

Evaluation of DNA damage caused by anthropogenic pollutants in surface water in urban regions



Emerging pollutants and managing wastewater and waste

INTRODUCTION



RESULTS

We detected caffeine at all sites and diethyltoluamide at sites 1 and 4 (qualitative data not shown). At all sites *Escherichia coli* and Total Coliforms were detected.

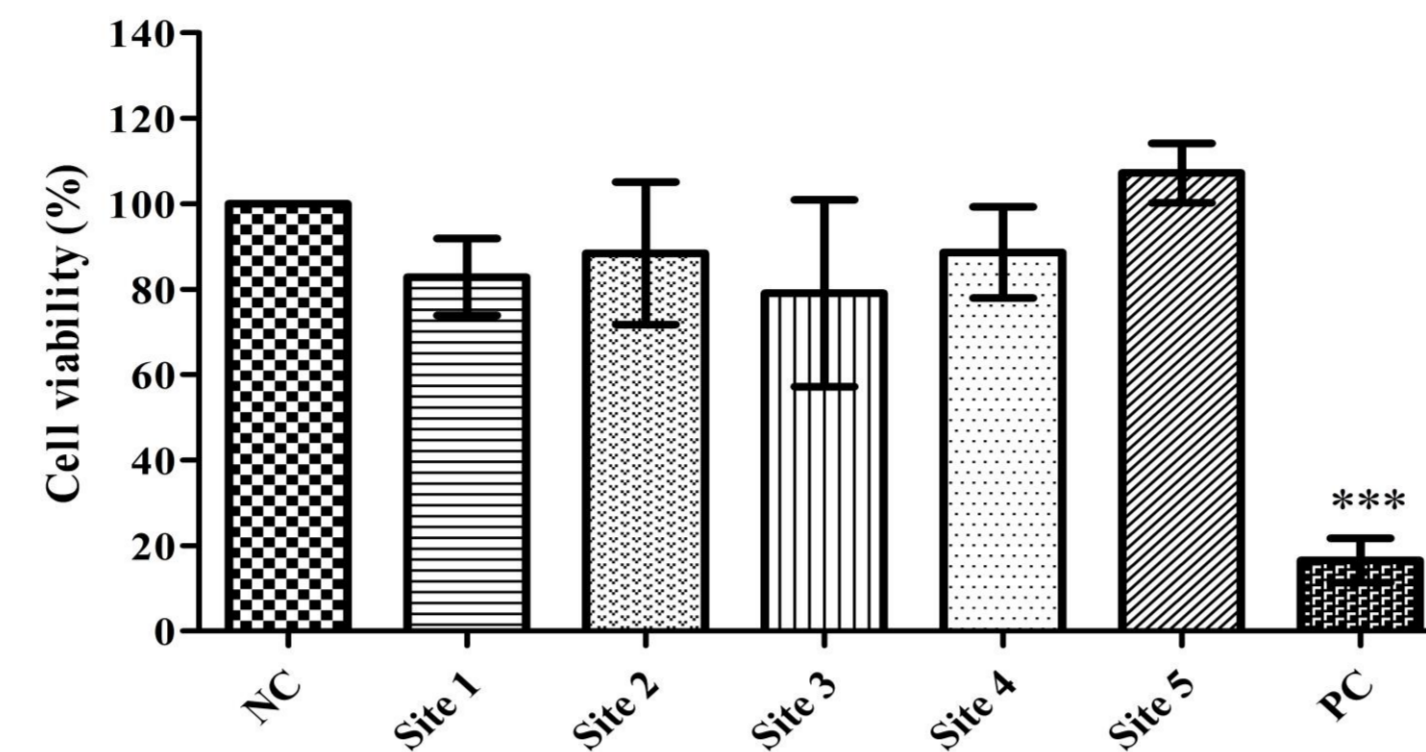


Fig. 2. Evaluation of cell viability by the MTT Assay on HepG2 cells exposed for 3 hours to surface water samples, Negative Control (NC; culture medium), and Positive Control (PC; DMSO 20%). The bars represent the mean \pm standard error. *** Significant difference in relation to the negative control at $p < 0.001$ (ANOVA, Dunnett's test).

