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Genotoxic and Mutagenic Assessment Induced by Vinasse, Before and After Being Subjected to Bio-oxidation and Fenton Processes

Evaluación genotóxica y mutagénica inducida por vinaza, antes y despues de ser sometidas a procesos de bioxidación y de Fenton

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ABSTRACT

Background: Colombia is joining global initiatives to mitigate climate change through bioethanol production, as it has large sugar cane plantations and sugar mills, particularly in the Valle del Cauca region. One of the main by-products of the bioethanol industry is vinasse, which consists mainly of water, organic solids and heavy metals. Some of the compounds present in vinasses, such as melanoidins and phthalates, show genotoxic, mutagenic and carcinogenic activity in onion cells, tilapia and aquatic organisms. Various methods, such as bio-oxidation and Fenton reaction, have been used to reduce the organic load of vinasses. Among the most commonly used assays to study genotoxicity and mutagenicity are single cell gel electrophoresis (comet assay) and the Ames test. **Objective**: In this study, the genotoxicity in human lymphocytes and the mutagenicity in Salmonella typhimurium induced by different dilutions of vinasse produced at the bioethanol production plant in Frontino, Antioquia, before and after being subjected to biooxidation and Fenton processes, were evaluated. **Methods**: Genotoxicity was evaluated by the comet assay in human lymphocytes, and mutagenic activity was evaluated by the Ames test using Salmonella typhimurium strains TA98 and TA100, with and without the addition of microsomal enzymes (S9). Both tests were applied to each type of vinasse considered in this study, including raw vinasse (RV), bio-oxidised vinasse (BV) and Fenton oxidised vinasse (FV). Results: The results showed that at RV doses above 3%, viability decreased to values between 70% and 88%, whereas for BV and FV, viability remained above 93% and 94%, respectively. Vinasse was also found to have a dose-dependent effect on genotoxicity. However, no mutagenic activity was observed in any of the Salmonella strains evaluated, indicating that vinasse does not induce mutations. **Conclusion**: The importance of addressing vinasse pollution and treatment methods to reduce its toxicity is emphasised. However, further research is needed to fully understand the risks associated with vinasse exposure and to develop effective mitigation strategies.

Keywords: Vinasse, bioethanol, lymphocytes, comet assay, bio-oxidation

RESUMEN

Antecedentes: Colombia se está sumando a las iniciativas mundiales para mitigar el cambio climático mediante la producción de bioetanol, ya que cuenta con grandes plantaciones de caña de azúcar e ingenios azucareros, sobre todo en la región del Valle del Cauca. Uno de los principales subproductos de la industria del bioetanol es la vinaza, que se compone principalmente de agua, sólidos orgánicos y metales pesados. Algunos de los compuestos presentes en las vinazas, como las melanoidinas y los ftalatos, muestran actividad genotóxica, mutagénica y carcinogénica en células de cebolla, tilapia y organismos acuáticos. Para reducir la carga orgánica de las vinazas se han utilizado diversos métodos, como la biooxidación y la reacción de Fenton. Entre los ensayos más utilizados para estudiar la genotoxicidad

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y la mutagenicidad se encuentran la electroforesis en gel unicelular (ensayo cometa) y el test de Ames. **Objetivo**: En este estudio se evaluó la genotoxicidad en linfocitos humanos y la mutagenicidad en Salmonella typhimurium inducidas por diferentes diluciones de vinazas producidas en la planta de producción de bioetanol de Frontino, Antioquia, antes y después de ser sometidas a procesos de biooxidación y Fenton. **Métodos**: La genotoxicidad se evaluó mediante el ensayo cometa en linfocitos humanos, y la actividad mutagénica se evaluó mediante la prueba de Ames utilizando las cepas TA98 y TA100 de Salmonella typhimurium, con y sin la adición de enzimas microsomales (S9). Ambas pruebas se aplicaron a cada tipo de vinaza considerado en este estudio, incluyendo la vinaza cruda (RV), la vinaza bio-oxidada (BV) y la vinaza oxidada por Fenton (FV). Resultados: Los resultados mostraron que a dosis de RV superiores al 3%, la viabilidad disminuyó a valores entre el 70% y el 88%, mientras que para la BV y la FV, la viabilidad se mantuvo por encima del 93% y 94%, respectivamente. También se observó que la vinaza tenía un efecto dosis-dependiente sobre la genotoxicidad. Sin embargo, no se observó actividad mutagénica en ninguna de las cepas de Salmonella evaluadas, lo que indica que la vinaza no induce mutaciones. **Conclusiones**: Se subraya la importancia de abordar la contaminación por vinaza y los métodos de tratamiento para reducir su toxicidad. Sin embargo, es necesario seguir investigando para comprender plenamente los riesgos asociados a la exposición a la vinaza y desarrollar estrategias de mitigación eficaces.

