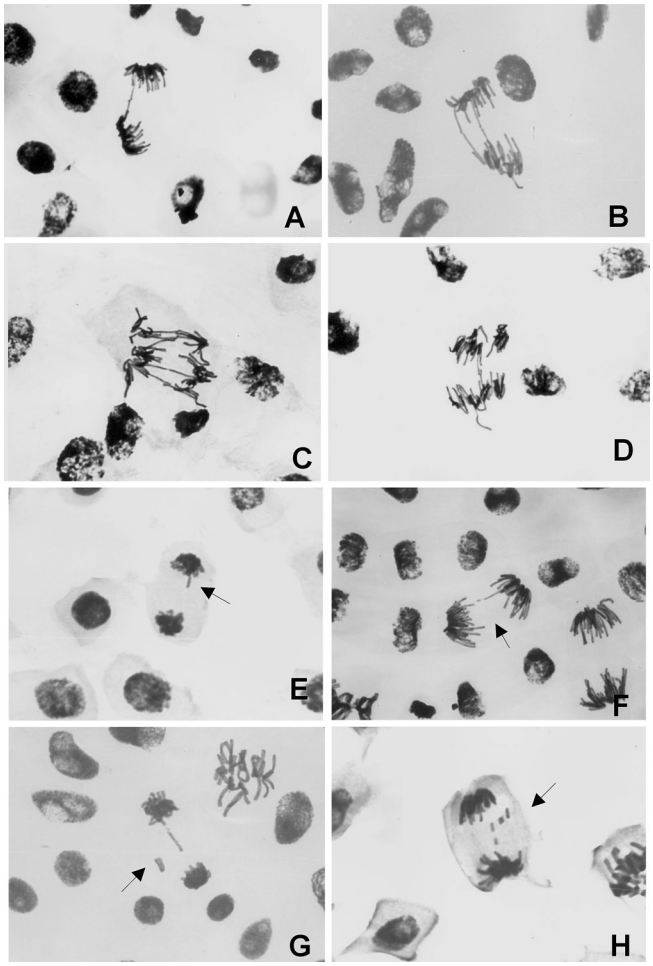

Fig. 2. Anaphase of the *Allium cepa* root tip cells treated with samples of water contaminated with chromium. A and B, anaphases with bridges, C and D, multipolar anaphases, E, cell with laggard chromosomes, F, G and H, cells with breaks demonstrating chromosome fragments.

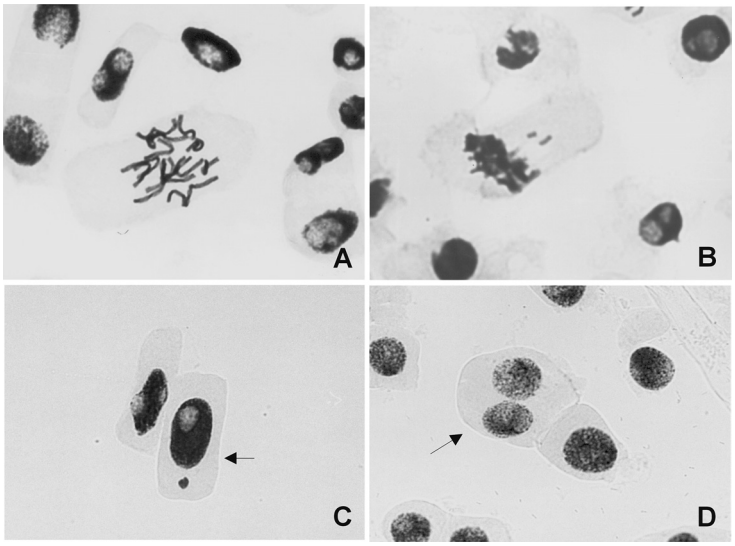

Fig. 3. Aberrant cells observed in *Allium cepa* root tip cells treated with samples of water contaminated with chromium. A, C-metaphase, B, chromosome adherence with breaks, showing chromosome fragments, C, cell with a micronucleus, D, binucleate cell.

treatment) with 9.23% and the PB-L site (site of effluent disposal) with 8.42%. These results indicate a mutagenic effect of the collected water samples probably caused by the chromium residues present in the tannery effluent and confirm by another reported, who demonstrated the genotoxic potential of chromium present in water contaminated with this metal (Liu *et al.* 1982, Sahi *et al.* 1998).