Table 1: Results of the physicochemical analysis of surface water samples. ^aAccording to CONAMA Resolution 357 - Class II. ^b<DL: below Detection Limit of 0.94 mg L⁻¹. ^cBOD: Biochemical Oxygen Demand. ^dCOD: Chemical Oxygen Demand. ^eConsidering intermediate environment. ^f<DL: below Detection Limit of 0.10mg L⁻¹.

Parameters	COLLECTION SITES					Recommended Values ^a
	Site 1	Site 2	Site 3	Site 4	Site 5	
pH	7.44	6.35	6.78	6.39	6.48	6.00- 9.00
Conductivity ($\mu\text{S cm}^{-1}$)	767	166.60	553	409	725	Not applicable
Hardness (mg L^{-1})	25.66	15.40	20.53	19.01	35.93	Not applicable
Chlorides (mg L^{-1})	76.54	22.61	56.01	44.18	114.01	Max. 250.00
Dissolved oxygen (mg L^{-1})	<DL ^b	6.33	<DL ^b	<DL ^b	5.65	Not less than 5.0
BOD ^c ($\text{mg O}_2 \text{ L}^{-1}$)	101.90	9.80	60.70	55.70	11.80	Max. 5.00
COD ^d ($\text{mg O}_2 \text{ L}^{-1}$)	552	1.05	362	1.05	342	Not applicable
Nitrates (mg L^{-1})	2.00	1.20	2.00	1.90	1.80	Max. 10.00
Nitrites (mg L^{-1})	0.09	0.15	0.02	0.02	0.14	Max. 1.00
Total ammoniacal nitrogen (mg L^{-1})	16.24	2.80	12.32	9.52	6.16	Max. 3.70
Total Phosphorus (mg L^{-1})	0.80	0.30	0.03	0.06	0.12	Max. 0.05 ^e
Oils and Greases (mg L^{-1})	88	460	34	98	14	Absent
Total Dissolved Solids (mg L^{-1})	136	120	164	186	206	Max. 500
Total Suspended Solids (mg L^{-1})	26	20	14	18	26	Not applicable
Sulfates (mg L^{-1})	101.30	11.50	61.20	49.90	43.10	Max. 250.00
Hydrogen Sulfide (mg L^{-1})	0.27	<DL ^f	0.33	0.29	<DL ^f	Max. 0.002
Total Alkalinity (mg L^{-1})	103.88	29.40	74.48	54.88	58.80	Not applicable
Turbidity (UNT)	61.60	9.49	31.30	31.30	8.39	Max. 100.00
True Color (mg Pt L^{-1})	100	50	50	50	50	Max. 75

OBJECTIVES

Evaluate the presence of emerging pollutants in surface water samples under the influence of different anthropogenic effluents, as well as the cytotoxic, genotoxic, and mutagenic potential of these samples in HepG2 cells.