Palabras Claves: Vinaza, bioetanol, linfocitos, ensayo cometa, biooxidación.

INTRODUCTION

Mitigating the negative effects of climate change is a global concern, and biofuels are an alternative to address this issue (1). In this regard, Colombia embraces these global initiatives by implementing bioethanol production, given that regions like Valle del Cauca have large sugarcane cultivation areas and host 14 sugar mills (2). One of the by-products of the ethanol production process is vinasse, which has a dark color and consists mainly of water (93%) and organic and mineral solids (7%), and in some cases, heavy metals (3, 4, 5, 6, 7).

It has been shown that some of the compounds present in vinasse, such as metals, melanoidins, phthalates, benzenopropanoic acid, and 2-hydroxyisocaproic acid, induce genotoxic, carcinogenic, and mutagenic activity in different biological models (8, 9, 10). Vinasse samples were found to exhibit genotoxic and mutagenic potential in *Allium cepa L* (11), which prompted researchers to seek strategies to reduce the harmful effects of vinasse. For example, different dilutions of vinasse showed a positive linear association in the Ames test using *Salmonella typhimurium* strains TA-98 and TA-100, and after biological oxidation treatment, the mutagenicity of vinasse was found to decrease significantly (12). Another study evaluated the toxicity of vinasse in the tilapia species *Oreochromis niloticus L*, finding toxic and cytotoxic potential in these samples (13). In the same species, the toxicity and genotoxicity were determined when dilutions of raw vinasse were analyzed using comet and micronucleus tests; upon performing chemical treatment on the vinasse, an effective alternative for reducing toxicity was found (14, 15).

Among the most commonly used assays for the study of genotoxicity and mutagenicity are the single-cell gel electrophoresis test, also known as the comet assay (16), and the Ames test (17). These assays are not only valuable for providing information but are also characterized by their high sensitivity and low cost.

Therefore, in this research, genotoxicity in human lymphocytes and mutagenicity in *Salmonella typhimurium* strains TA-98 and TA-100 induced by raw vinasse (RV), vinasse subjected to bio-oxidation (BV), and vinasse chemically oxidized using Fenton reactions (OV) were evaluated.

MATERIALS AND METHODS

Vinasse Samples

Five liters of vinasse were obtained from the biorefinery plant located in the municipality of Frontino (Antioquia), 172 km from Medellín (6°46′43″N, 76°07′53″W), Colombia.

Three types of vinasse were obtained and sequentially processed to enhance the reduction of organic matter, starting with raw vinasse (RV) obtained as a by-product of the distillation process at the biorefinery plant. A portion of RV underwent biological oxidation (BV) with Saccharomyces cerevisiae yeast under anaerobic conditions. Initially, the pH was adjusted between 6.5-7.5; then, 10 mL of RV were mixed with 182 g of sludge inoculum, adjusted to 500 mL with tap water, and placed in a water bath at 37°C for 10 days. Once the bio-oxidation process was completed, a sample of BV was taken and subjected to Fenton treatment (FV); for this, the pH was adjusted to 4.0, then a mixture of 8 g $FeSO_4$ and 80 mL of H_2O_2 (50%) per liter of vinasse was added (18), and the pH of the final effluent was adjusted to 6.5 to precipitate iron salts (19). Each of the three types of vinasse underwent a physicochemical analysis to measure the C/N ratio, carbon percentage, solids, and heavy metals. Variables such as pH and chemical oxygen demand (COD) were measured for all three types of samples following the protocol for Colombia (20, 21).

