

Sugarcane Vinasse, a Residue of Ethanol Industry: Toxic, Cytotoxic and Genotoxic Potential Using the *Allium cepa* Test

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Received 2 March 2016; accepted 4 April 2016; published 7 April 2016

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Abstract

The search for fuels to replace petroleum consumption has caused an increase in the production of biofuels worldwide. The ethanol, which comes from sugarcane, is an energy resource with low polluting potential, but its production generates other environmental problems. On average, 10 to 15 liters of vinasse are generated while preparing each liter of ethanol. Vinasse is the final by-product of the biomass distillation, mainly for the production of ethanol, from different cultures such as sugarcane. Because excessive quantities of vinasse are produced, alternatives have been required for use, for example as fertilizer, in a process known as fertigation. These excessive amounts of vinasse applied in soils have generated adverse effects on soil properties and to the organisms. This study carried out the toxic, cytotoxic and genotoxic potential of sugarcane vinasse obtained from two different harvests (Samples I and II), using the *Allium cepa* organism test. *A. cepa* seeds were exposed to raw vinasse (RV) and diluted in different concentrations: control soil + raw vinasse (SV); vinasse diluted in water at 50% + control soil (V 50%); vinasse diluted in water at 25% + control soil (V 25%); vinasse diluted in water at 12.5% + control soil (V 12.5%). The chemical characterization of vinasse samples showed a low pH and high concentration of potassium. The results demonstrate that the two RV samples tested are toxic, since no seeds germination was observed. The cytotoxic potential was observed in the sample II of SV and V (50%). All groups evaluated in samples I and II, induced chromosomal alterations, statistically significant compared with negative control. An increase in frequency of micronuclei in meristematic cells was observed in the SV (Sample I) and all groups evaluated in samples II. Based on the results it is concluded that the genetic material of the test-system was damaged when exposed to sugarcane vinasse,

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suggesting that one should be very careful in the use of this waste that has been used sometimes indiscriminately in soils.

Keywords

Micronucleus, Chromosome Aberrations, Agroindustrial Residue

1. Introduction

In recent years worldwide, it has increased the demand for “green” fuel (biofuels) as an alternative to fossil fuels [1]. Biofuels are obtained from different crops such as sugarcane, eucalyptus, corn, soybean, poplar etc. [2]. Sugarcane as a biofuel crop has expanded in the last decade and today is ethanol obtained from sugarcane unites biofuel more employees [3]. Brazil’s increased production, turning into the largest producer of sugarcane in the world, devotes roughly 50% ethanol [4]. This crop has received much attention as one of the most socioeconomically important. Sugarcane/ethanol industry results in lots of waste as the vinasse. For each liter of ethanol, 10 to 15 liters of sugarcane vinasse are produced [5] [6]. Vinasse is the final by-product of the biomass distillation, mainly of the sugar-ethanol industry. This is dark brown slurry, unpleasant odor, acidic pH and a high organic content [7]. Because of its high pollution potential and the large quantities produced, alternatives for its use have been studied. Among them, it includes the use as a fertilizer in fertigation of the own sugarcane culture, by the application of raw vinasse in the soil, sometimes indiscriminately, causing major problems. Studies have shown that the vinasse application in soil may cause unbalance of nutrients, leaching of metals, salinization etc. [8].

The soil is an essential component of ecosystems and is the main substrate used by plants, playing multiple roles, such as regulation of the distribution, drainage, and infiltration of rainfall and irrigation [9]. Despite its importance, only few recent studies have focused on soil contamination compared those on water and air. For studies of soil contamination, higher plants are used worldwide, considered more sensitive and simpler than those using animals. According to Ma *et al.* (1995) [10], plants as direct receptors of pollutants, provide an important tool for genetic tests and environmental monitoring. They are considered excellent genetic models for detecting environmental mutagens [11] [12].

A. cepa is one of the best test-systems already developed, due to its high sensitivity to chemical agents and good correlation with mammal test-systems [13] [14]. The sensitivity of tests with *A. cepa* has been reported as superior in 82% of the results obtained with rodents [15]. Tests with *A. cepa* have received special attention, mainly after being adapted to evaluate the effects of pollutants in the soil and water, such as metals [16], chemical compounds from industrial effluents [17] and pesticides [18] [19].