METHODOLOGIES

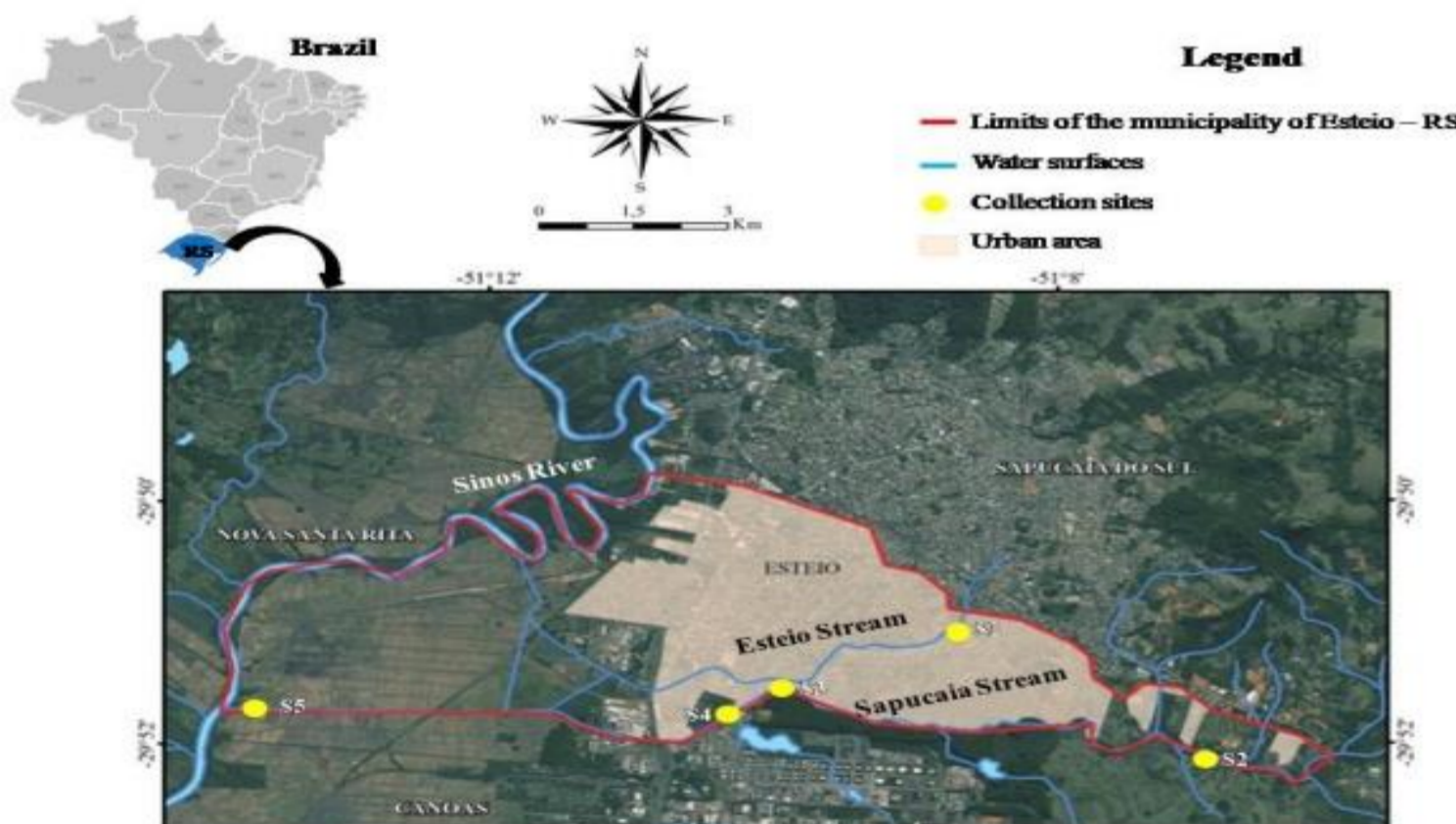


Fig. 1: Map of collection sites.