Lymphocyte Culture

Five milliliters of heparinized whole blood were collected in the morning. Subsequently, the blood was centrifuged on a Hystopaque density gradient. After centrifugation (2,000 rpm, 25°C, 30 min), lymphocytes were collected and washed three times with phosphate-buffered saline (PBS) at 1,200 rpm for 7 minutes.

Cytotoxicity Test Using Trypan Blue Exclusion Method

To determine sublethal doses of vinasse, 5E+4 lymphocytes with over 95% viability were taken and mixed with the vinasse sample to be evaluated. The mixture was then incubated at 37°C for 1 hour. After incubation, a fraction of the incubated sample was treated with 0.4% trypan blue. This mixture was left for 15 minutes at room temperature. Finally, viability was calculated by observing under a Neubauer chamber. Twenty serial dilutions (1:1) were performed with each vinasse sample, starting from a concentration of 100% down to 0.00019%.

Genotoxic evaluation using single cell gel electrophoresis (Comet assay)

In this test, various sublethal concentrations of each of the three types of vinasse were considered: 0.5%, 0.75%, 1.0%, 1.25%, 1.75%, and 3.0%. A negative control, which was PBS, and a positive control, hydrogen peroxide (H_2O_2 50 μM), were also included. To detect DNA damage, alkaline single cell gel electrophoresis (comet assay) was used following the protocol proposed by Tice (22).

Mutagenicity assay

In this study, *Salmonella typhimurium* strains TA 98 and TA 100 were used to evaluate the mutagenic activity of vinasse extracts.

A cytotoxicity curve of the vinasse extract was performed with serial dilutions starting from the stock (100%) down to 20 dilutions (0.0002%). The concentrations used in all three types of vinasse for mutagenic evaluation in both strains were 3.125%, 6.25%, 12.5%, 25.0%, 50.0%, and 100.0%. Each experiment was conducted with and without the addition of rat liver metabolic enzymes, S9 fraction.

The protocol described by Maron and Ames (23) was followed. Each sample was treated with 1E+7 bacteria, with and without the presence of activating enzymes contained in a male rat liver homogenate (S-9 mix). A positive response criterion was considered when there was a significant increase in mutation compared to the negative control in all three independently conducted experiments.

Statistical Analysis

A descriptive statistical analysis of the collected data was conducted, calculating the arithmetic mean and standard deviation from three independent assays for each analyzed variable: tail length and number of revertants. These measures provide a statistical summary reflecting measures of central tendency and dispersion, essential for understanding the overall distribution of the data. To delve into the relationship between variables and explore the nature and strength of these interactions, linear regressions were implemented. A significance level of p-value less than 0.05 was accepted as significant, following standards established in the scientific literature. Due to non-compliance with normality and homoscedasticity assumptions, non-parametric statistical methods were employed. In particular, an aligned rank transform analysis of variance (ANOVA) was used, suitable for comparing variables among different types of vinasse, their concentrations, and the interaction between vinasse type and concentration. Additionally, a second-degree polynomial regression analysis was applied to address the variability observed in the slopes of the previous linear regressions. This analysis allows for modeling nonlinear relationships by including polynomial terms, providing a more complex and detailed representation of the dynamics between the variables.

RESULTS

Physicochemical analysis of vinasse

The physicochemical analysis of vinasse was conducted by the Interdisciplinary Group of Molecular Studies, where the following parameters were analyzed: pH, conductivity, carbon-to-nitrogen ratio, chemical oxygen demand (COD), and the metals cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), nickel (Ni), and arsenic (As). An acidic pH of 3.84 was found for VC, 6.3 for VB, and 6.5 for VO; these pH values are characteristic of this type of sample. Similarly, the sample exhibited a high content of organic material represented by a COD (chemical oxygen demand) of 30,420 mg/L for VC, 11,000 mg/L for VB, and 6,860 mg/L for VO. A removal of organic matter in terms of COD was observed for the vinasse treated by bio-oxidation,

with 63.8%, and for the bio-oxidized vinasse treated with chemical oxidation, 77.4% was recorded. The metals Cd, Cr, Ni, Pb, Hg, and As were not detected in the raw vinasse sample. Finally, the C/N ratio result of 192.6 indicates a low nitrogen content in the sample.

