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Cellular damages in the *Allium cepa* test system, caused by BTEX mixture prior and after biodegradation process

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ABSTRACT

Petroleum and derivatives have been considered one of the main environmental contaminants. Among petroleum derivatives, the volatile organic compounds benzene, toluene, ethylbenzene and xylene (BTEX) represent a major concern due to their toxicity and easy accumulation in groundwater. Biodegradation methods seem to be suitable tools for the clean-up of BTEX contaminants from groundwater. Genotoxic and mutagenic potential of BTEX prior and after biodegradation process was evaluated through analyses of chromosomal aberrations and MN test in meristematic and F_1 root cells using the *Allium cepa* test system. Seeds of *A. cepa* were germinated into five concentrations of BTEX, non-biodegraded and biodegraded, in ultra-pure water (negative control), in MMS 4×10^{-4} M (positive control) and in culture medium used in the biodegradation (blank biodegradation control). Results showed a significant frequency of both chromosomal and nuclear aberrations. The micronucleus (MN) frequency in meristematic cells was significant for most of tested samples. However, MN was not present in significant levels in the F_1 cells, suggesting that there was no permanent damage for the meristematic cell. The BTEX effects were significantly reduced in the biodegraded samples when compared to the respective non-biodegraded concentrations. Therefore, in this study, the biodegradation process showed to be a reliable and effective alternative to treat BTEX-contaminated waters. Based on our results and available data, the BTEX toxicity could also be related to a synergistic effect of its compounds.

1. Introduction

Petroleum and derivatives have been considered one of the major environmental contaminants. Due to their worldwide and massive production, transportation and utilization as primary energy source and raw material for several products like plastic, solvents, pharmaceuticals, cosmetics, fuel, synthetic rubber among others, many researchers have been attempting to evaluate the potential impacts of these compounds in both environment and human beings.

Environmental contamination by petroleum and derivatives spills has been commonly reported as a consequence of accidents in loading, discharging, transportation, production of byproducts and combustion (Pedrozo et al., 2002).

BTEX is an acronym that stands for benzene, toluene, ethylbenzene, and xylene. These are volatile organic compounds commonly found in petroleum derivatives that have been particularly studied once they present toxic properties, mobility in the environment and high solubility in water. These features make BTEX a major concern in water pollution, particularly in groundwater contamination (Lovley, 1997; Anneser et al., 2008).

Benzene is recognizably carcinogenic to humans, likely to cause leukaemia and some types of lymphoma (Bird et al., 2005). Nonetheless, *in vitro* studies suggest that benzene is slightly mutagenic or non-mutagenic, although it might cause chromosomal breakages and interfere with the chromosomal segregation (Dean, 1985; Waters et al., 1988; Bird et al., 2005; Chen et al., 2008). It may be present in the water as a result of industrial effluents or spills (Irwin et al., 1997).

According to Hammer (2002), toluene is neurotoxic and teratogenic but the presence of genotoxic and mutagenic effects are still in debate.

Chronic exposures to ethylbenzene are related to harmful effects in the respiratory system and kidneys (NTP, 1999; ATSDR, 2004). According to Henderson et al. (2007), this compound seems to present no genotoxic effects in organisms, but several works about this assumption are also controversial.

Regarding the xylene, chronic exposure to this compound is associated to harmful effects in the nervous system, liver and kidneys (ATSDR, 2004; USEPA, 2005). However, according to the World Health Organization (WHO, 1997), this chemical substance is neither genotoxic nor carcinogenic to both humans and laboratory animals.

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Little is known about the mechanism of action and the toxic effects of BTEX, including possible additive, antagonist or synergistic effects of these substances in target organisms. Most of the information about the interaction of such compounds is restricted to their binary combinations (ATSDR, 2004).

Bioremediation processes are very useful tools to remove environmental organic pollutants. It relies on the endogenous microbiological community from the contaminated environment, kept under controlled aeration and supplied with nutrients, to degrade the natural environment pollutant and/or convert it into less harmful compounds to the organisms (Bamforth and Singleton, 2005).

According to Maila and Cloete (2005), the use of bioindicators in the removal of organic pollutants might be a complement to the current available methods once it provides, based on their biological response, a better assessment of the efficacy of the technological processes applied to improve the environmental conditions. Amongst the methodologies used to analyze the efficiency of biodegradation processes, those that can identify the genotoxic and mutagenic effects of metabolites derived from biodegradation would be mostly recommended (Plaza et al., 2005).

