

Use of *Tradescantia pallida* (Rose) DR Hunt var. *purpurea* Boom (Commelinaceae) as biomonitor and bioaccumulator in water sources which is not indicated for population's supply

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Abstract. The aim of this study was to analyze the quality of water from different waterspouts, which is frequently consumed by urban population, without control and without supervision. The samples are from country towns of (I) Cabreúva, (II) Cajamar and (III) Jundiaí, respectively, all situated in São Paulo State, in Brazil. For this analysis, we used the Trad-MCN methods, with the *Tradescantia pallida* (Rose) DR Hunt var. *purpurea* Boom (Comelinaceae), to identify and quantify the presence of micronucleus and estimate the mutagenesis rate. The microbiological and physical-chemical analysis (turbidity, phosphate, pH, temperature, ammonium, and coliforms) are obtained by reagents for aquarium testing and Ecokit II Alfakit®. Cabreúva's waterspout presented bigger mutagenic power when compared to others, and it was the only sample containing fecal coliforms. All the experimental samples presented the toxicity. We observed the big expression of the micronucleus when the *T. pallida purpurea* was incubated within the three samples. The coliforms values observed in the samples were above of those permitted by law. The authorities must better inspect the water source and request the people not to consume the water from these places.

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Introduction

Water is one of the most important natural resources found in nature, and all human beings depend on it in order to survive and to carry out innumerable tasks or chores, as those in industry, navigation, agriculture, electric power generation and recreation. Through the hydrological cycle, water becomes a renewable resource. However, not all the water found in nature is appropriated for human consumption, since a great amount of water on the planet is saline, and part of fresh water is ice, thus, remaining 0,003%, that is 265.400 trillion tons of water are suitable to be used by living beings. Hence, there is a small amount of water for consumption, and, from this fraction, a good part of it, is inappropriate, due to anthropogenic pollution (Braga et al., 2002).

Along with continuous population growth, the demand for natural resources has increased significantly and some people have been searching for alternative water sources for consumption, such as waterspouts and wells (Braga et al., 2002; Silva et al., 2013). Alternative water sources are sought by the population, due to water supply crisis, due to economic factors. However, they do not know the risk of groundwater consumption, which might be contaminated (CETESB, 2013; Sampaio, 2014). Various diseases, such as cholera, hepatitis, typhoid fever, bacillary dysentery, diarrhea, and others, might be transmitted through water (Richter and Azevedo Netto, 2003).

According to CONAMA (the Environment National Council) resolution No. 357/2005 (Brasil, 2005) and Government Health's Decree 2.914/2011 (Brasil, 2011), the water appropriate for human consumption may be according some microbiological, chemical, and physical parameters limits. CONAMA 357/2005, though of the physicals and chemicals parameters, classifies water in some categories. According to this resolution, springs fall within the classification of surface fresh waters as class 1, being it the best classification; however, it is not ensured the absence of toxicants or pathogenic microorganisms (Lopes et al., 2010).

Tradescantia pallida (Rose) DR Hunt var. *purpurea* Boom (Commelinaceae) is a vegetable which adapts itself easily, and which develops itself throughout the year. It is a small vegetable that has some big pairs of chromosomes. When exposed to some chemical and physical agents, these chromosomes suffer mutation, and the genetic changes evaluations are conducted based on somatic mutations within the micronucleus expression (Carvalho, 2005).

Turbidity occurs when suspended solid particles from materials such as clay, sand, sewers, and industrial effluents are found in the water. Increased turbidity affects the water treatment and harms water organisms and some recreational activities (ANA, 2013).

Phosphate presence in the water can be related to the detergents, soaps, cleaning products that come from domestic effluents, and from pesticides and fertilizers (Nascimento and Barbosa, 2016).

Water pH may affect the metabolism of water species. According to CONAMA 357/2005 Resolution, the water pH should be between 6 and 9, to ensure the protection of water life. Water temperature influences physical and chemical water parameters, among them superficial tension and viscosity. Water organisms have a thermal tolerance limit. The increase of water temperature causes damages in their growth and reproduction. Effluents with high temperature cause significant damage to such organisms (ANA, 2013).

Ammonia is easily found in shallows and groundwater, so it is considered an important water pollution parameter. Normally it is found in small amounts in the water, because it is absorbed by the soil or oxidized to nitrite and nitrate. However, if it's found in high amounts, it indicates a pollution source (Alaburda and Nishihara, 1998).