Cytotoxicity

The results show cell viability in toxicity assays with values above 85% for all three types of vinasse. It is noteworthy that at RV doses above 3%, viability decreased to between 70% and 88%. In contrast, BV and FV maintained viabilities above 93% and 94%, respectively (Table 1).

Table 1. Cytotoxicity induced in human lymphocytes by crude vinasse (RV), yeast-biooxidized vinasse (BV), and biooxidized vinasse treated with Fenton oxidation (FV). PBS was used as the negative control and pure DMSO as the positive control.

Concentration (%)	RV	BV	FV
	Mean	Mean	Mean
0.00019	94.0 ± 2.0	95.7 ± 4.9	97.3 ± 4.6
0.00038	93.3 ± 4.9	94.0 ± 3.6	95.3 ± 3.1
0.00076	92.7 ± 4.0	94.3 ± 1.5	97.7 ± 1.5
0.00152	94.7 ± 2.1	95.0 ± 4.6	94.7 ± 3.5
0.00305	92.3 ± 0.6	93.0 ± 2.6	96.0 ± 2.6
0.00610	92.7 ± 4.2	95.7 ± 2.1	95.7 ± 2.5
0.01220	90.0 ± 2.6	93.3 ± 0.6	94.3 ± 1.5
0.02441	94.0 ± 4.6	95.3 ± 2.9	96.7 ± 3.2
0.0488	92.3 ± 3.5	96.7 ± 4.0	94.3 ± 2.1
0.09765	95.3 ± 4.5	95.0 ± 2.6	97.3 ± 3.8
0.19531	92.7 ± 2.3	96.0 ± 2.6	95.7 ± 3.5
0.39063	92.0 ± 3.0	94.0 ± 1.7	93.7 ± 2.5
0.78125	89.3 ± 1.2	95.7 ± 2.3	98.0 ± 1.0
15.625	90.0 ± 2.0	97.3 ± 3.1	95.0 ± 3.0
3.125	87.7 ± 3.1	95.0 ± 1.7	97.7 ± 1.5
6.25	89.3 ± 2.1	92.7 ± 1.5	96.7 ± 3.2
12.5	86.7 ± 0.6	95.7 ± 2.1	97.7 ± 2.5
25	85.3 ± 1.5	96.0 ± 1.0	95.7 ± 5.1
50	74.7 ± 1.5	94.3 ± 1.5	97.3 ± 2.1
100	69.7 ± 0.6	95.0 ± 2.6	95.0 ± 1.0

Genotoxicity

The results of the different evaluations of genotoxicity in human lymphocytes for the three types of samples are depicted in Figure 1. The analysis of variance shows significant differences observed in concentration, indicating that higher concentrations lead to greater damage to the cell nucleus, represented by tail length (µm). Likewise, there are differences among treatments at concentrations above 1.25%, primarily due to the values recorded in raw vinasse, which are lower compared to the other two types of vinasse.

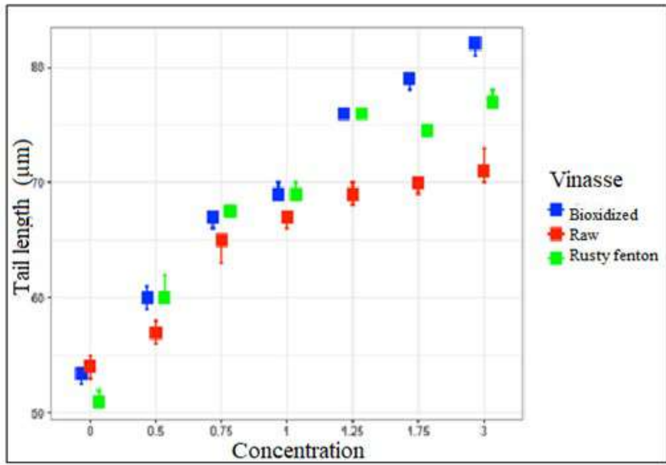

Figure 1. Tail Length (µm) in human lymphocytes induced by raw vinasse (RV), bioxidized vinasse (BV), and bioxidized vinasse subjected to chemical oxidation by Fenton reaction (FV). Number of cells analyzed at each concentration = 300; p-value < 0.05.

