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Cytotoxic potential of waters of the streams Mandacaru, Maringá, Miosótis and Nazareth in the urban area of Maringá, Paraná State, Brazil

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ABSTRACT. Streams Mandacaru, Maringá, Miosótis and Nazareth in the urban area of Maringá, Estado do Paraná, Brazil are affected by the local population's polluting practices. Meristemic cells of *Allium cepa* L. roots were used as test system to evaluate the cytotoxic effects of the streams' water. Onion roots were prepared by Feulgen reaction and stained with Schiff's reagent. Statistical analysis of the mitotic index was undertaken with the chi-square test. Results did not show significant cytotoxic effect of water samples. Results may dependent on climate variations, concentration of harmful substances, and on the time and samples sites evaluated. A deeper awareness of the population should be underscored for the proper use, preservation and maintenance of soil and water, with particular emphasis to this region.

Keywords: cytotoxicity, stream water, *Allium cepa* L.

Investigação do potencial citotóxico das águas dos ribeirões Mandacaru, Maringá, Miosótis e Nazareth, localizados na área urbana de Maringá, Estado do Paraná, Brasil

RESUMO. Os Ribeirões Mandacaru, Maringá, Miosótis e Nazareth estão localizados na zona urbana da cidade de Maringá, Estado do Paraná, e por isso, recebem influência das práticas poluentes da população local. Com o objetivo de avaliar os efeitos citotóxicos das águas desses ribeirões, foi utilizado como sistema-teste de células meristemáticas de raízes de *Allium cepa* L. As raízes de cebola foram preparadas pela reação de Feulgen e coradas com o reativo de Schiff. Os índices mitóticos foram analisados estatisticamente utilizando o teste do Qui-quadrado. Os resultados obtidos não mostram efeito citotóxico significativo das águas amostradas avaliadas. Os resultados obtidos podem ser dependentes das variações climáticas, da concentração das substâncias nocivas, e da época e local das amostragens avaliadas. Além disso, vale destacar a necessidade de ser realizada, principalmente na área de estudo, conscientização da população em geral, no sentido de uso, preservação e manutenção do solo e das águas.

Palavras-chave: citotoxicidade, água de ribeirão, *Allium cepa* L.

Introduction

Increase in human population and an accelerated industrialization process have caused unprecedented changes in water quality. Water has always been considered a renewable natural resource. Nevertheless, environmentalists have recently been warning on water waste and contamination caused by deforestation, pollutants, the release of debris in a greater amount than the river's speed and capacity to break them up, and by mining discharges which dilute the hazardous elements and heavy metals, such as mercury, lead and zinc (MORAES; JORDÃO, 2002).

Brazil has 12% of all fresh water on Earth, with an immense wealth of surface water courses and underground water. The city of Maringá in the northwestern region of the state of Paraná, Brazil, lies 596 m above sea level, at 23º25'S and 51º57'W. Several streams, running within its urban area, discharge water into the river Pirapó which, in turn, supplies the population with fresh water (BORSATO; MARTONI, 2004).

Results from bacteriological and chemical analysis supplied by the Agriculture and Environment Department show that, according to CONAMA 357 (Decree 357 of the National Environmental Council) reference tables, certain streams in Maringá, especially streams Mandacaru, Maringá, Miosótis and Nazareth, are contaminated with total coliform and thermotolerant, oils, grease, heavy metals and organic nitrogen.

Harmful consequences on people exposed to contaminated water are numerous and may cause the development of allergic reactions through direct contact, such as water ingestion, or through indirect contact, such as inhalation of toxic gas products or by ingestion of animals or plants that accumulated harmful substances such as pollutants in the water. People may also develop diseases caused by pathogens such as cholera, schistosomiasis and other water-brought diseases with great damage to human organisms (such as cancer), detected only after a long period of exposure. Frequently, harmful effects will become apparent only in future generations, such as malformation in babies and other congenital diseases caused by genotoxic substances (MORAES; JORDÃO, 2002).