In this context, in order to obtain more information about the possible effects of sugarcane vinasse on plants, the present study evaluated the toxic, cytotoxic and genotoxic potential of the raw sugarcane vinasse and diluted added to the soil, of two different harvests (Samples I and II), using the *A. cepa* test.

2. Material and Methods

2.1. Tested Substance—Sugarcane Vinasse

Vinasse samples (I and II) were collected at a sugarcane processing facility, located in São Paulo State, Brazil (22°21'25"S/47°23'03"W). The samples were maintained in a cold storage chamber (4°C), at the Department of Biochemistry and Microbiology of the UNESP (São Paulo State University), Rio Claro, São Paulo, to minimize bacterial degradation until beginning of experiments.

2.2. Test-Organism

The biological material used on the evaluation of sugarcane vinasse toxicity was the seeds of *A. cepa* (Liliaceae), from the same lot and variety (Baia Periforme). They were stored in the dark at a temperature between 6°C and 10 °C until use.

2.3. Control Substrate

The soil used for the application of sugarcane vinasse samples, termed control soil (CS), was obtained on the UNESP Rio Claro Campus, São Paulo (22°24'36"S/47°33'36"W). For the bioassays, soil samples were homogenized, dried at ambient temperature and sieved with 4-mm mesh sieves and subjected to chemical characterization.

2.4. Chemical and Physico-Chemical Analysis

Physicochemical analysis of the control soil (CS), raw sugarcane vinasse (RV), metals analysis of sugarcane vinasse samples and polycyclic hydrocarbons in control soil and the combinations of control soil and sugarcane vinasse samples, it was performed for guidelines of soil quality (mg/Kg) and groundwater quality in São Paulo State, according to the current legislation—CETESB 195/2005-E—to Environmental Sanitation Technology Company (Companhia de Tecnologia de Saneamento Ambiental-CETESB). For the CS samples, 16 priority aromatic polycyclic hydrocarbons established by the Environmental Protection Agency (EPA) were quantified following EPA 8270D method.

The maximum dosage of vinasse used was determined according to the current legislation P4.231 of the CETESB (2005).

2.5. Treatment Groups

Treatments were prepared with two different samples (I and II) of sugarcane vinasse, different controls (CS, NC and PC) and all bioassays were conducted in duplicate.

Groups 1: Negative control (NC)—ultrapure water.

Groups 2: Control soil (CS).

Groups 3: Raw vinasse (Samples I and II) (RV).

Groups 4: Raw vinasse (Samples I and II) + control soil (SV).

Groups 5: Vinasse (Samples I and II) diluted in water at 50% + control soil (V 50%).

Groups 6: Vinasse (Samples I and II) diluted in water at 25% + control soil (V 25%).

Groups 7: Vinasse (Samples I and II) diluted in water at 12.5% + control soil (V 12.5%).

Groups 8: Positive control (PC), aneugenic herbicide Trifluralin® (TRIF) (CAS N01582-09-8) at a concentration of 0.019 mg/mL [18].

2.6. *Allium cepa* Assay

To evaluate the toxic, cytotoxic and genotoxic potential of the different groups, *A. cepa* seeds were used according to a modified version of Grant's protocol (1982) [13]. All treated groups with the different concentrations and samples (I and II) of sugarcane vinasse (RV, SV, V 50%, V 25% and V 12.5%), were placed in Petri dishes with 100 seeds each. As well as, the negatives controls groups (NC and CS) and positive control groups (TRIF). The germination of all treatments was monitored between 4 to 5 days at 22°C, until root tips reached 1.5 cm in length. Root tips were collected and fixed in Carnoy's solution (3:1 ethanol/glacial acetic acid v:v) and stored at 4°C until use. For slide preparation, roots were prepared with the Feulgen reaction [20], followed by three baths with distilled water to remove the fixative excess. After the baths, roots were hydrolyzed with HCl 1 N for 8 minutes at 60°C, and later rinsed with distilled water and stained with Schiff reagent in the dark for 2 h. The root meristems and the F₁ region were sectioned in a drop of acetic carmin (2%), coverslipped and gently pressed with the aid of a metal knife. Coverslips were removed with liquid nitrogen and after drying, slides were mounted with syntetic resin for later examination under light microscope. On each slide, 1000 meristem and F₁ cells of *A. cepa* were examined (totalling approximately 5000 cells per treatment) under a light microscope.