Allium cepa represents one of the most used organisms in biomonitoring studies. Several tests to identify the presence of potentially genotoxic and mutagenic chemical compounds have been successfully performed with this plant. Meristematic and F_1 cells of *A. cepa* present some features that are suitable for cytogenetic studies, thereby being recommended to evaluate both chromosomal aberrations and micronuclei assays for environmental pollutants evaluation (Ateeq et al., 2002; Matsumoto and Marin-Morales, 2004; Fernandes et al., 2007; Caritá and Marin-Morales, 2008; Leme and Marin-Morales, 2008).

The aim of the present work was to evaluate the putative genotoxic and mutagenic effects of different concentrations of BTEX and their biodegraded products through analyses of chromosomal aberrations and micronuclei (MN) in meristematic and F_1 cells of *A. cepa* roots, as well as test the efficacy of biodegradation in reducing the harmful effects of BTEX.

2. Material and methods

2.1. Tested hydrocarbons

To perform the experiments, we tested the BTEX mixture that comprises benzene (purity of 99% – CAS No. 71-43-2), toluene (purity of 99% – CAS No. 108-88-3), ethylbenzene (purity of 99.80% – CAS No. 100-41-4) and xylene (purity of 99%, mixture of isomers – CAS No. 1330-20-7).

Five different BTEX samples were prepared. The first sample (BTEX 1) was prepared considering the water solubility indexes for each BTEX compound (benzene: 1780 mg L^{-1}, toluene: 535 mg L^{-1}, ethylbenzene: 152 mg L^{-1} and xylenes: 135 mg L^{-1} – this value corresponds to the solubility of the less soluble isomer, *m*-xylene). The other two samples were obtained by diluting BTEX 1 by 10 (BTEX 2) and 100 (BTEX 3). BTEX 4 was prepared according to the highest acceptable concentrations of each BTEX compound in drinking water, as established by the Environmental Sanitation Technology Company of São Paulo State/Brazil – CETESB (2004) (benzene: 5 μg L^{-1}, toluene: 170 μg L^{-1}, ethylbenzene: 200 μg L^{-1} and total xylenes: 300 μg L^{-1}). The fifth sample (BTEX 5) was prepared simulating the mixture in values below the acceptable threshold for drinking water (benzene: 1.25 μg L^{-1}, toluene: 42.5 μg L^{-1}, ethylbenzene: 50 μg L^{-1} and total xylenes: 75 μg L^{-1}).

The negative control comprised ultra-pure water and the positive control methyl methanesulfonate – MMS (CAS No. 66-27-3) at 4×10^{-4} M.

These same five samples of BTEX were submitted to a biodegradation process by bacteria selected from a raw effluent of a petroleum refinery. The method used to perform the biodegradation was the one described by Mazzeo et al. (2010). After this process, the biodegraded samples presented the following concentration: BTEX 1 (benzene: 322.25 mg L^{-1}, toluene: 63.7 mg L^{-1}, ethylbenzene: 14.8 mg L^{-1} and xylene: 4.04 mg L^{-1}), BTEX 2 (benzene: 20.7 mg L^{-1}, toluene: 2.5 mg L^{-1}, ethylbenzene: 0.3 mg L^{-1} and xylene: 0.4 mg L^{-1}), BTEX 3 (benzene: 0.08 mg L^{-1}, toluene: 0.03 mg L^{-1}, ethylbenzene: 0.01 mg L^{-1}, xylene: absent), BTEX 4 (benzene: 0.035 mg L^{-1}, toluene: 0.016 mg L^{-1}, ethylbenzene: absent, xylene: absent), BTEX 5 (all absent).

The negative control was performed with ultra pure water and the positive control with methyl methanesulfonate – MMS (CAS No. 66-27-3), in a concentration of 4×10^{-4} M. Culture medium of the bacteria used in the samples' degradation was used as a blank.

2.2. Test-organism

Seeds of *A. cepa* ($2n = 16$ chromosomes) of a same stock and variety were used to evaluate the genotoxic and mutagenic BTEX effects of the five tested samples.

2.3. Chromosome aberration and micronucleus tests

The seeds of *A. cepa* were germinated in glass jars (500 mL), with Teflon membrane caps, to prevent the compounds to volatilize into the atmosphere, assuring that the BTEX samples would remain the same during the entire experimental period.