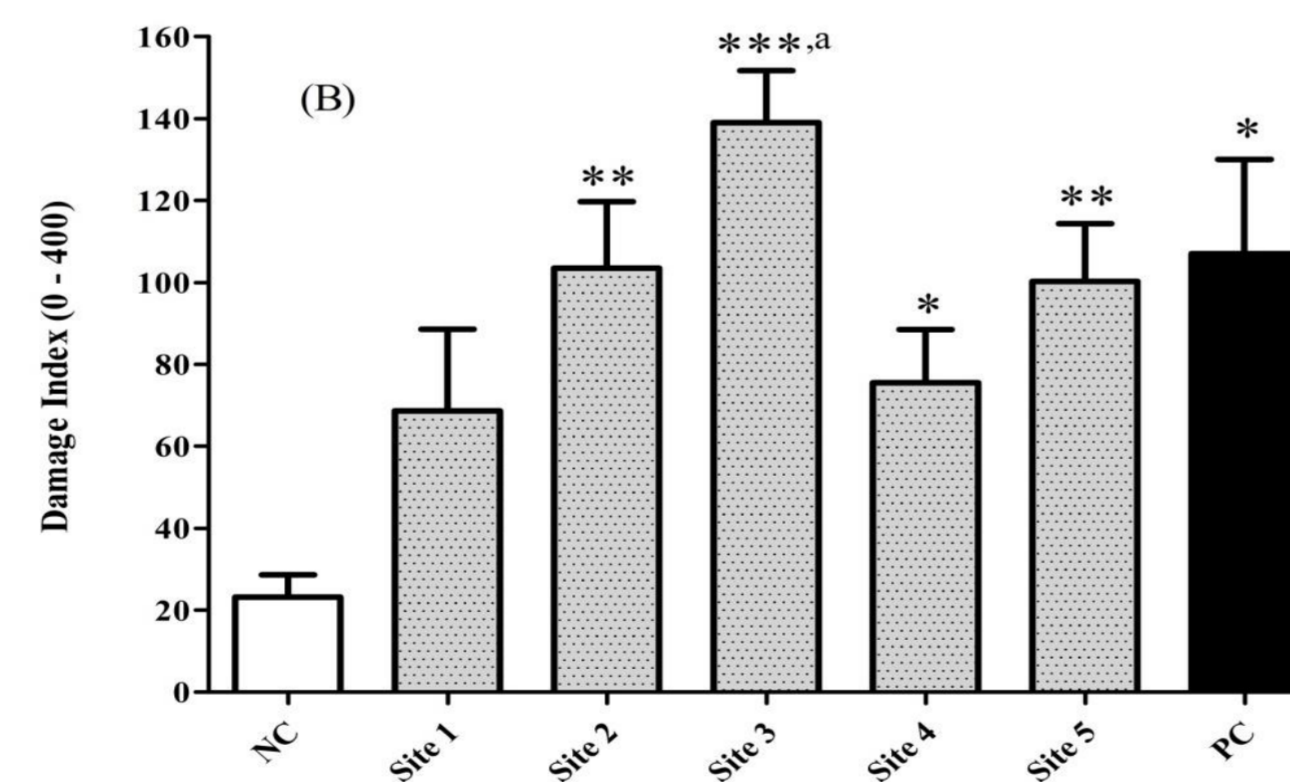
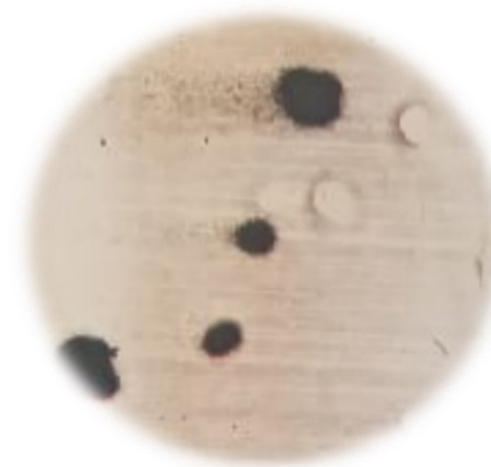