Genotoxic substances are substances that inscribe chemical changes in DNA and alter the stability of cell processes, particularly cell division. Their effects are mainly detected through changes in the growth and reproduction of living organisms. However, they are cytotoxic when they interfere with the viability of the cell in general (SILVA et al., 2003).

Quantifying the reduction of cell division in the meristemic cells of *A. cepa* L. roots is a reliable means to determine quickly the presence of toxic substances in the environment for the monitoring of pollution levels in natural environments and to evaluate the levels of water pollution is. Results of *A. cepa* L. test may indicate the presence of cytotoxic and genotoxic or mutagenic sub-modalities in the environment, which endangers the survival of living organisms (MATSUMOTO et al., 2006).

The above-mentioned test is used routinely in laboratories around the world and is considered a valuable tool in determining the environmental contamination by chemicals tested and provided for in databases.

This study evaluated the cytotoxic potential of waters from streams Mandacaru, Maringá, Miosótis and Nazareth by the test system of meristemic cells of *Allium cepa* L. root through cell division rates.

Material and methods

The cytotoxicity of water from source and downstream of the streams Mandacaru, Maringá, Miosótis and Nazareth (Figure 1) was evaluated as a test system using the roots of *Allium cepa* L. Sampled water, collected from all streams, was kept in a refrigerator (4°C), for 48 hours, till use.

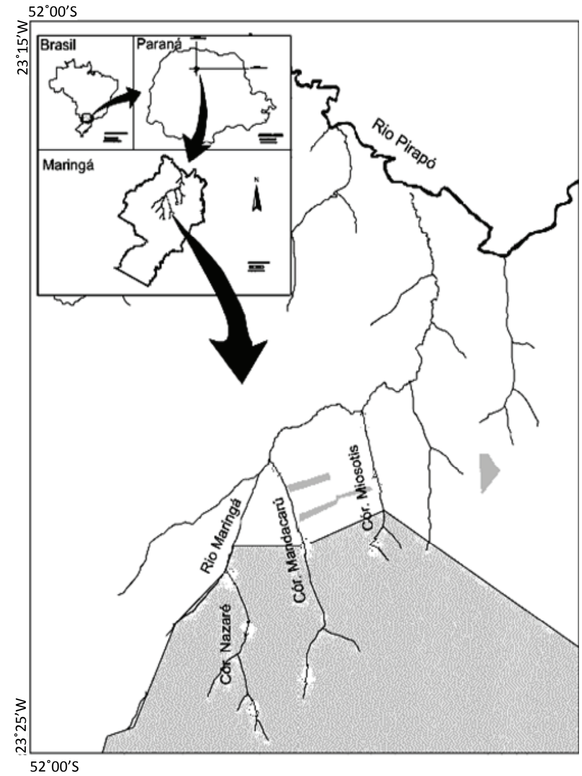

Figure 1. Location of streams analyzed, Mandacaru, Maringá, Miosótis and Nazareth, and their area of influence on urban regions (in gray). Modified from Cunico et al. (2006).

Three negative controls (CO1, 2 and 3), undertaken according to the date of collection of water samples from streams, were developed in filtered water to compare different samples of water with their respective controls.

Experiment was carried out according to methodology originally introduced by Levan (1949, apud FISKESJÖ, 1985). Onion bulbs produced roots in flasks with water at room temperature; they were then aerated and placed in the dark. Before each treatment, three roots were collected and fixed (3 methanol: 1 acetic acid) as control (Co). The roots of these bulbs were then placed in the collected water samples during 24h. After treatment, six roots were withdrawn from each onion and fixed (Tr). Remaining roots were washed and the bulbs again placed in water for 24 hours, since the time of mitosis in onion is 13.5 hours at 25°C, to recover from any chromosomal damage, or in the mitotic spindle of cellular division, which may have occurred. Remaining roots were retrieved and fixed (Re). The onions in the negative control group remained in filtered water throughout the entire sampling period (CO^-).