The assays were performed by adding 60 mL of each non-biodegraded and biodegraded BTEX sample, MMS (positive control), ultra-pure water (negative control) and blank solution (biodegradation control) in separated jars. All assays were carried out in duplicate. The seeds were placed to germinate onto polypropylene membranes moistened with each one of the mentioned solutions. After reaching 2 cm in length the roots were collected and fixed in Carnoy's fixative 3:1 (ethanol: acetic acid, v/v) for 6–12 h. Afterwards, they were transferred to a new Carnoy's fixative and stored at 4 °C before utilization.

The slide preparation with the meristematic and F_1 root cells followed the procedure described by Leme and Marin-Morales (2008).

2.4. Analysis of results

In order to evaluate cell damages, 10 slides per treatment were prepared. Approximately 500 cells were analyzed per slide, totalizing 5000 cells per test in each treatment.

The genotoxic potential was determined according to the observation and quantification of any chromosomal and nuclear abnormality in the meristematic cells of all treatments. The evaluation of mutagenic effects was carried out by scoring micronucleated cells of both meristematic and F_1 regions in all slides of all treatments.

The obtained results were compared to the negative control using the Mann–Whitney statistical test ($p < 0.05$).

The frequencies of alterations between non-biodegraded and biodegraded samples of each BTEX sample were compared in order to evaluate the efficacy of the biodegradation process in the reduction of the BTEX-related genotoxic and mutagenic damages. In this case, comparisons were done based on the mean values of non-biodegraded samples and the exact values from the biodegradation test, once two repetitions were performed in the non-biodegraded samples and just one test was carried out for the biodegraded samples.

Table 1
Frequencies of chromosomal and nuclear aberrations observed in meristematic cells of *Allium cepa* exposed to different non-biodegraded and biodegraded BTEX concentrations and the comparison of the frequency of genotoxic damages between non-biodegraded BTEX samples and their corresponding biodegraded samples.