Mutagenicity evaluated by the Ames test

The mutagenicity results in *Salmonella Typhimurium* TA-98 and TA100 with and without S9, for the three types of samples, are described in tables 2 and 3. According to the mutagenicity criterion for a compound, it should exceed double the revertants compared to the negative control. As can be seen, no dose of vinasse in strain TA98 without S9 and with S9 reached between 40 and 50 revertants, values that would indicate the mutagenic action of any of the vinasse types; the highest value recorded was 26.3 revertants for VO at the 6.25% dose with S9 (Table 2).

Table 2. Mutagenic activity in Salmonella typhimurium TA98 induced by raw vinasse (RV), biotreated vinasse (BV), and biotreated vinasse subjected to chemical oxidation by Fenton reaction (FO), with or without addition of microsomal enzymes (S9). Data represent the average of three independent experiments, each performed in duplicate.

	TA-98					
	RV		**BV**		**FV**	
Dose	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
0	21.3 ± 1.2	25.0 ± 0.8	20.3 ± 2.3	22.3 ± 2.8	19.0 ± 4.6	19.2 ± 3.5
3.125	21.0 ± 1.6	24.3 ± 0.8	21.7 ± 1.9	22.0 ± 2.8	25.5 ± 3.5	23.2 ± 6.0
6.25	21.7 ± 1.4	24.0 ± 1.4	22.1 ± 2.6	24.5 ± 1.0	25.8 ± 3.3	26.3 ± 5.5
12.5	20.7 ± 1.3	23.7 ± 2.1	20.1 ± 2.3	22.5 ± 2.6	25.0 ± 3.9	22.8 ± 2.0
25	20.3 ± 0.8	24.3 ± 1.0	22.7 ± 2.3	24.5 ± 1.0	24.0 ± 4.1	22.0 ± 4.0
50	20.7 ± 1.5	23.0 ± 1.5	20.3 ± 2.8	22.8 ± 1.6	22.8 ± 3.6	24.7 ± 3.0
100	21.3 ± 1.2	22.7 ± 1.4	21.7 ± 5.0	23.5 ± 1.0	23.8 ± 2.9	23.3 ± 3.0

The average revertant results in both strains without S9 for the different types of vinasse are reaffirmed with linear regression analysis applied to the data. For strain TA98, although a p-value <0.05 and a negative correlation coefficient of 63% were obtained for RV, no mutagenic activity was recorded because the criterion of doubling the number of revertants was not met. For BV and FV, the p-values were 0.76 and 0.70, with correlation coefficients less than 10% respectively, indicating that the data did not exhibit a linear behavior and trended towards zero mean.

Regarding strain TA100 (Table 3), the results indicated that vinasses did not induce mutations. Linear regression analyses for strain TA100 without S9 showed that in RV, the data trended towards zero with a correlation coefficient of only 6%; whereas in BV and FV, these coefficients were 46% and 50%, respectively, the former showing an inverse trend and the latter a direct proportion. In these two types of vinasse, the p-values were less than 0.05; however, as described earlier, none of the doses doubled the number of revertant colonies compared to the number recorded in the negative control, which should have shown an average of revertants between 166.6 and 192.4. The highest value was in FV at the 12.5% dose with S9, with an average of 108.3 revertants.

Table 3. Mutagenic activity in Salmonella typhimurium TA100 induced by raw vinasse (RV), biotreated vinasse (BV), and biotreated vinasse subjected to chemical oxidation by Fenton reaction (FV), with or without addition of microsomal enzymes (S9). Data represent the average of three independent experiments, each performed in duplicate.