The total coliforms include the species that live in the water, soil, vegetable and human or animal feces. It is a limited parameter to evaluate the sanitary quality water. They are used to evaluate treated and distributed waters. *E. coli* is a bacterium of the coliforms group, which ferments lactose and mannitol, producing acid and gas in the 44,5 +/- 0,2 °C in 24 hours. It is a common indicator of faecalis because its habitat is in the human and homeothermic animals' intestine (Ministério da Saúde, 2005).

At Cabreúva City, the Decree No. 315, from November 21st, 2006 establishes an agreement between the town council and the Sabesp (the company which is responsible for the water treatment), however there is great demand from the population to evaluate the city spouts (Cabreúva, 2006). As at Cabreúva, Cajamar, although Municipal Law No. 1.882 from May 17th, 2012 establishes the contract between the town of Cajamar and the water concessionaire Sabesp, which is responsible for water supply in the city, there is a population demand in order to evaluate a spout that exists in the city, which is known as a greater spout compared to other cities (Cajamar, 2012). In Jundiaí, even with the city having DAE (Department of Water and Sewage), which takes care of all of the water supply and effluent treatment, there is also a great demand for the spout analysis in Malota, district of Jundiaí (Jundiaí, 1999).

The aim of this study was to analyze water quality of the spouts in Cabreúva, Cajamar and Jundiaí cities through Trad-MCN, physical-chemical and microbiological methods.

Materials and methods

Waterspouts

At this work, we used three waterspouts from three different cities, which are: Cabreúva (I), Cajamar (II) and Jundiaí (III). The first one is located on Vereador José de Moraes Road, km 3 (point I - approximate decimal coordinates -23.287269, -47.131763) (Figure 1A and 1B); the second one on Avenue Dr. João Antônio Abdala Avenue, s/nº - Bairro Vila Nova (point II - approximate decimal coordinates: -23.344511,-46.862513)

(Figure 1C and 1D); the third one on Malota Avenue, s/nº, Bairro da Malota (point III - approximate decimal coordinates: -23.21270191, -46.90641853) (Figure 1E and 1F).



Figure 1. A and B: point I (Cabreúva spout); C and D: point II (Cajamar spout); E and F: point III (Jundiá spout). Source: images A, C and E by Google Maps (2017).

***T. pallida purpurea* bioassay**

According to Mielli et al. (2009), the inflorescences of the *T. pallida purpurea* was used in the bioassay. *T. pallida purpurea* cuttings are collected in Botanical Garden Jundiaí, after receiving municipal authorization.

One hundred *T. pallida purpurea* stems was used (approximately 5-8 cm) containing the buds, which were going through the meiosis prophase, between the pachytene and the diplotene. This phase is very important because the plant becomes more sensitive to mutation. Inflorescences which ranged in size from 5 to 8 cm were placed in tap water for a 24 h adaptation phase. In the intoxication phase, the stems were incubated with samples from each source for 8 h. Positive control was carried out by incubation in formaldehyde 1:1000 (mutagenic agent), white control in distilled water and negative control in tap water. Then all stems were placed in tap water for 24 h due to a recovery phase. Samples and control buds were cut and put into solution of alcohol 98% 3:1 acetic acid, for fixation.

After 48 h of slides preparation, the buds were placed on glass slides and acetocarmine and orcein were added. Ten slides were made to each (controls and samples), and randomly chosen group of 300 tetrads were counted from each slide, at a magnification of 400X with a light microscope. The micronuclei were expressed as percentage (total number of micronuclei per 100 tetrads).

Microbiological and physical-chemical parameters analysis

Water turbidity was measured by Secchi Disc; the phosphate with the Phosphate Azôo Test®. The pH and ammonium were obtained by Labcon Test®. All physical and chemical analyzes were made *in loco*. Coliforms were measured by Ecokit II Alfakit®.

Statistical analysis

It was used chi-square test, which is a probability test to identify a dispersion value for two nominal variants, verifying the association between quantitative variants, considering differences greater than 95% between the samples ($P < 0.05$).

Results and discussion

A total of 300 tetrads were analyzed in each sample, and results are shown in the Table 1. Physical-chemical and microbiological assays are show in the Table 2.

