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Research Article

Genotoxicity biomonitoring of sewage in two municipal wastewater treatment plants using the *Tradescantia pallida* var. *purpurea* bioassay

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Abstract

The genotoxicity of untreated and treated sewage from two municipal wastewater treatment plants (WTP BN and WTP SJN) in the municipality of Porto Alegre, in the southern Brazilian state of Rio Grande do Sul, was evaluated over a one-year period using the *Tradescantia pallida* var. *purpurea* (Trad-MCN) bioassay. Inflorescences of *T. pallida* var. *purpurea* were exposed to sewage samples in February (summer), April (autumn), July (winter) and October (spring) 2009, and the micronuclei (MCN) frequencies were estimated in each period. The high genotoxicity of untreated sewage from WTP BN in February and April was not observed in treated sewage, indicating the efficiency of treatment at this WTP. However, untreated and treated sewage samples from WTP SJN had high MCN frequencies, except in October, when rainfall may have been responsible for reducing these frequencies at both WTPs. Physicochemical analyses of sewage from both WTPs indicated elevated concentrations of organic matter that were higher at WTP SJN than at WTP BN. Chromium was detected in untreated and treated sewage from WTP SJN, but not in treated sewage from WTP BN. Lead was found in all untreated sewage samples from WTP SJN, but only in the summer and autumn at WTP BN. These results indicate that the short-term Trad-MCN genotoxicity assay may be useful for regular monitoring of municipal WTPs.

Key words: effluent, micronucleus, mutagenicity, pollution, Trad-MCN.

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Introduction

The sewage produced in urban centers contains numerous pollutants from domestic and industrial sources, and treated sewage may contain complex mixtures of organic and inorganic compounds that may not be degraded during treatment (Rank and Nielsen, 1998). Several studies have investigated the genotoxicity of industrial effluents (Houk, 1992; Ruiz *et al.*, 1992; Nielsen and Rank, 1994; Rodrigues *et al.*, 2010) and the water bodies that receive them (Ohe *et al.*, 2004; Mitteregger *et al.*, 2007; Umbuzeiro *et al.*, 2007). The sewage sludge from municipal treatment plants has also been tested for toxicity and genotoxicity (Hopke *et al.*, 1984; Ottaviani *et al.*, 1993; Rank and Nielsen, 1998; Mielli *et al.*, 2009). However, liquid effluents from municipal sewage plants have been evaluated using chemical analyses primarily to meet legal obligations, and less attention has been paid to their genotoxic effects on organisms (Grisolia *et al.*, 2005).

In 2006, the Brazilian state of Rio Grande do Sul issued a regulation to establish criteria for the release of domestic and industrial liquid effluents into the environment based on their toxicity, genotoxicity and type of sewage (CONSEMA, 2006a). Over the next 8-12 years, the state environmental control agencies will define the test organisms and protocols to be used in monitoring these effluents.

The *Tradescantia* micronucleus (Trad-MCN) assay, a sensitive test for evaluating genotoxicity (Crebelli *et al.*, 2005; Rodrigues *et al.*, 1997), is based on the small chromatin masses derived from chromosomal breakages or aneuploidy during meiosis of the pollen mother cells (Ma *et al.*, 1984). Clone #4430, a hybrid of *T. subacaulis* Bush and *T. hirsutiflora* Bush, has been successfully used for bioassays (Ma *et al.*, 1994). On the other hand, *Tradescantia pallida* (Rose) D. R. Hunt var. *purpurea* Boom is an appropriate alternative for genotoxicity testing because it is well adapted to and extensively cultivated in tropical and subtropical regions (Batalha *et al.*, 1999).

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In this work, we used the Trad-MCN assay to evaluate and compare the genotoxicities of untreated and treated sewage from two municipal wastewater treatment plants (WTPs) over a one-year period.