	TA-100					
Dose	**RV**		**BV**		**FV**	
	-S9	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
0	83.7 ± 1.2	87.8 ± 1.0	96.2 ± 4.8	89.5 ± 2.8	89.0 ± 1.5	89.5 ± 2.6
3.125	84.2 ± 1.2	24.3 ± 0.8	93.8 ± 4.2	90.3 ± 2.9	107.0 ± 16.2	114.5 ± 15.9
6.25	84.0 ± 1.2	88.0 ± 1.4	93.3 ± 4.9	91.2 ± 1.7	104.0 ± 15.4	107.3 ± 15.9
12.5	83.0± 1.8	87.7 ± 2.0	92.7 ± 5.4	92.3 ± 4.5	107.0 ± 11.5	107.7 ± 14.5
25	82.8 ± 1.9	88 ± 0.8	92.7 ± 3.5	95.5 ± 5.2	117.0 ± 8.3	102.5 ± 15.7
50	82.8 ± 1.8	88.3 ± 1.4	91.0 ± 4.1	92.2 ± 2.8	103.0 ± 12.8	98.7 ± 8.9
100	83 ± 1.8	87.8 ± 0.8	91.2 ± 4.3	91.8 ± 4.1	111.0 ± 14.7	111.0 ± 15.5

The analyses for strain TA100 with S9 presented very low correlation coefficients for all three types of vinasse, namely -0.37, 0.45, and 0.21 for RV, BV, and FV respectively (data not shown). Among these, only BV yielded a p-value less than 0.05; however, 45% of the data is not sufficiently representative to assert the mutagenic effect of this type of vinasse. This is explained by the fact that results at all evaluated doses did not show a number of revertants doubling the negative control.

Comparison among the types of vinasse showed a p-value less than 0.05, suggesting statistically significant differences in the number of revertants among the different types of vinasse. When comparing metabolic activity, the p-value is greater than 0.05, indicating no statistically significant differences in the number of revertants due to metabolic activity.

A statistically significant difference was observed between raw vinasse and biotreated vinasse subjected to chemical oxidation by Fenton reaction with a p-value of 0.0137 in strain TA-100 without S9, indicating that the means of the number of revertants are significantly different between these two types of vinasse. No significant differences were found between biotreated vinasse and raw vinasse (p= 0.3812), nor between biotreated vinasse and biotreated vinasse subjected to chemical oxidation by Fenton reaction (p= 0.1074).

DISCUSSION

Lymphocytes showed lower viability above concentrations of 3% in samples of raw vinasse (RV). This was evidenced by the trypan blue assay, which indicates damage to the cell membrane; thus, this test serves as an indicator of cell integrity (24, 25). In contrast, lymphocytes exposed to samples of biotreated vinasse (BV) and biotreated vinasse subjected to chemical oxidation by Fenton reaction (FV) did not show any toxicity, as they presented results similar to untreated cells. This may be related to the decrease in COD values resulting from the sequential treatment of biotreatment and chemical oxidation by Fenton reactions applied to the vinasse, achieving a reduction of 35.9% in BV and 22.4% in FV; totaling a reduction of 58.3% in COD. Another possible explanation is the presence of nitrogenous compounds, which through microbial action produce nitric oxide in raw vinasse; this compound interacts with hemoproteins such as guanylate cyclase, catalase, and proteins of the mitochondrial respiratory chain (26, 27). Consequently, treatments involving biotreatment reduce the cytotoxic effect of vinasse on human lymphocytes.

Regarding genotoxicity, the results at each concentration of vinasse compared to the negative control show an increasing trend of genotoxic damage as these concentrations increase (Figure 1). Similar results were reported in blood samples of fish evaluated using the comet assay, where authors considered vinasse dilutions of 1%, 2.5%, and 5%, all showing damage except at the 1% dilution (18). Regarding the types of vinasse treatments, BV and FV showed no differences in genotoxicity results between them; however, these two types of vinasse differed significantly from RV, exhibiting longer comet tails. One possible explanation is the presence of melanoidins, which are not removed by yeast in anaerobic treatment (BV) and by Fenton reactions (FV). Melanoidins have been reported to have genotoxic effects on human lymphocytes (28), although this can be somewhat contradictory, given the well-known antioxidant property of melanoidins. Further understanding is needed because factors such as pH, substrate origin, temperature, and solvent, among others, can alter their behavior (29, 30).