Onion roots were prepared by Feulgen reaction and stained with Schiff's reagent. Analysis of slides was undertaken in "blind test" by 400x light microscope to calculate the mitotic index (MI-%). Five bulbs were used for each negative control and treated with water samples from each stream to determine MI. Further, 1,000 cells per bulb, totaling 5,000 cells per group, were analyzed. Statistical analysis was undertaken by chi-square test ($\alpha = 0.05$).

Water samples (from source and downstream sites of the four streams) were analyzed and compared for their physical and chemical parameters, namely pH, chemical oxygen demand (COD), biochemical oxygen demand (BO_5D), presence/absence of oils and greases, and atomic absorption, with the limit parameters of CONAMA 357 (Decree 357 of the National Environmental Council) for the above-mentioned streams. The physical and chemical reports were issued by Mary Abrão de Campos, coordinator of the Sanitation Laboratory, Department of Civil Engineering, State University of Maringá, and by biochemical José Roberto Franca de Abreu, Coordinator of the Maringá section of the Paraná Waterworks (Sanepar).

The characteristics of collection sites and samples in the Mandacaru stream were: source - apparently clean water at the time of collection; the area features scanty riparian vegetation; fingerlings on the site and watercress plantation nearby - and downstream - the water was collected some 5 m from the site of the Sanepar sewage discharge; no riparian vegetation; strong bad smell and heavy pollution; the area features pastureland and drained lowlands. With regard to the Maringá stream: source - sampling site with no riparian vegetation; extensive erosion; much littering and household garbage - and downstream - collecting site with no riparian vegetation; the surrounding area is used for agriculture. With regard to the Miosótis stream: source - bad-smelling water; no riparian vegetation; surroundings with extensive pollution - and downstream - reddish water; site with small quantities of garbage; extensive erosion; presence of pigs, no bad smell, riparian vegetation extant. With regard to the Nazareth stream: source - apparently clean water; scanty vegetation; planting of bean crops in the vicinity - and downstream - slightly cloudy water; no riparian vegetation at the site; presence of livestock in the vicinity; erosion.

Results and discussion

The *A. cepa* test, which uses the meristemic cells of onion roots, determines the cytotoxic and mutagenic effects of various substances and is a standard procedure for rapid tests in detecting toxins and pollutant levels in the environment. Current study evaluated the cytotoxic effects of water from streams Mandacaru, Maringá, Miosótis and Nazareth located in urban area of Maringá, Paraná State, Brazil.

Figure 2 shows the results of mean mitotic index (MI) obtained from meristemic cells of *A. cepa* L. root. The chi-square test evaluated average values of MI and compared results obtained at different sampling times 0, 24 and over 24h, Control, Treatment and Recovery, respectively, in the same experimental group, between the different experimental groups and between the later and results for negative controls at the corresponding time of sampling.