Assays	Treatment	NC	PC	BLANK	BTEX 1	BTEX 2	BTEX 3	BTEX 4	BTEX 5
Chromosomal aberrations									
Adherence	Non-biodegraded	0.10 ± 0.10	0.33 ± 0.26	–	0.31 ± 0.21[a]	0.60 ± 0.32[a]	0.56 ± 0.27[a]	0.69 ± 0.40[a]	0.60 ± 0.21[a]
	Biodegraded	0.15 ± 0.15	0.39 ± 0.19	0.23 ± 0.17	0.29 ± 0.18	0.33 ± 0.26	0.35 ± 0.31[c]	0.29 ± 0.22[c]	0.14 ± 0.16[c]
Laggard	Non-biodegraded	0.00 ± 0.00	0.02 ± 0.06	–	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06	0.00 ± 0.00
	Biodegraded	0.02 ± 0.06	0.08 ± 0.14	0.12 ± 0.14	0.02 ± 0.06	0.12 ± 0.16	0.08 ± 0.14	0.08 ± 0.13	0.10 ± 0.10
C-metaphase	Non-biodegraded	0.00 ± 0.00	0.00 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06
	Biodegraded	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06	0.00 ± 0.00
Chromosomal loss	Non-biodegraded	0.00 ± 0.00	0.00 ± 0.00	–	0.12 ± 0.14	0.02 ± 0.06	0.04 ± 0.08	0.22 ± 0.29	0.02 ± 0.07
	Biodegraded	0.02 ± 0.06	0.06 ± 0.10	0.00 ± 0.00	0.12 ± 0.10[b]	0.08 ± 0.10	0.08 ± 0.10	0.14 ± 0.16	0.06 ± 0.09
Chromosomal bridge	Non-biodegraded	0.04 ± 0.07	0.19 ± 0.13[a]	–	0.08 ± 0.10	0.29 ± 0.25[a]	0.12 ± 0.10	0.08 ± 0.14	0.08 ± 0.14
	Biodegraded	0.00 ± 0.00	0.17 ± 0.17	0.06 ± 0.13	0.08 ± 0.13	0.06 ± 0.09[c]	0.19 ± 0.18	0.06 ± 0.09	0.09 ± 0.13
Chromosomal break	Non-biodegraded	0.02 ± 0.06	0.23 ± 0.18[a]	–	0.18 ± 0.19	0.25 ± 0.19[a]	0.02 ± 0.06	0.14 ± 0.19	0.04 ± 0.08
	Biodegraded	0.00 ± 0.00	0.27 ± 0.28[b]	0.02 ± 0.06	0.06 ± 0.09	0.06 ± 0.09[c]	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06
Multipolarity	Non-biodegraded	0.00 ± 0.00	0.00 ± 0.00	–	0.00 ± 0.00	0.08 ± 0.13	0.00 ± 0.00	0.02 ± 0.06	0.02 ± 0.06
	Biodegraded	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06	0.02 ± 0.06	0.02 ± 0.06	0.03 ± 0.07	0.02 ± 0.06	0.00 ± 0.00
Nuclear abnormalities									
Nuclear buds	Non-biodegraded	0.29 ± 0.30	1.25 ± 0.48[a]	–	3.55 ± 1.07[a]	2.62 ± 0.72[a]	1.48 ± 0.58[a]	3.04 ± 1.14[a]	1.22 ± 0.42[a]
	Biodegraded	0.52 ± 0.33	1.34 ± 1.02	0.65 ± 0.37	1.42 ± 0.70[b,c]	0.94 ± 0.30[b,c]	1.13 ± 0.70[b,c]	0.97 ± 0.75[c]	0.91 ± 0.41
Polynucleated cell	Non-biodegraded	0.00 ± 0.00	0.00 ± 0.00	–	0.14 ± 0.13[a]	0.06 ± 0.13	0.10 ± 0.25	0.00 ± 0.00	0.06 ± 0.19
	Biodegraded	0.00 ± 0.00	0.02 ± 0.06	0.08 ± 0.10	0.10 ± 0.14	0.14 ± 0.16	0.08 ± 0.10	0.06 ± 0.09	0.02 ± 0.06
Polyploid cell	Non-biodegraded	0.00 ± 0.00	0.00 ± 0.00	–	0.04 ± 0.08	0.02 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Biodegraded	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mini cell	Non-biodegraded	0.00 ± 0.00	0.02 ± 0.06	–	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Biodegraded	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lobulated nuclei	Non-biodegraded	0.00 ± 0.00	0.08 ± 0.13	–	0.14 ± 0.24	0.09 ± 0.16	0.10 ± 0.14	0.02 ± 0.06	0.04 ± 0.08
	Biodegraded	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total of Aberrations	Non-biodegraded	0.44 ± 0.30	2.12 ± 0.55[a]	–	4.55 ± 1.34[a]	4.03 ± 0.75[a]	2.40 ± 0.69[a]	4.23 ± 1.21[a]	2.10 ± 0.74[a]
	Biodegraded	0.71 ± 0.30[b]	2.33 ± 1.0[b]	1.17 ± 0.48	2.15 ± 0.8[b,c]	1.74 ± 0.5[b,c]	1.94 ± 0.7[b,c]	1.62 ± 0.61[b,c]	1.33 ± 0.42[c]

NC, negative control; PC, positive control.
[a] Statistically different from negative control ($p < 0.05$).
[b] Statistically different from blank ($p < 0.05$).
[c] Significant difference between biodegraded and non-biodegraded concentrations ($p < 0.05$).

3. Results

3.1. Genotoxic induction

The types and frequencies of the observed chromosomal and nuclear aberrations are shown in Table 1 and Fig. 1.

Results based on the quantification of chromosomal and nuclear aberrations in meristematic cells of *A. cepa* indicated significant genotoxic effects related to chromosomal adherence and nuclear buds in all non-biodegraded samples. The second highest sample (BTEX 2) led to a significant increase in the number of chromosomal bridges and breaks. A significant frequency of polynucleated cells was observed in the highest tested sample (BTEX 1). The total amount of aberrations was significant in all tested samples when compared to the negative control.

After biodegradation, significant differences in relation to the blank (biodegradation control) were detected only for the presence of nuclear buds (BTEX 1, 2, 3) and chromosomal loss (BTEX 1). The total number of aberrations was significantly higher in the three highest concentrations, when compared to the blank.

The comparison of total chromosomal and nuclear abnormalities between BTEX samples prior and after the biodegradation process revealed a significant decrease of aberrations for all tested samples. Among the significant alterations observed in the non biodegraded samples (adherence, chromosomal bridge, chromosomal break and nuclear buds) when compared to their respective degraded sample, presented a statistically significant decrease, indicating a reduction of the genotoxic effects after biodegradation process (Table 1).