The equation shown in Figure 2 indicates that the comet tail length (y) can be calculated as a function of the concentration of raw vinasse (x). As an example, extrapolation is presented by substituting three values of x into the formula: for X= 4, Y= 654; X=5, Y= 51.84; and X=6, Y= 31.6. This shows a reduction in damage at higher concentrations, which apparently contradicts the linear regression analysis. To investigate this further, the first derivative was calculated to determine the concentration value with the maximum response of the dependent variable. It was observed that the concentration value of 2.47 corresponds to the highest response damage in the comet assay. This indicates that the linear regression analysis indeed shows a direct relationship between the two variables, but only up to a concentration of 2.47; at higher concentrations, the response variable decreases, as illustrated earlier.

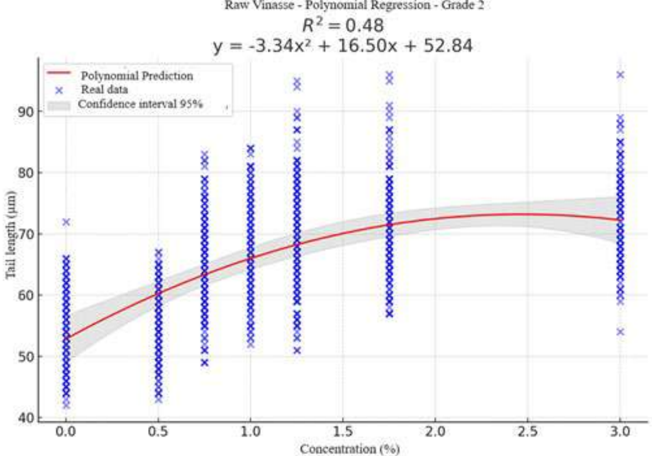

Figure 2. Polynomial regression of genotoxicity induced in human lymphocyte DNA by raw vinasse (RV). The controls used are: negative (PBS) and positive (hydrogen peroxide H_2O_2 at 50 µM). The blue points represent the experimental data, while the red line shows the fit of the second-degree polynomial model. The gray shaded area indicates the 95% confidence interval. Both the coefficients of the equation and the intercept are significant ($p < 0.05$).

Similarly, analyses were conducted for Biotreated Vinasse (Figure 3), where the value of the first derivative corresponded to 2.67.

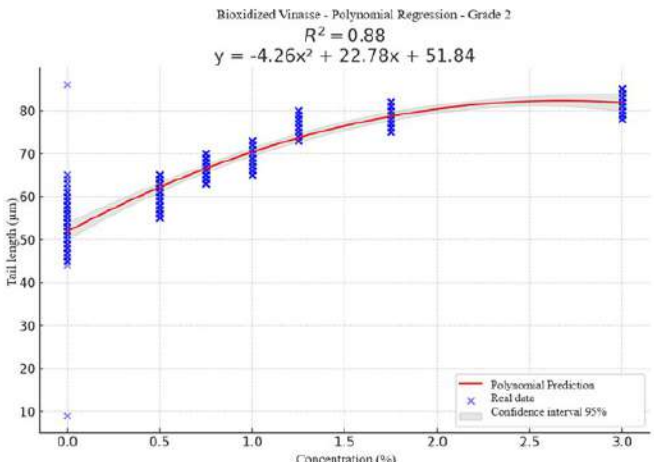

Figure 3. Polynomial regression of genotoxicity induced in human lymphocyte DNA by bioxidized vinasse (BV). The controls used are: negative (PBS) and positive (hydrogen peroxide H_2O_2 at 50 µM). The blue points represent the experimental data, while the red line shows the fit of the second-degree polynomial model. The gray shaded area indicates the 95% confidence interval. Both the coefficients of the equation and the intercept are significant ($p < 0.05$).

Finally, the analysis for oxidized vinasse (Figure 4) shows that the value of the first derivative was 2.35.