Materials and Methods

Sampling sites and collection of sewage samples

Untreated and treated sewage samples from two WTPs were evaluated. The plants are located in the hydrographic basin of Guaíba Lake, in the southern Brazilian state of Rio Grande do Sul, and receive wastewater from the municipality of Porto Alegre; both are operated by the Municipal Department of Water and Sewage (DMAE). WTP SJN is located in a densely populated urban area with commercial and industrial activities. The sewage is treated by biological oxidation using an activated sludge process followed by aerated lagoon treatment at a mean flow rate of 246 L/s (range: 146-323 L/s). WTP BN is located in a less populated urban residential area and the sewage is treated in conventional, Australian-type stabilization lagoons at a mean flow rate of 23 L/s (range: 8-60 L/s).

In February, April, July and October 2009 (representing summer, autumn, winter and spring, respectively), untreated and treated sewage samples were collected from both sampling sites, transported to the laboratories where the experiments were done and preserved according to criteria established by the Brazilian Association of Technical Standards (ABNT – NBR 9898).

Physicochemical analyses

Untreated and treated sewage samples from both WTPs were collected for physicochemical analyses at the intervals indicated above. For each sewage sample collected the biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), total nitrogen (TN), total phosphorus (TP) and heavy metal content were measured. The parameters tested included some of those used in monitoring the efficiency of the WTPs operated by the DMAE, in accordance with the standard recommendations of the State Foundation for Environmental Protection (FEPAM) and the quality criteria of the State Environmental Council (CONSEMA, 2006a,b). The samples were analyzed using the methods described by the APHA (2005). Metals were analyzed by using inductively-coupled plasma optical emission spectrometry (ICP-OES) (USEPA, 2001). All analyses were done in the research division of DMAE, in Porto Alegre. Rainfall data were obtained from the Defesa Civil – Rio Grande do Sul (Defesa Civil, 2010).

Trad-MCN bioassay

Samples of *Tradescantia pallida* var. *purpurea* were grown in pots (37 cm x 20 cm x 20 cm) containing 4 kg of commercial soil from the same batch, in a non-polluted area of Feevale University. The plants were watered three times a week. Once a week, 100 mL of 1/3-strength Hoagland solution was applied to each pot and 100 mL of an N-P-K fertilizer solution (10-10-10, w/w/w) was applied once a month.

Plant exposure, inflorescence fixation and storage, slide preparation and data analysis were done using a modified version of the protocol described by Ma *et al.* (1984). Cuttings collected from the plant stocks bearing young inflorescences were maintained partially immersed in distilled water for 24 h in groups of 15 to 20. After this period of adaptation, only turgid cuttings were exposed to the following samples for 8 h: (a) untreated sewage, (b) treated sewage, (c) 0.1% formaldehyde solution (positive control) and (d) distilled water (negative control). Treatment was followed by a 24 h recovery period in distilled water. The inflorescences were fixed in 3:1 (v/v) ethanol/acetic acid for 24 h and stored in 70% ethanol. One bud per inflorescence was dissected and the anthers were squashed in 1% acetocarmine stain on a slide. Only preparations with early tetrads were included in the analysis. Seven slides were prepared for each sample. The slides were coded and the number of MCN in a random set of 300 tetrads per slide was scored under 400x magnification (Olympus CX4 microscope). The MCN frequencies were calculated by dividing the total number of micronuclei by the total number of tetrads scored, and the results were expressed as MCN/100 tetrads. All of the experiments were done in the Laboratory of Plant Biotechnology at Feevale University.

Statistical analysis

The Trad-MCN bioassay data were analyzed using the software package SPSS 17.0 after initial natural logarithm (ln) data transformation to obtain a normal distribution of variables and homogenous variances. The MCN frequencies of the samples in each of the four months were subsequently compared by one-way ANOVA followed by the Tukey test for multiple comparisons. Values of $p < 0.05$ indicated significance.

Results

Tables 1 and 2 show the physicochemical properties of the samples collected from WTP SJN and WTP BN for genotoxicity evaluation during the four seasons of 2009. The biochemical oxygen demand (BOD) met legal criteria, except at WTP SJN in the summer and winter. Total suspended solid (TSS) values were above the maximum permitted, except in the winter at WTP BN and WTP SJN and in the summer at WTP SJN. WTP BN satisfactorily removed total nitrogen (TN) in the summer and autumn, whereas the removal at WTP SJN was unsatisfactory in all seasons. The total phosphorus (TP) concentrations were higher than recommended at both WTPs in all seasons except at WTP BN in the spring.