3.2. Mutagenic induction

The mutagenic effects, evaluated by the MN test, were recorded by scoring both meristematic and F1 root cells of *A. cepa* (Table 2 and Fig. 1).

In the meristematic cells, the BTEX samples 1, 2, 4 and 5 induced an increase in MN frequency when compared to the negative control. On the other hand, no significant differences were observed between the BTEX tested samples and the negative control for F_1 regions' cells. No significant frequency of MN was recorded for the biodegraded samples in both meristematic and F_1 cells (Table 2).

When MN frequencies of non-biodegraded BTEX samples were compared to the corresponding biodegraded ones, a significant decrease in the biodegraded sample 4 was detected in meristematic cells (Table 2). No differences in the MN frequency were observed between the non-biodegraded samples and the biodegraded samples for the F_1 cells.

4. Discussion

Due to the intensive utilization of BTEX in several human activities (Hutchins et al., 1991; Barreto et al., 2007), this compound is commonly found in the environment and mainly associated to groundwater contamination (Lovley, 1997).

Although some studies have been performed with the components of BTEX mixture separately, conclusive assessment on the genotoxic and mutagenic effects of the whole BTEX mixture is still quite limited (ATSDR, 2004).

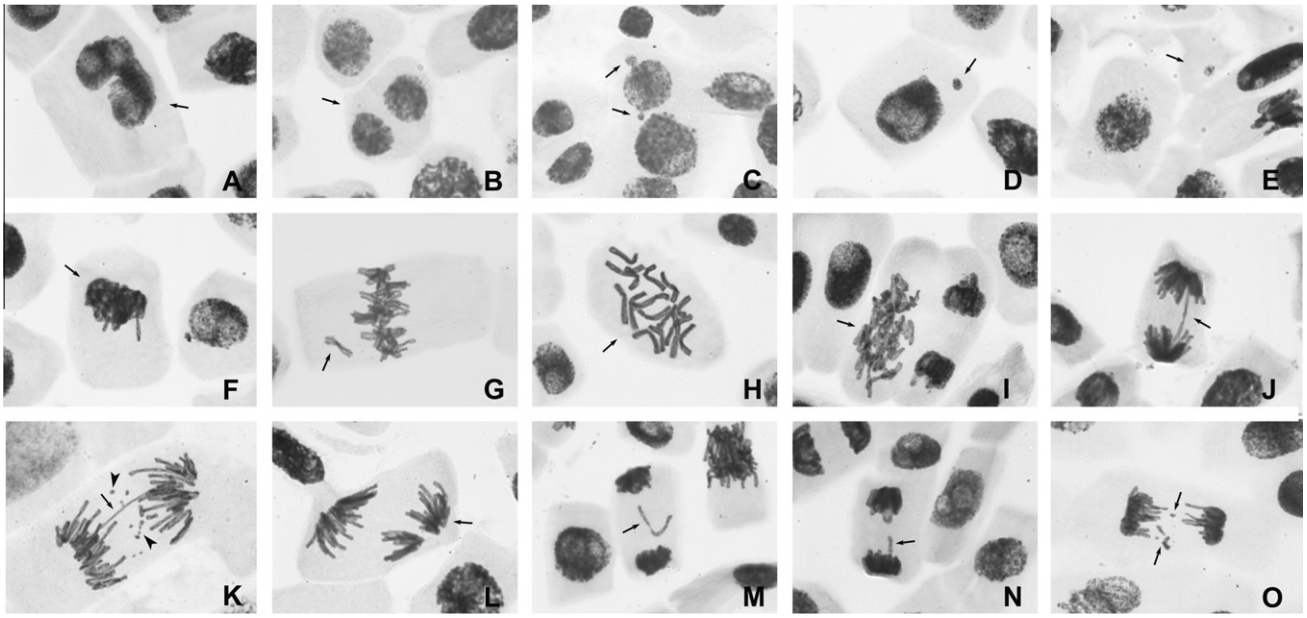

Fig. 1. Chromosomal and nuclear aberrations observed in meristematic cells of *Allium cepa* exposed to different BTEX samples. (A) bilobulated nucleus (arrow); (B) polynucleated cell (arrow); (C) cell bearing two nuclear buds (arrows); (D) cell with micronucleus (arrow); (E) mini cell (arrow); (F) metaphase with adherence (arrow); (G) metaphase with chromosomal loss (arrow); (H) C-metaphase; (I) polyploid metaphase with adherence (arrow); (J) anaphase with chromosomal bridge (arrow); (K) anaphase with chromosomal bridge (arrow) and breaks (arrowhead); (L) multipolar anaphase; (M) telophase with chromosomal loss (arrow); (N) telophase with chromosomal laggard (arrow); (O) telophase with chromosomal break (arrow).