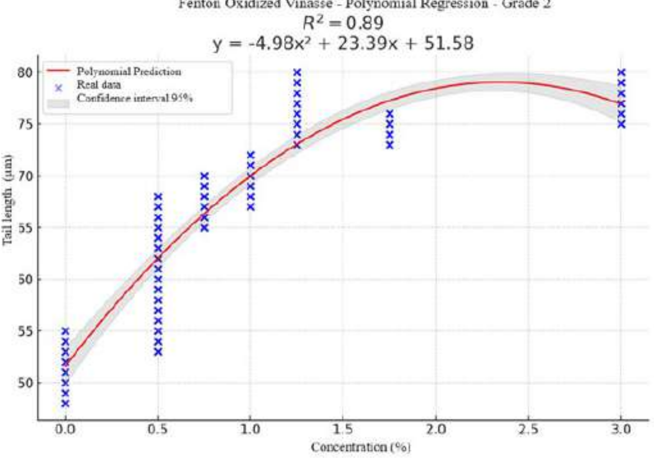

Figure 4. Polynomial regression of genotoxicity induced in human lymphocyte DNA by biotreated vinasse subjected to subsequent chemical oxidation by Fenton reaction (FV). The controls used are: negative (PBS) and positive (hydrogen peroxide H_2O_2 at 50 µM). The blue points represent the experimental data, while the red line shows the fit of the second-degree polynomial model. The gray shaded area indicates the 95% confidence interval. Both the coefficients of the equation and the intercept are significant ($p < 0.05$).

Considering that this study shows an increase in damage associated with vinasse concentration, it has been observed that certain phthalates and phenolic compounds induce genotoxic damage (31); similarly, furan, found in this type of waste, has been shown to be a potent carcinogen in rats and mice (32, 33, 34). Considering that polycyclic aromatic hydrocarbons (PAHs) may be present in vinasse (35) and aquatic ecosystems (36), and they are generally found environmentally in complex mixtures interacting with other compounds (37), the genotoxic response found in this study could also be attributed to these compounds, given their known genotoxic capacity (38).

The absence of mutagenic response was evident in all three types of vinasse analyzed in the present study for strains TA98 and TA100 with and without the addition of microsomal enzymes (S9). This indicates that the vinasses considered in this evaluation do not contain compounds that induce mutation detected by gain, loss, or substitution of nitrogenous bases (22). Another possible reason is that there might indeed be mutagenic compounds present, but the presence of melanoidins could block such mutagenic activity, due to their reported antioxidant capacity (31, 39). Furthermore, melanoidins, being recalcitrant molecules, were not affected by treatments in biotreated vinasse and vinasse oxidized with Fenton chemical reactions (32, 40). On the other hand, it should also be considered that the compounds present in these

samples may be aneugenic, meaning they have effects on structures of the mitotic apparatus in eukaryotic cells or on proteins such as histones, which are not found in prokaryotic cells (33, 41, 5). These results are consistent with another study where no mutagenic effect was found with strain TA100 with or without S9. However, specifically with strain TA98, mutagenic effects were indeed found, reaching 5 times the revertant colonies compared to the control at the 50% dose of raw vinasse (17). In the same study, it was found that vinasse treatments had a protective effect, as no mutagenicity was recorded; biotreated vinasse and vinasse oxidized with Fenton showed a number of revertant colonies similar to the negative control at the 50% dose.

The third aspect to consider is that mutagens present in vinasses could induce mutations through mechanisms other than base substitutions, losses, or gains.

CONCLUSIONS

The cytotoxicity of raw vinasse was determined to be at a concentration of 3% in human lymphocytes, and it was observed that biotreatment (BV) and chemical oxidation (FV) with Fenton reactions have a protective effect on these cells at the same concentrations.

Genotoxicity showed a dose effect, with increased genotoxic response at higher concentrations in all three types of vinasse. In this evaluation, there was no improvement in the genotoxic response with vinasse treated by biotreatment and chemical oxidation.

The vinasses evaluated in this study do not have a mutagenic effect on strains TA98 and TA100; that is, the compounds present in the sample do not cause gain or loss of nitrogenous bases, nor base substitutions. Therefore, the protective effect of vinasse treatment by biotreatment or chemical oxidation with Fenton reactions could not be confirmed.

Conflicts of interest: The authors declare no conflicts of interest

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