Tables 1 and 2 also show the heavy metal concentrations in the sewage samples. Chromium (Cr) was detected in untreated and treated sewage from WTP SJN in all seasons except the winter, when it was undetectable in treated sewage. In contrast, at WTP BN, low concentrations of this metal were detected in untreated sewage samples only in the summer and autumn, and no chromium was found in treated sewage. Lead (Pb) was found in all untreated sewage samples from WTP SJN, but was only found at WTP BN in the summer and autumn.

The MCN frequencies of untreated sewage samples from WTP BN were significantly higher than those of the negative control in the summer and autumn, whereas treated sewage did not differ significantly from the negative control throughout the monitoring period (Figure 1). In contrast, the MCN frequency in plants exposed to untreated sewage from WTP SJN was significantly higher than in the negative control in all seasons, except spring. At this WTP, treated sewage samples had high MCN frequencies that were equal to or greater than those in positive control samples, except in the spring. The MCN frequencies in untreated sewage from both WTPs were lower in the spring than in other seasons and did not differ from the negative control. Except in the spring, when all samples had low MCN frequencies, treated sewage from WTP SJN was significantly more genotoxic than treated sewage from WTP BN.

Discussion

Effluents from municipal sewage treatment plants have been tested for mutagenicity using the *Salmonella*/microsome test (Meier *et al.*, 1987), the polychaete *Platynesis dumerilli* (Jha *et al.*, 1997), *Allium cepa* (Nielsen and Rank, 1994; Grisolia *et al.*, 2005) and fish (Grisolia *et*

Table 1 - Physicochemical characteristics of the WTP BN sewage samples in four seasons of 2009.

Parameter[1]	February 2009 (Summer) Sewage		April 2009 (Autumn) Sewage		July 2009 (Winter) Sewage		October 2009 (Spring) Sewage		Emission pattern[2]
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	
BOD (mg/L)	169.0	54.8	104.0	52.6	125.0	36.7	52.0	20.0	70.0
COD (mg/L)	ne[3]	ne	209.4	114.2	242.8	130.6	111.0	135.0	260.0
TSS (mg/L)	92.0	104.0	520.0	414.0	71.0	44.0	74.0	82.0	80.0
TN (mg/L)	46.20	11.07	49.92	10.49	47.36	17.33	16.52	8.73	15.0
TP (mg/L)	6.05	2.43	7.77	3.73	7.43	2.42	3.17	1.69	2.0
Pb (mg/L)	0.0030	nd[4]	0.0072	0.0037	nd	nd	nd	nd	0.2
Cu (mg/L)	0.0160	nd	0.0161	0.0004	0.0099	0.0043	0.0088	0.0023	0.5
Cr (mg/L)	0.0002	nd	0.0006	nd	nd	nd	nd	nd	0.5
Cd (mg/L)	nd	nd	nd	nd	nd	nd	nd	nd	0.1
Hg (mg/L)	nd	nd	0.0001	0.0001	0.0001	0.0020	nd	0.0014	0.1
Zn (mg/L)	0.0509	0.0028	0.0567	0.0030	0.0442	0.0082	0.0360	0.0128	2.0

[1]BOD = biochemical oxygen demand, COD = chemical oxygen demand, TSS = total suspended solids, TN = total nitrogen, TP = total phosphorus, Pb = lead, Cu = cupper, Cr = chromium, Cd = cadmium, Hg = mercury, Zn = zinc. [2]According to CONSEMA (2006b). [3]ne = not evaluated. [4]nd = not detected.

Table 2 - Physicochemical characteristics of the WTP SJN sewage samples in four seasons of 2009.