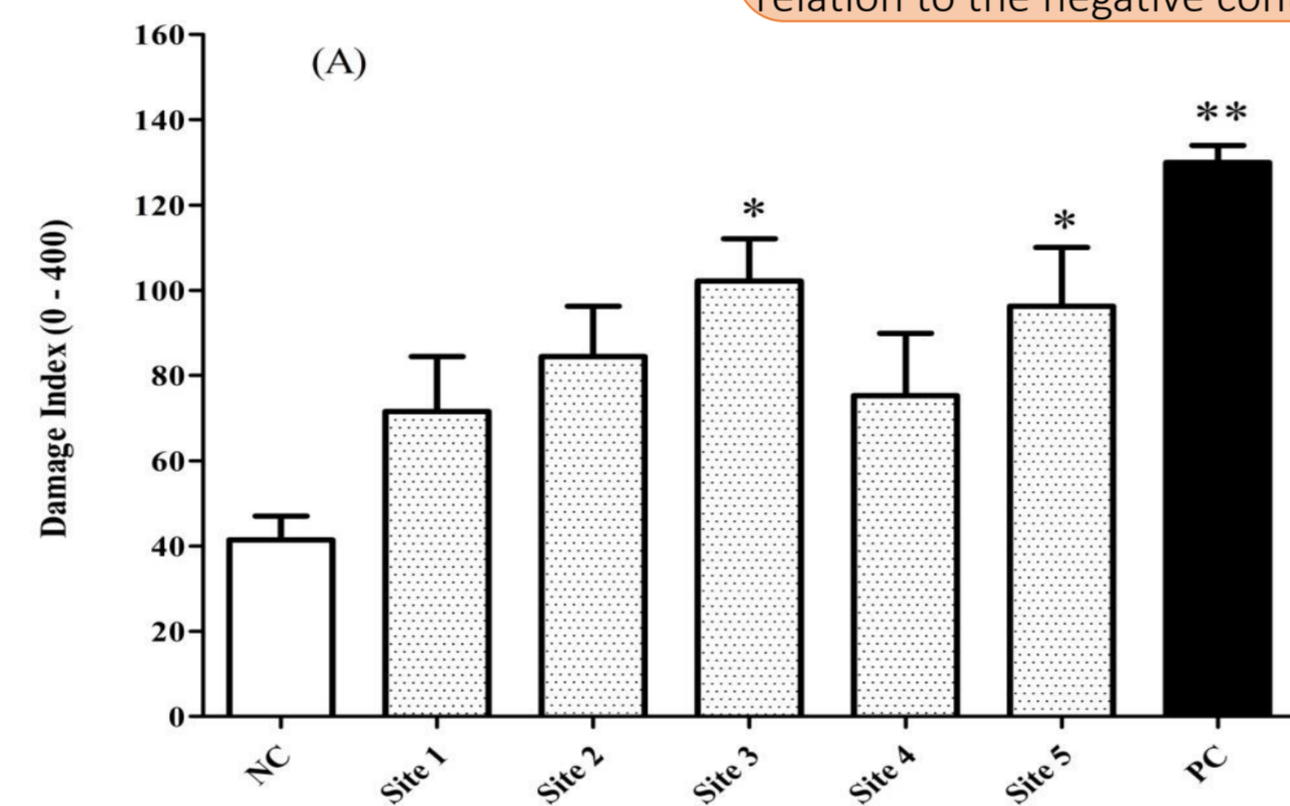