Table 1. Frequency of micronuclei in *T. pallida purpurea*. Data are expressed as absolute and percent values.

Sample	Absolute value	% micronuclei
Cabreúva	32	10,67%
Cajamar	25	8,33%
Jundiaí	14	4,67%
Positive control (formaldehyde)	31	10,33%
Negative control (tapwater)	3	1%
White control (distilled water)	1	0,33%

Table 2. Physical-chemical and microbiological analysis.

Parameter	Cabreúva	Cajamar	Jundiaí
Turbidity	<25 NTU	<25 NTU	<25 NTU
pH	6,4	6,2	6,2
Temperature	21 C°	20 C°	22 C°
Phosphate	0 mg/l	0 mg/l	0,1 mg/l
Amonium	0ppm	0,001ppm	0,001ppm
Total coliform	800 CFU	0 CFU	1200 CFU
<i>E. coli</i>	400 UFC	0 UFC	0 UFC

In this work, the Trad-MCN bioassays results showed a very important difference between control (negative and white) and samples group. The sample (I) Cabreúva's source shows micronuclei amount bigger than positive control (formaldehyde), suggesting the presence of mutagenic agents in the water. The other samples also show a bigger micronucleus amounts, when compared to negative and white controls, but Cajamar's (II) water samples showed more micronuclei than Jundiaí's (III) water sample. These results suggest that water sources analyzed are contaminated with mutagenic substances. In this present study, we did not aim at identifying such substances, owing to the need of more accurate assays.

Statistical analysis detected significant differences between samples (I) from the spout at Cabreúva and (II) the spout at Cajamar, compared with negative and neutral controls ($P < 0.05$), and did not detect significant statistically difference at the comparison of the same samples to the ones from the positive control ($P > 0.05$), indicating that the water from the experimental sample (I) from Cabreúva and from the experimental sample (II) from Cajamar have substances which are capable of causing mutagenesis in living beings, although it has not been the objective of the present study identifying such substances, which could only be done with very specific analyzes.

Significant statistical differences were also detected between the experimental sample (III) from Jundiaí and all controls ($P > 0.05$), which indicates that the sample does not present the high levels of positive control mutagenesis, but it is not as innocuous as tap water or distilled water.

Other bioassays with Trad-MCN have identified previous mutagenic substance and presented similar results as the present study. Araújo et al. (2014) observed the presence of the micronuclei in the *T. pallida purpurea*, when it was exposed to pollution from urban effluents, and obtained values as ($< 0,05$), thus evidencing the genotoxicity from the effluent; Alves et al. (2003) performed the biomonitoring of the laboratory's air and Botanic Institute Herbarium, where the naphthalene was used as insecticide, they found the *T. pallida purpurea* micronuclei presence 2.3%-8.4% at laboratory and 7.4% at herbal. In our study we detected the 4.67% and 10.67% micronuclei in experimental samples.

The physical-chemical and microbiological analyzes results presented nonconformity with CONAMA 357/2005 Resolution, that defined the limited 200 UFC (colony forming unit) to total or fecal coliforms. At this work, we observed and found 400 UFC in the sample (I), from Cabreúva's source, attesting that this water is not appropriated to human consumption. It is important to highlight that the resolution determines the value about six samples collected over a year; however, for the present assay, we have collected only one. Regarding the other parameters, there were no nonconformities.

The Decree 2.914/2011 provides for vigilance control and procedures to ensure water with quality for human consumption and its standard of potability (Brasil, 2011). In its chapter V, the decree determines that in the case of coliforms presence, one should take corrective actions until satisfactory results are reached. In the water samples from

Cabreúva's (I) and Jundiaí's (III) it was observed 800 and 1.200 UFC total coliforms, respectively.

According to the values obtained to microbiological and mutagenicity obtained in this work, we suggest a greater control by the authorities in these water sources, to prevent its use by the population. It is necessary another assay to determine the mutagenic factors, and the source of pollution.

Conclusions

The Trad-MCN assay was effective to detect the mutagenic factors presence in the water. Our results show that the water sources I, II and III contained genotoxicants, evidenced by the great micronuclei number. Besides that, the microbiological assay shows a great number of the total and fecal coliforms, in disagreement with the relevant legislation.

It is important to emphasize that the population consume these waters based on its turbidity. The authorities must inspect these water sources, alert and prevent the population from consuming water from these places.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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