Parameter[1]	February 2009 (Summer) Sewage		April 2009 (Autumn) Sewage		July 2009 (Winter) Sewage		October 2009 (Spring) Sewage		Emission pattern[2]
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	
BOD (mg/L)	325.0	52.0	221.0	17.3	182.0	45.0	184.0	27.0	40.0
COD (mg/L)	639.0	ne[3]	227.9	39.4	419.2	96.4	340.0	81.0	150.0
TSS (mg/L)	424.0	14.0	474.0	400.0	128.0	37.0	438.0	71.0	50.0
TN (mg/L)	49.37	18.18	43.33	18.63	36.10	21.71	20.20	10.63	10.0
TP (mg/L)	12.00	1.49	5.45	4.80	5.35	3.46	2.19	1.24	1.0
Pb (mg/L)	0.03500	nd[4]	0.00020	nd	0.01600	0.00063	0.01218	nd	0.2
Cu (mg/L)	0.15240	0.00365	0.01610	0.00560	0.02325	0.00575	0.03998	0.00840	0.5
Cr (mg/L)	0.08815	0.00084	0.00240	0.00150	0.00350	nd	0.00604	0.00051	0.5
Cd (mg/L)	0.00130	nd	nd	nd	nd	nd	nd	nd	0.1
Hg (mg/L)	0.00080	0.00010	0.00010	0.00010	nd	nd	0.00140	0.00130	0.1
Zn (mg/L)	0.43560	0.03064	0.04280	0.03610	0.05875	0.30000	0.17693	0.03953	2.0

[1]BOD = biochemical oxygen demand, COD = chemical oxygen demand, TSS = total suspended solids, TN = total nitrogen, TP = total phosphorus, Pb = lead, Cu = cupper, Cr = chromium, Cd = cadmium, Hg = mercury, Zn = zinc. [2]According to CONSEMA (2006b). [3]ne = not evaluated. [4]nd = not detected.

al., 2005). However, *Tradescantia* is apparently more sensitive to genotoxic substances than *Allium cepa*, a widely used bioindicator of water quality (Crebelli *et al.*, 2005). In contrast, there have been no reports on the use of *T. pallida* var. *purpurea* to test untreated and treated sewage from WTPs. *Tradescantia pallida* var. *purpurea* has been successfully used to evaluate genotoxicity by *in situ* exposure in a Brazilian river (Umbuzeiro *et al.*, 2007) and by long-term exposure to treated sludge samples from WTPs in the State of São Paulo (Mielli *et al.*, 2009), with the latter study showing that this organism is as sensitive as clone #4430.

For samples from WTP BN, the high genotoxicity of untreated sewage was not observed in treated sewage. In contrast, both untreated and treated sewage samples from WTP SJN had high MCN frequencies. In general, the values of the physicochemical parameters for treated sewage from WTP BN were below the legal emission limits, whereas the corresponding values for WTP SJN were higher. The total nitrogen and total phosphorus concentrations of samples from WTP SJN were above the legal limits. Of all the metals analyzed, chromium was detected in more seasons and at higher concentrations in samples from WTP SJN than in those from WTP BN. These differences in the physicochemical characteristics between the two WTPs may have influenced the MCN frequencies, which were higher for WTP SJN.

Industrial effluents containing chemicals other than those included in our tests may have been released unexpectedly into the sewer system, which was built to treat only domestic sewage. In large metropolitan areas, sewage from different sources may form complex mixtures that have marked effects on organisms (White and Rasmussen, 1998), and sludge from domestic sewage is less genotoxic than that from other sources (Hopke *et al.*, 1984). Heavy metals in different substrates can induce micronuclei in *Tradescantia* (Majer *et al.*, 2002). However, in the present study, we did not identify which chemicals were responsible for genotoxicity. Further studies should investigate how the complex mixtures of pollutants influence micronucleus formation.

In the spring, the MCN frequencies of untreated and treated sewage from both WTPs were not significantly different from the negative control. These findings may be explained by the cumulative volume of rainfall during the four days before sampling. In the first three seasons of the year, rainfall before sampling was zero or very close to zero, but was much higher (37.6 mm) in the spring and may have diluted the sewage. These findings indicate that monitoring should be done at various time points throughout the year. Furthermore, tests should include both untreated and