Fig. 3. DNA damage using the alkaline Comet Assay was measured as DNA strand breaks (A) or oxidative damage (B; enzyme-modified Comet Assay; + FPG - sensitive sites) in HepG2 cells. Negative Control (NC, culture medium) and Positive Control (PC, 4NQO, 0.040 μM). The bars represent the averages \pm standard error. *Significant difference in relation to the respective NC at $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. ^aSignificant difference in relation to the Site 1 and 4 at $P < 0.05$ (ANOVA, Tukey's test).

Table 2: Results of the analysis of inorganic elements present in Sapucaia and Esteio streams (RS, Brazil). ^and: not detected.

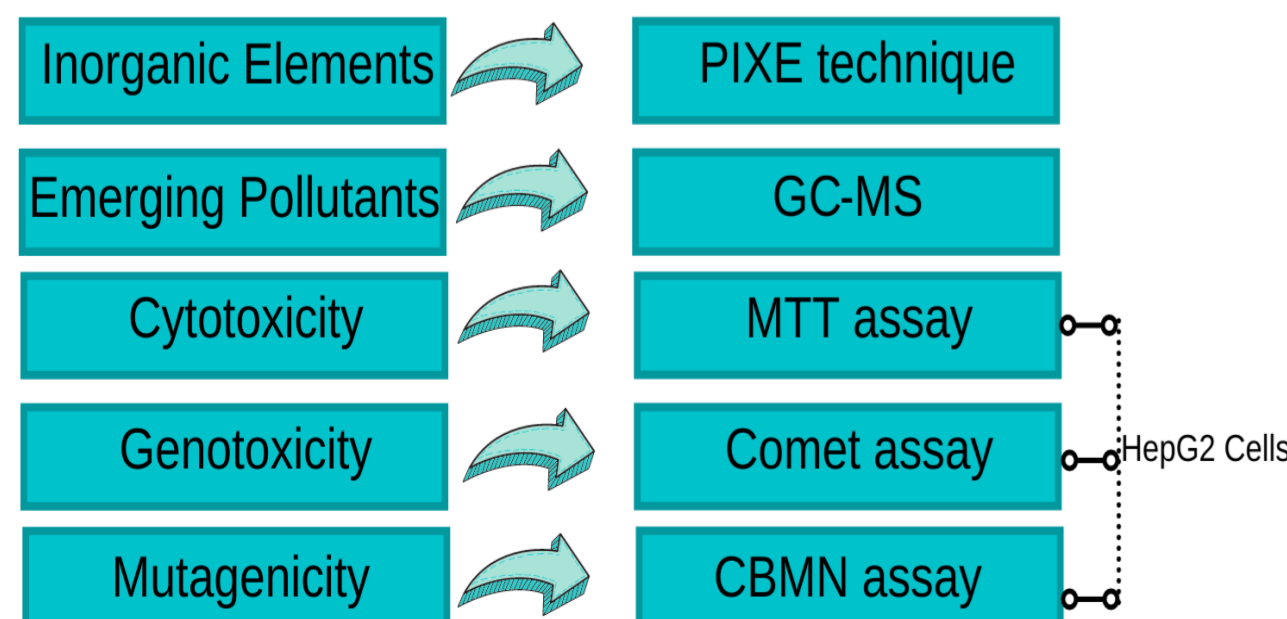
Inorganic Elements (ng/cm^2)	COLLECTION SITES				
	Site 1	Site 2	Site 3	Site 4	Site 5
Magnesium (Mg)	nd ^a	nd ^a	683.7	665.1	208.9
Aluminum (Al)	5537	3045	18686	12996	3901
Silicon (Si)	18387.4	7978.4	91420.4	55552.4	9838.4
Phosphorus (P)	1163	260	1845	2167	1277
Sulfur (S)	1027.3	nd ^a	1846.3	2036.3	273.8
Chlorine (Cl)	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a
Potassium (K)	1320.7	334.2	7584.7	2514.7	429.3
Calcium (Ca)	2264	nd ^a	5637	5320	2363
Titanium (Ti)	1413.1	417.3	4015.1	3648.1	547.1
Chrome (Cr)	10.5	3.8	34.2	57.3	18.3
Manganese (Mn)	166.6	1157	554.9	725.5	3699
Iron (Fe)	9534.7	5256.7	33452.7	24190.7	11722.7
Nickel (Ni)	2.8	nd ^a	nd ^a	nd ^a	1.9
Copper (Cu)	35.8	nd ^a	109.6	156	nd ^a
Zinc (Zn)	122.3	32.4	265.5	413.1	108.6
Strontium (Sr)	nd ^a	nd ^a	167.5	nd ^a	82.5
Zirconium (Zr)	nd ^a	nd ^a	367.7	136.9	nd ^a

Table 3: Evaluation of the frequency of Binucleated cells (BN) with Micronuclei (MN), Nucleoplasmic Bridges (NPB), and Nuclear Buds (NBUD) in the CBMN assay in HepG2 cells exposed to surface water samples and negative and positive control. Results expressed as mean \pm standard error. ^aCulture medium. ^bmethyl methanesulphonate (MMS -100 μM). *Significant difference in relation to the negative control at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (ANOVA, Dunnett's test).

Parameters	Negative Control ^a	COLLECTION SITES					Positive Control ^b
		Site 1	Site 2	Site 3	Site 4	Site 5	
DNA damage (1,000 cells BN)							
MN	6.75 \pm 1.11	11.50 \pm 0.29*	14.75 \pm 1.65**	13.75 \pm 1.31**	9.25 \pm 1.18	14.50 \pm 1.32**	17.00 \pm 1.00***
NPB	0.00 \pm 0.00	0.25 \pm 0.25	0.00 \pm 0.00	0.25 \pm 0.25	0.25 \pm 0.25	0.50 \pm 0.29	0.33 \pm 0.33
NBUD	7.00 \pm 0.58	11.75 \pm 1.11*	16.25 \pm 0.75***	19.50 \pm 0.50***	17.50 \pm 0.50***	17.00 \pm 1.00***	17.00 \pm 2.08***

CONCLUSIONS

Although the samples in the concentration used were not cytotoxic, water samples from all sites induced DNA damage in HepG2 cells. However, it is difficult to attribute these damages to a specific substance since the factors are a complex mixture of different compounds. Despite this, it is observed that both urban and industrial contributions had a similar effect in the cells evaluated. Thus, we emphasize the importance of conducting biomonitoring as well as the detection of different pollutants in aquatic environments.



Juliana Picinini, PhD student, Lutheran University of Brazil (ULBRA)

Renata F. Oliveira, Ana Letícia H. Garcia, Gabriele N. da Silva, Viviane C. Sebben, Guilherme M. S. de Souza, Johnny F. Dias, Dione Corrêa, Juliana da Silva