2.7. Toxic, Cytotoxic and Genotoxic Effects on Meristematic Cells of *A. cepa*

The toxicity was evaluated based on the seed germination index, obtained by the ratio between the germinated seeds number and all the seeds exposed to germination.

Cytotoxicity was assessed based on the quantification of morphological cell alterations indicating cell death and on the mitotic index (MI), characterized by the total number of dividing cells in the cell cycle following the equation: $MI = (\text{number of dividing cells} / \text{total number of observed cells}) \times 100$.

In the evaluation of the genotoxicity, cells with chromosome alterations were quantified and it was calculated of chromosomal aberration index (CAI) by the formula $CAI = (\text{number of cells with CA} / \text{total number of observed cells}) \times 100$.

2.8. Micronuclei in Meristematic and F₁ Region Cells of *A. cepa*

The micronuclei (MN) were counted in meristematic region cells and for the F₁ region cells. The observation of MN in the F₁ region cells permits examine possible damage fixation.

2.9. Statistic Analysis

The mean and standard deviation was calculated from the mitotic, chromosomal aberrations index and MN in meristematic and the F₁ region cells. The data do not follow a normal distribution (Shapiro-Wilk) and Kruskal-Wallis test show differences between groups. All groups were compared to the NC by the non-parametric Mann-Whitney test, with the program Statistical Package for the Social Sciences for Windows, version 15.0, (SPSS Inc., Chicago, IL, EUA).

3. Results

3.1. Fertility Analysis of the Control Substrate

To ensure the correct application of sugarcane vinasse, similar to that applied in the field, the following parameters of fertility and the agronomic potential of the control soil (CS) were measured: pH, organic matter (OM), residual phosphorus (P res), potassium (K), calcium (Ca), magnesium (Mg), exchangeable aluminum (H+Al), sum of bases (SB), cation exchange capacity (CTC), base saturation (V%) and Ca/Mg and Mg/K ratios (**Table 1**). The control soil was classified as clay, slightly acidic, with low levels of organic matter and heavy metals.

3.2. Chemical Characterization of Raw Sugarcane Vinasse Samples

The results of the physicochemical analyses of the control soil and sugarcane vinasse samples are presented in **Table 2**. The pH of sugarcane vinasse was low in both samples, while biochemical oxygen demand (BOD), chemical oxygen demand (COD) and potassium levels were high.

Metal analyses of the control soil and sugarcane vinasse samples are presented in **Table 3**. Arsenic and copper concentrations in the control soil are above the reference levels for soil quality established by CETESB (195/2005-E), but below the limits for intervention in agricultural areas, which are 35 mg/Kg for arsenic and 200 mg/Kg for copper. The concentrations of barium, copper, chrome, mercury, molybdenum, nickel and zinc were below the maximum concentration allowed (MCA).

3.3. Characterization of Organic Compounds in Soil Samples

None of the 16 priority aromatic polycyclic hydrocarbons (APHs) determined by the Environmental Protection Agency (EPA) was found in the study samples, as shown in **Table 4** for both samples.

3.4. *Allium cepa* Assay

The results from the *A. cepa* assay, in which the cells were exposed to sugarcane vinasse samples and the negative and positive controls, are shown below in **Table 5** (Sample I) and **Table 6** (Sample II).

Table 1. Data on the control soil fertility.

Samples	pH	g/dm ³	mg/dm ³	mmol/dm ³ TFSA						%	Ratios	
	Ca/Cl ₂	OM	P res	K	Ca	Mg	H + Al	SB	CTC	V	Ca/Mg	Mg/K
Crop I	6.2	18	3	0.8	2	1	88	3.9	91.9	4.2	2	1.25
Crop II	5.1	17	3	1.7	9	6	30	16.6	47.1	3.5	-	-

TFSA: dried soil air; OM: organic matter; P res: residual phosphorus; SB: sum of bases; CTC: cation exchange capacity; V: base saturation.