Table 2
Frequencies of micronuclei (MN) observed in meristematic and F_1 cells of *Allium cepa* exposed to different non-biodegraded and biodegraded BTEX samples and the comparison of the frequency of mutagenic damages between non-biodegraded BTEX samples and their corresponding biodegraded samples.

Assays	Treatment	MN	MN F_1
NC	Non-biodegraded	0.04 ± 0.08	0.79 ± 0.65
	Biodegraded	0.11 ± 0.13	0.77 ± 0.43
PC	Non-biodegraded	3.47 ± 1.48[a]	5.30 ± 2.89[a]
	Biodegraded	1.94 ± 0.62[b]	5.29 ± 1.91[b]
BLANK	Non-biodegraded	–	–
	Biodegraded	0.18 ± 0.21	0.39 ± 0.50
BTEX 1	Non-biodegraded	0.30 ± 0.27[a]	1.00 ± 0.92
	Biodegraded	0.12 ± 0.14	0.56 ± 0.63
BTEX 2	Non-biodegraded	0.27 ± 0.23[a]	0.67 ± 1.24
	Biodegraded	0.16 ± 0.16	0.96 ± 0.83
BTEX 3	Non-biodegraded	0.10 ± 0.14	1.68 ± 1.26
	Biodegraded	0.25 ± 0.21	0.69 ± 0.48
BTEX 4	Non-biodegraded	0.28 ± 0.25[a]	1.01 ± 0.70
	Biodegraded	0.10 ± 0.14[c]	0.46 ± 0.49
BTEX 5	Non-biodegraded	0.28 ± 0.26[a]	0.44 ± 0.46
	Biodegraded	0.14 ± 0.13	0.55 ± 0.79

MN, micronuclei; NC, negative control; PC, positive control.
[a] Statistically different from negative control ($p < 0.05$).
[b] Statistically different from blank ($p < 0.05$).
[c] Significant difference between biodegraded and non-biodegraded concentrations ($p < 0.05$).

According to Leme and Marin-Morales (2008), the organism *A. cepa* proved to be very sensitive to detect toxic effects of hydrocarbons in petroleum, which was also confirmed in the present study.

Genotoxicity assays, comprising different BTEX concentrations, showed that this mixture can induce several types of both nuclear and chromosomal aberrations in meristematic cells of *A. cepa*. The most frequent chromosomal aberration in all tested samples was chromosomal adherence. According to Fiskesjö and Levan (1993), Marcano and Del-Campo (1995), Marcano et al. (1998) and Türkoglu (2007), the chromosomal adherence is an indicator of toxic effects on the genetic material that can lead to irreversible damages to the cells, including cell death. This aberration is also associate to the formation of chromosomal bridges and, eventually, chromosomal breaks (Marcano et al., 2004; Leme et al., 2008), thereby explaining the significant frequencies of both alterations in the second highest sample herein tested.

Regarding the nuclear alterations, the most frequent abnormality was the occurrence of nuclear buds. According to Serrano-García and Monteiro-Montoya (2001), nuclear bud is an acknowledged genotoxic alteration and its formation is related to the initiation of the nuclear envelope formation prior to the total migration of the chromosomes to the poles and, consequently, their incorporation into the nuclei. According to these authors nuclear buds may be derived from chromosome breaks, bridges and chromosomes rearrangements caused by clastogenic agents, which hinder the proper reorganization of the chromatin in the nucleus. According to Shimizu et al. (2000), nuclear buds may also be the result of cellular activities that promote the elimination of the amplified genetic material.

Chromosomal damages have been reported in workers exposed to low levels of benzene (Green, 1988), and similar results were confirmed in evaluations with other animals (USEPA, 1985). Studies performed by Rank and Nielsen (1994) showed that benzene increased significantly the frequency of chromosomal aberrations in meristematic root cells of *A. cepa*, exposed to concentrations of 100 μM. The other BTEX components are thought to do not present significant genotoxic effects, although several studies provided controversial and confusing results, particularly about the toxic effects in plants (Dean, 1985; WHO, 1986, 1997). Therefore, we hypothesize that the genotoxic effects caused by BTEX in the meristematic cells of *A. cepa*, might be related to the genotoxicity of benzene and a synergistic action among the components in the mixture as well.