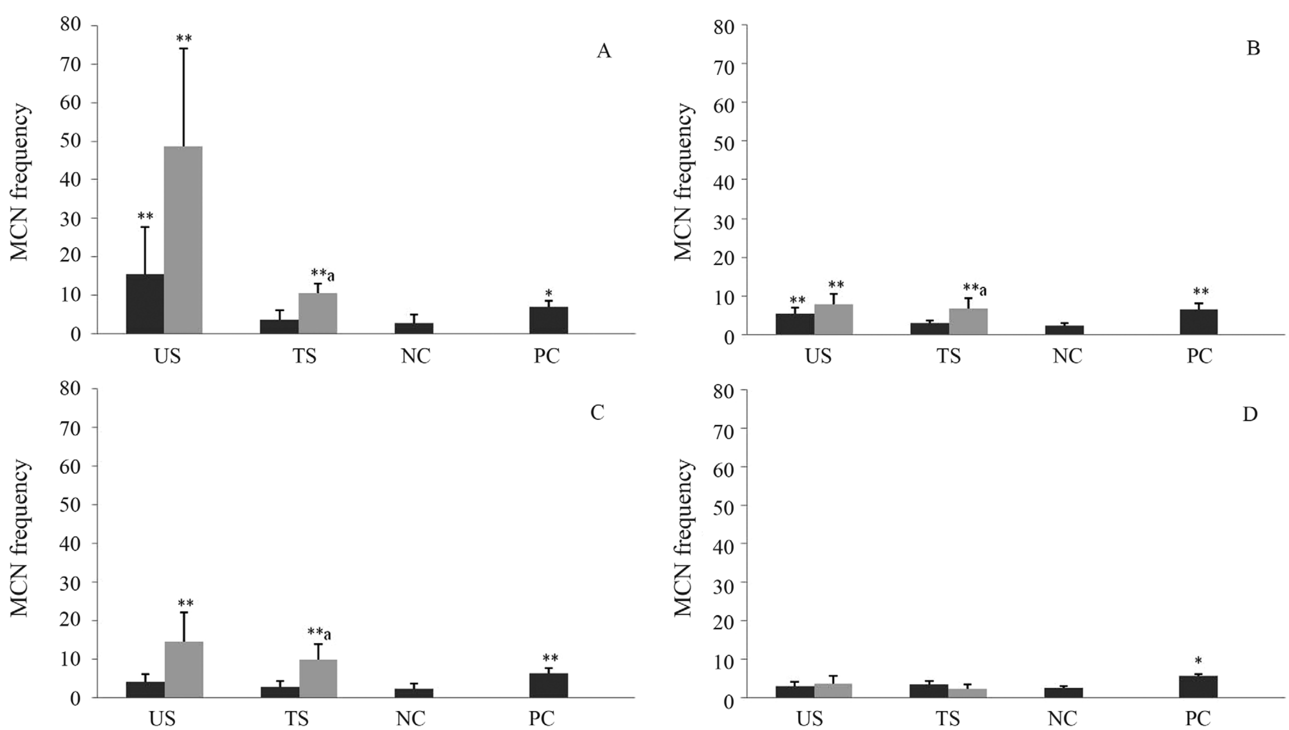

Figure 1 - Frequency of micronuclei (MCN) in tetrads of *Tradescantia pallida* var. *purpurea* obtained during monitoring of untreated and treated sewage samples from two wastewater treatment plants (black column = WTP BN; gray column = WTP SJN) in the summer (A), autumn (B), winter (C) and spring (D) of 2009. NC = negative control, PC = positive control, TC = treated sewage and US = treated sewage. The columns are the mean ± SD. Asterisks indicate significant (*p < 0.05) and highly significant (**p < 0.001) differences compared to the negative control (ANOVA followed by the Tukey test). The letter "a" indicates a significant difference (p < 0.001) compared to WTP BN treated sewage in the same month (ANOVA followed by the Tukey test).

treated sewage to determine whether the test organism is a suitable bioindicator for the samples under evaluation. Otherwise, it may be unclear whether negative results are indicative of a lack of genotoxicity or simply reflect the low sensitivity of the bioindicator used to test the samples.

Our results indicate that this short-term assay is a useful tool for assessing the genotoxicity of sewage. Future studies should evaluate its efficiency in the regular monitoring of municipal WTPs.

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