Table 2. Physicochemical analysis of the control soil and raw sugarcane vinasse samples.

Parameters	Sample I		Sample II		Method
	CS (mg/Kg)	RV (mg/Kg) (V)	CS (mg/Kg)	RV (mg/Kg) (V)	
Ammonia (mg/L)		<QL		<QL	USEPA 440/5-85-001
Calcium (mg/L)	29.3	719	42.8	671	SM21 3120 B
COD (mg/L)		5046		7941	SM21 5210 B
BOD (mg/L)		13380		25225	SM21 5220 D
Hardness (mg CaCO ₃ /L)		2493		276	SM21 2340 B
Total phosphate (mg/L)	317	1.3		NE	SM21 4500-P C
Potassium (mg/L)	437	2056	<0.008	3401	SM21 3120 B
Non-filtrable residue (mg/L)		2765		1800	SM21 3120 D
Sodium (mg/L)	<QL	50.2		114	SM21 3120 B
Sulfate (mg/L)		710	<0.5	2993	SM21 4500- SO ₄ ²⁻ E
Organic Carbon	12.6		32.3		SSSA Cap40
Electric Conductivity (µs/cm)	115	13530	97.9	15110	SM21 3120B
Total Sulfur	151	1219	123	1681	SM21 3120B
Total Phosphorus	182		317	207	SM21 3120B
Total Magnesium	<QL	237	<QL	264	SM21 3120B
Nitrate (mg/Kg)	4.4	1.3	8.14	1.49	SM21 4500- NO ₃ ⁻ E
Nitrite (mg/Kg)	0.06	0.008	0.043	0.03	SM21 4500- NO ₂ ⁻ B
Amoniacal Nitrogen (mg/Kg)	31.8		49.6		SM21 4500 NH ₃ E
Nitrogen Kjeldal (mg/Kg)	476	276	922	171	SM21 4500-Norg B
Nitrate (mg/Kg)	4.4	1.3	8.14	1.49	SM21 4500- NO ₃ ⁻ E
Nitrite (mg/Kg)	0.06	0.008	0.043	0.03	SM21 4500- NO ₂ ⁻ B
pH	6.2	3.9	5.1	4.37	EPA 4095 C
Total Potassium	406	2056	<QL	3401	SM21 3120B
Total Sodium	<QL	50.2	<LQ	114	SM21 2540B
Total Solids	0.86		0.93		SM21 2540B
Total Volatile Solids	IV		0.08		SM21 2540B
Solid content	0.86		0.93		SM21 2540B
Moisture (g/g)	0.14		0.06		SM21 2540B

CS: control soil; RV: raw vinasse; IV: inconsistent value; NE: data not evaluated; QL: quantification limit; SM: standard methods of the water and wastewater; RV: guidelines of soil quality (mg/Kg) and groundwater quality in São Paulo State, according to CETESB (195/2005-E); EPA: Environmental Protection Agency, US.

Table 3. Metals analysis of the control soil and raw sugarcane vinasse samples.

Parameters	Sample I		Sample II		Method	VR (mg/Kg)
	CS (mg/Kg)	RV (mg/Kg) (V)	CS (mg/Kg)	RV (mg/Kg) (V)		
Arsenic	16.8		<QL	<QL	SM21 3120B	3.5
Barium	5.91	0.41	<QL	<QL	SM21 3120B	75

Continued

Cadmium	<QL	<LQ	<0.16	<QL	SM21 3120B	<0.5
Lead	49.3	<QL	42.7	<QL	SM21 3120B	17
Copper	37.2	0.35	76.5	0.76	SM21 3120B	35
Chromium	31.2	0.04	108	3.56	SM21 3120B	40
Total Sulfur	151	1219	123	1681	SM21 3120B	
Mercury	<QL	0.0019	0.065	<QL	EPA 470A	0.05
Molybdenum	3.64	0.008	9.6	<QL	SM21 3120B	<4
Nickel	13	0.03	24.2	<QL	SM21 3120B	13
Selenium	<QL	<QL	52.1	<QL	SM21 3120B	0.25
Total Sodium	<QL	50.2	<LQ	114	SM21 2540B	
Zinc	23.2	1.66	96	<QL	SM21 3120B	60

CS: control soil; RV: raw vinasse; SM: standard methods of the water and wastewater; EPA: Environmental Protection Agency, US; QL: quantification limit; RV: guidelines of soil quality (mg/Kg) and groundwater quality in São Paulo State, according to CETESB (195/2005-E)

Table 4. Analysis of aromatic polycyclic hydrocarbons in the samples of control soil and the combinations of control soil + vinasse.