Bioassays carried out in stamen hair of *Tradescantia* by Van't Hof and Schairer (1982) showed that a concentration of 4.000 mg L^{-1} of gaseous benzene caused a significant increase in the frequency

of mutations in this plant, confirming the mutagenic potentiality in this test-organism.

The significant occurrence of micronuclei in meristematic cells of *A. cepa* indicates that BTEX is capable of causing mutagenic effects in this test-organism. Micronuclei might be formed by acentric fragments (clastogenic action), chromosomal losses or malformation of the mitotic fuse (aneugenic action) (Sudhakar et al., 2001), or even by the elimination of amplified genetic material (Fernandes et al., 2007; Fenech et al., 2011).

In our experiment, we believe that micronuclei were formed by the induction of chromosomal breaks during cell division, as well as by the expulsion of excess genetic material by nuclear buds, which will be later eliminated in the form of a micronucleus.

According to Whysner et al. (2004) and Mondrala and Eastmond (2010) benzene is considered an inhibitor of the enzyme topoisomerase II, responsible for the reconnection of fragments of DNA during the replication process, which results in the clastogenic effect of benzene. Moreover, Alberts et al. (2008) affirm that the inhibition of topoisomerase II leads to a tangle of the daughter chromosomes, which remain intertwined and therefore unable to separate after replication. Thus, if such damage is not repaired, replication of a damaged DNA may lead to the amplification of the genetic material. The amplification promoted by benzene and its metabolites was confirmed by Ji et al. (2009) when they verified the endoreduplication in TK6 cells, after exposure to these compounds. This excess material, originated from the amplification, is eliminated from the nucleus as a micronucleus (Fernandes et al., 2007).

However, results based on mutagenicity assays indicated that benzene is slightly or non-mutagenic (Dean, 1985; Waters et al., 1988; Bird et al., 2005). Therefore, the present results can be explained by a synergistic action of BTEX mixture, whose effects strengthen the mutagenic action of its components. Our results obtained from the BTEX mixture corroborate those reported by Roma-Torres et al. (2006), which analyzing workers exposed to BTX (benzene, toluene and xylene), showed that this mixture was able to induce both mutagenic and genotoxic effects, as well as chromosomal aberrations, micronuclei and DNA damages in leukocytes from the peripheral blood.

Since the F_1 cells are derived from mitotic division of meristematic cells (Ma and Xu, 1986), the lack of significant MN frequencies in the F_1 region of *A. cepa* roots, independently on the tested sample, means that the harmful effects observed in the meristematic cells were not transferred or fixed to the F_1 cells. The presence of cellular alterations in meristematic cells and the lack of correspondence of MN in F_1 cells might be related to cell inviability, caused by the recorded disturbances in meristematic cells exposed to BTEX, like the high index of chromosome adherence, which probably prevented the progress of the damages to the F_1 region.

The BTEX biodegradation process by bacteria was efficient in reducing damages observed in the genetic material of *A. cepa* cells. The comparisons between the obtained results with the tested BTEX samples and their corresponding biodegraded samples showed a significant decrease of both genotoxic and mutagenic damages. However, when the biodegraded treatments were compared to the blank, the frequency of the total of nuclear and chromosomal aberrations remained as significant as those from non-biodegraded assays in the case of the three highest samples. This might be explained by the fact that BTEX was still present in the biodegraded samples in enough concentration to cause genotoxic effects in the cells.

5. Conclusions

Based on the obtained results with the test-organism *A. cepa*, BTEX proved to be potentially genotoxic and mutagenic for the meristematic cells, even when low concentrations were tested.

The species *A. cepa* represents an efficient test-organism to evaluate the toxic effects induced by petroleum hydrocarbons, such as BTEX. Chromosomal aberrations assay and MN test with this organism showed to be sensitive tools to detect BTEX-induced DNA damages.

Analyses of genotoxicity and mutagenicity in meristematic and F1 cells of *A. cepa* indicated that the biodegradation process by bacteria was efficient in the biodegradation of aqueous BTEX mixture, once it decreased both mutagenic and genotoxic effects, thus showing that the harmful compounds in the mixture were degraded into non-toxic metabolites to the cells.

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