A high frequency of aberrant cells was observed during the 2002 rainy period for samples obtained from the PB-T site and submitted to continuous (9.33%) and chronic discontinuous (9.64%) treatments, for samples obtained from the PB-A site and submitted to the acute discontinuous treatment (5.88%), and for samples obtained from the PC site and submitted to recovery treatment (8.23%).

Alterations probably induced by the action of chromium present in the water samples were observed during both the dry and rainy periods, with these changes occurring in both interphase and dividing cells. Abnormalities observed in interphase cells included cells with micronuclei and binucleate cells, with the former being the most frequent alteration. Mitotic irregularities included irregular anaphases (multipolar and anaphase bridges), chromosome breaks and losses, C-metaphases and chromosome adhesions (Table 2, Figs. 2, 3), with anaphase irregularities being the most frequent. These findings agree with other reported (Liu *et al.* 1982, Sahi *et al.* 1998), who described the potential of chromium to induce mutagenic effects (C-mitosis, anaphase bridges, and chromosome adhesions and fragments) in *A. cepa* roots.

Chromatid or chromosome breaks might be related to the formation of intrachrosomome bridges as well as to the occurrence of chromosome fragments (Fiskesjö 1993). It is also possible that the bridges result from chromosome adhesions which, in this case, are multiple and persist until telophase (Giacomelli 1999). Our data agree with this statement since we noted sequences of cell divisions showing adhesion-mediated bridges which later suffered chromosome rearrangements in which fragments were duplicated to the same chromosome and segregated to the same cell pole, thus resulting in a cell with unbalanced chromosomes (Fig. 4). Many chromosome fragments were scattered within the cell. These fragments probably resulted from simple or double breaks as well as from anaphase bridges that later suffered breaks.

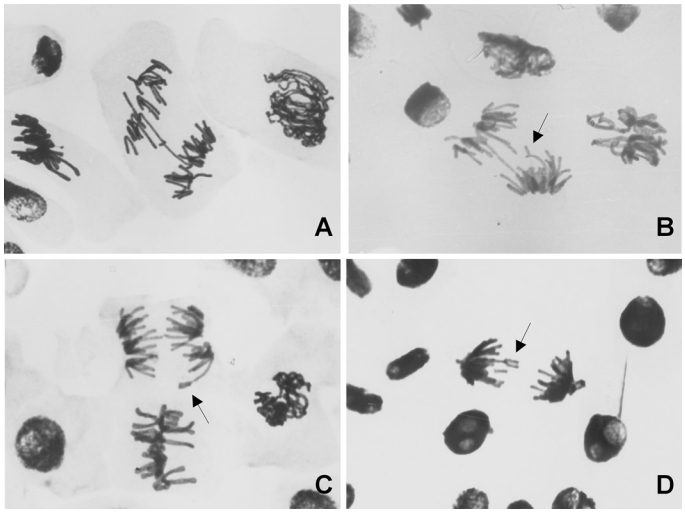

Fig. 4. Anaphases of cells submitted to germination in samples of water contaminated with chromium residues. A, Anaphase with bridges showing telomere adherence, B, C and D, anaphases with chromosomes duplicated in the telomeric portions due to chromosome adherence taking place in the previous phase of the cell cycle.

The chromosome rearrangements observed in the present study might be the events responsible for the cumulative mutagenic effect, as described above for the effect of chromium with respect to alterations in the mitotic index. Thus, the clastogenic effect of chromium might lead to cumulative changes in the cell division of cell generations following those of cells exposed to the metal.

Although the Sapucaizinho river in the Municipality of Patrocínio Paulista/SP is considered to be a good quality river whose water is used for different activities, the permitted concentrations of heavy metal residues in effluents emitted by the leather tanning industries should be revised by the authorities in charge. Assessment of the river water using the *A. cepa* test system clearly demonstrated the mutagenic risk (danger) potential at some collection sites, especially during the dry period when the frequency of clastogenic effects of these water samples was higher. Since chromium is a heavy metal found in tannery effluents, we believe that the alterations observed in the present study resulted from the action of this metal on *A. cepa* cells. All effects were observed in water samples with a chromium concentration below that permitted by the legislation of the State of São Paulo (1995) and the World Health Organization (0.05 mg/l) (Table 2), indicating that this parameter should be revised in terms of its potential to induce genotoxicity.

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