Parameters	Sample		Method	Concentration allowed in the soil (mg/Kg)	
	CS	SV		CB	CO
Acenaphthene (µg/Kg)	<QL	<QL	EPA 8270 D	-	-
Acenaphthylene (µg/Kg)	<QL	<QL	EPA 8270 D	-	-
Anthracene (µg/Kg)	<QL	<QL	EPA 8270 D	-	-
Benzo (a) anthracene (µg/Kg)	<QL	<QL	EPA 8270 D	0.025	0.025
Benzo (a) pyrene (µg/Kg)	<QL	<QL	EPA 8270 D	0.052	0.052
Benzo (a) fluoranthene (mg/Kg)	<QL	<QL	EPA 8270 D	0.38	-
Benzo (a) perylene (mg/Kg)	<QL	<QL	EPA 8270 D	0.57	
Benzo (a) fluoanthene (µg/Kg)	<QL	<QL	EPA 8270 D	0.38	0.38
Chrysene (mg/Kg)	<QL	<QL	EPA 8270 D	8.1	-
Dibenzo (a,h) anthracene (mg/Kg)	<QL	<QL	EPA 8270 D	0.08	-
Phenanthrene (µg/Kg)	<QL	<QL	EPA 8270 D	3.3	3.3
Fluoranthene (µg/Kg)	<QL	<QL	EPA 8270 D	-	-
Fluorenone (µg/kg)	<QL	<QL	EPA 8270 D	-	-
Indeno (1,2,3-cd) pyrene (µg/Kg)	<QL	<QL	EPA 8270 D	0.031	0.031
Naphthalene (µg/Kg)	<QL	<QL	EPA 8270 D	0.12	0.12
Pyrene (µg/Kg)	<QL	<QL	EPA 8270 D	-	-

CS: control soil; SV: control soil + vinasse CB: guidelines (prevention) for soils in São Paulo State according to CETESB 9195/2005-E); CO: maximum concentration allowed in the soil, according to CONAMA (375/2006); EPA: Environmental Protection Agency, US; QL: quantification limit.

Table 5. Mean and standard deviation of the mitotic, chromosomal aberrations index in 5000 meristematic cells of *A. cepa* and MN in meristematic and the F₁ region cells, after exposure to ultrapure water (negative control), trifluralin (positive control), control soil (CS), control soil + vinasse (Sample I) and three concentrations of vinasse (Sample I).

Parameters analysed/5000 cells				
Groups	MI	CAI	MN (M)	MN (F ₁)
NC	29.1 ± 3.7	0.2 ± 0.2	0.2 ± 0.4	0.4 ± 0.5
CS	29.9 ± 1.7	0.4 ± 0.2	0.4 ± 0.5	0 ± 0
SV	29.4 ± 2.6	3.8 ± 0.6*	1.2 ± 0.4*	0.4 ± 0.5
V (50%)	41.7 ± 13.4	1.4 ± 0.6*	0.4 ± 0.5	2.2 ± 1.5
V (25%)	34.5 ± 3.3	1.6 ± 0.7*	0.6 ± 0.9	0.8 ± 0.8
V (12.5%)	42.4 ± 10.5	1.3 ± 0.4*	0.6 ± 0.5	1 ± 1
TRIF	19.4 ± 1.4*	10.2 ± 1.6*	10.4 ± 1.3*	2.6 ± 1.1*

NC: negative control; TRIF: trifluralin-positive control; CS: control soil; SV: control soil + vinasse (Sample I); V: vinasse (Sample I); MI: mitotic index; CAI: chromosomal aberration index; MN (M): micronuclei in meristematic cells; MN (F₁): micronuclei in cells of the region F₁. *p* < 0.05. Values statistically significant, compared to negative control with the Mann Whitney test.

Table 6. Mean and standard deviation of the mitotic, chromosomal aberrations index in 5000 meristematic cells of *A. cepa* and MN in meristematic and the F₁ region cells, after exposure to ultrapure water (negative control), trifluralin (positive control), control soil (CS), control soil + vinasse (Sample II) and three concentrations of vinasse (Sample II).

Parameters analysed/5000 cells				
Groups	MI	CAI	MN (M)	MN (F ₁)
NC	50.4 ± 7.7	0.3 ± 0.2	0.4 ± 0.5	0.2 ± 0.4
CS	57.2 ± 13.5	0.5 ± 0.5	0.8 ± 0.8	0 ± 0
SV	64.0 ± 7.9*	1.3 ± 0.6*	2.8 ± 1.5*	1.0 ± 0.7
V (50%)	63.2 ± 9.8*	2.6 ± 1.1*	3.0 ± 2.8*	1.0 ± 1.0
V (25%)	55.2 ± 7.9	2.2 ± 0.7*	2.6 ± 0.5*	0.2 ± 0.4
V (12.5%)	61.7 ± 3.7	3.7 ± 1.4*	1.8 ± 0.8*	2.2 ± 3.3
TRIF	37.8 ± 9.6	9.1 ± 3.3*	19.8 ± 13.1*	3.6 ± 1.8*

NC: negative control; TRIF: trifluralin-positive control; CS: control soil; SV: control soil + vinasse (Sample II); V: vinasse (Sample II); MI: mitotic index; CAI: chromosomal aberration index; MN (M): micronuclei in meristematic cells; MN (F₁): micronuclei in cells of the region F₁. *p* < 0.05. Values statistically significant, compared to negative control with the Mann Whitney test.

The germination in the treated groups (V 50%, V 25% and V 12.5%) and control groups (CS and NC) was over 90%. In the RV seeds did not germinate and in SV, the germination index was below 5%.

The MI was analyzed, which represented the number of dividing cells. In the Sample I, no significant differences and in the Sample II, the groups SV and V 50% showed significant differences were observed when comparing the treatments with the negatives controls (*p* < 0.05).

The genotoxic potential was evaluated by the CAI for all treatments (SV, V 50%, V 25% and V 12.5%) and was statistically significant when comparing with the negatives controls (*p* < 0.05). The CAs and nuclear abnormalities observed in the present study were visualized at all stages of the cell cycle. Several types of aberrations were considered [14]. The most frequent aberrations were metaphase with chromosome adherence, anaphase with chromosome loss and polyploid anaphase.

It was also quantified the presence of MN in meristematic and F₁ region cells (Table 5 and Table 6). The MN in meristematic cells was only statistically significant for treatments SV in Sample I, but in Sample II, all treatments were statistically significant when comparing with the negatives controls (*p* < 0.05). No differences statistically significant were observed comparing with the negatives controls (*p* < 0.05) for MN in F₁ region cells, in any of the samples evaluated.

4. Discussion

Several alternatives have been developed for the use of vinasse from sugarcane in Brazil, in order to the large volumes that are produced daily. Therefore, every day becomes more necessary the assessments of possible damage to exposed ecosystems. Therefore, this study intended to contribute to a better understanding of the toxicity that this residue derived from the ethanol industry can have on soil.

Thus, the initial chemical analysis of the soil is of great importance to identify and quantify the different chemical elements present in the soil samples used in this study. Also is very important to determine the chemical and physico-chemical characteristics of the vinasse sample study which vary depending on the harvest. This analysis allowed to reproducing the correct amount of application of vinasse in soils on the recommendation of the legislation (CETESB P4.231/2005).

The addition of sugarcane vinasse on the soil can bring harmful effects in the terrestrial ecosystems, for example in the seed germination and alterations in the genetic material of exposed organisms [6]. In the present study, the RV seeds did not germinate indicating that vinasse has a toxic potential for *A. cepa* seeds, probably due to the low pH. From an ecological perspective, it is important to take into account the pH of the substrate where seeds will germinate and grow, due to its direct effect on plants, in addition to nutrient release [21]. This result can also be due to the large quantity of potassium present in the vinasse samples, as hydric and saline stresses are correlated with the excess of soluble salts, reducing the potential of water in the soil and consequently, preventing the absorption of water by seeds in general [22]. According to Leonel and Rodrigues (1999) [23], when the levels of potassium nitrate were tested in citrus seeds, the results showed a toxic potential, inhibiting their germination. Studies conducted by Gazziero *et al.* (1991) [24] also showed that potassium nitrate did not promote the germination of *Sorghum halepense* seeds.

This study did not reveal a significant reduction in the MI at the evaluated vinasse samples, being this a parameter that allows for the estimation of the frequency of cellular division, used for identify the presence of cytotoxic pollutants in the environment [25]. The examination of meristematic cells exposed to different samples, revealed increase in the frequency of CAs in the *A. cepa* cells.

Chromosome loss and polyploidy are events that can derive from problems in cytoplasmic microtubules [18]. Metaphases with chromosome adherence indicate a toxic effect on chromosomes, characterizing an irreversible effect on the cell [26]. According to Liu *et al.* (1994) [27], chromosome adherence might be due to the presence of cadmium in some compounds that can cause cell alterations even in small quantities. Also according to [28], adherence is a common sign of toxic effects on the genetic material and may cause irreversible effects on the cell. One of the consequences of adherence may be the chromosomal loss [29]. In this case, chromosomes keeps united and when they separate may be broken and/or lost [30]. This phenomenon can lead to cases of aneuploidy and polyploidy [31]-[33].

Polyploid cells have a large chromosomal imbalance due to the diversion of chromosomal number. In this case, the chromosomes tend to be condensed [31] [34] promoting the adhesion of chromosomes and chromatids [18].

A substance capable of inducing the formation of micronucleus may be considered a clastogenic or aneugenic compound. The clastogenic action of a substance is demonstrated by the presence of micronuclei from chromosome breaks during the process of cell division. The aneugenic action, on the other hand, is characterized by the inactivation of the mitotic fuse, which results in loss of entire chromosomes that become absent in the main nucleus of the cell [35].

Genotoxicity studies are very important and have been several reports of damage to the genetic material from different organisms exposed to vinasse for example in *Drosophila melanogaster* [36], *Tradescantia pallida* [37] and *Saccharum* species hybrids [38]. According to the latter authors, these alterations were caused by the high concentrations of K, P, S, Fe, Mn, Zn and Cu and heavy metals such as Cd, Cr, Ni and Pb.

Souza *et al.* (2009) [39] in a study on the clastogenic/aneugenic potential of land farming soil from a petroleum refinery before and after addition of sugarcane vinasse reported a significant increase in chromosome aberrations in *A. cepa* seeds as chromosome breakages. This clastogenic effect is probably due to release of metal contained in land farming caused by sugarcane vinasse.

In the other hand, Christofolletti *et al.* (2013) [17] evaluated the toxic potential of biosolid, sugarcane vinasse and a combination of both residues using *A. cepa* assay. The authors also observed sugarcane vinasse genotoxicity for chromosome aberrations as for example, cells in metaphase with chromosome adherence, polyploid

metaphases and anaphases, anaphase with chromosome bridges and with chromosome loss and cells with nuclear buds.

Based on the results obtained, this evaluation indicates the importance of studies to assess the toxic, cytotoxic and genotoxic potential of different residues disposed in the environment. These residues may induce alterations that cause irreversible damage to organisms and ecosystems.

5. Conclusion

After the quantification of the seeds germination and chromosomal aberrations in the test system here applied, it was concluded that the sugarcane vinasse *in natura* and in different dilutions showed a toxic and genotoxic potential for the *A. cepa* species. Maybe the low pH, electric conductivity, and chemical elements present in sugarcane vinasse may cause changes in the chemical and physical-chemical properties of soils. Results of this study reinforce the need for more research to evaluate the biological effects of sugarcane vinasse discharged into the environment in different ecosystems compartments, as well as different levels of biological organization.

Acknowledgements

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processes 2009/53047-9, 2009/50578-3 and 2012/50197-2), for financial support and Thays Casimiro Fernandes for suggestions.

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