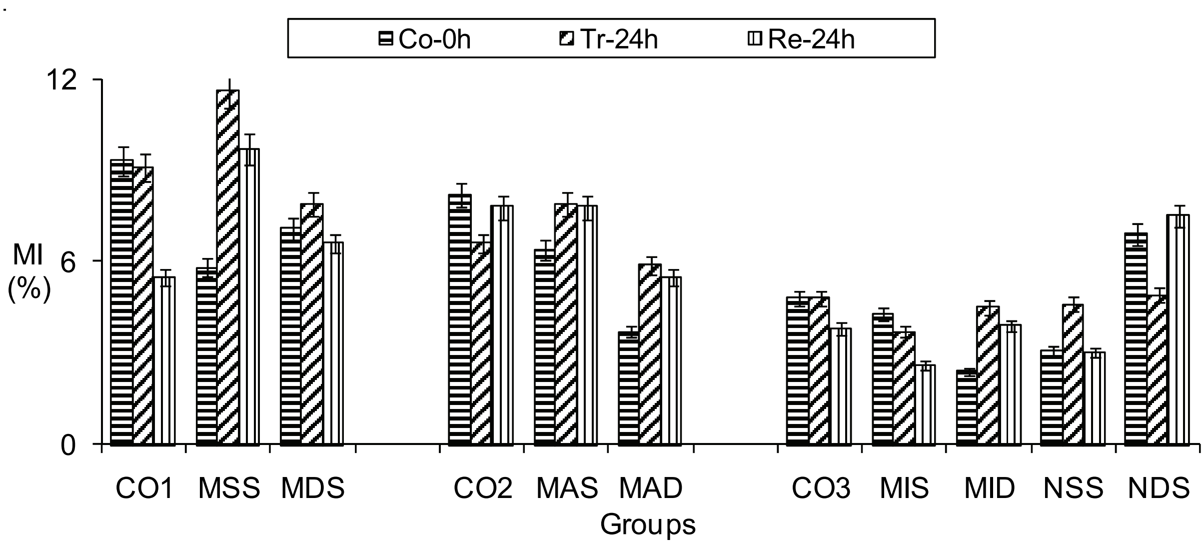

Figure 2. Mean mitotic index (MI) obtained for each control group and treated with water from streams in Maringá, Paraná State, Brazil, with meristemic cells of *Allium cepa* L. root.

Groups - Control: Control 1 (CO1), Control 2 (CO2), Control 3 (CO3), and Treatment: Mandacaru Source Site (MSS) and Downstream Site (MDS); Maringá Source Site (MAS) and Downstream Site (MAD); Miosótis Source Site (MIS) and Downstream Site (MID); Nazareth Source Site (NSS) and Downstream Site (NDS).

Time Sampling: Control (Co) = 0h, Treatment (Tr) = 24h, Recovery (Re) = 24h.

Total number of cells analyzed: 5,000 per group.

There were no significant changes in cell division rates in the eight water samples evaluated either when compared to treatments (24h) with their respective controls at 0h or when compared with MI recovery rates (24h). No changes were observed when results of three groups of water samples from streams were compared with sampling times of the corresponding negative control group.

Although statistical analysis did not show significant results with regard to the cytotoxic potential of water in the streams studied, it is worth noting that there are differences in the percentage between the mitotic indexes of treatments (Tr = 24h) with the source waters of streams Mandacaru, Maringá and Miosótis and their respective negative controls (Tr = 24h). The average percentage of mitotic index at the source waters of streams Mandacaru and Maringá increased by 28% and 20% respectively when compared with the average percentage of their control groups. This fact indicated that these waters may contain substances with mitogenic capacities, consequently causing an increased rate of cell division, which can lead to uncontrolled proliferation of cells. The source of Miosótis stream decreased by 22% the average percentage of the mitotic index when compared to control percentage. This fact indicated that the water contains substances that may be toxics, consequently inhibiting cell division of cells of *A. cepa* L. The data above suggest that the sources of the streams Mandacaru and Maringá, which are situated closer to the urban area of Maringá, Paraná State, as compared to the source of the Miosotis stream (Figure 1), undergo a greater discharge of pollutants of organic origin. Thus, a greater induction of cell growth in treated cells exists. These results may be due to high levels of oils, phosphate, aluminum, fecal coliform and BO_5D present at the source of the stream Maringá.

Lack of any chronic toxicity in water bodies is the first item in Articles 14 and 15 of CONAMA Decree 357, 2005, as a prerequisite for Class 1 and Class 2 types of fresh water bodies (SILVA et al., 2003).

In fact, waters of the above-mentioned classes are used as supplies for human consumption, recreation, fishing and irrigation. A negative result for the water's toxic effect is, however, only one of the twelve conditions prior to certifying its quality. However, this does not take into account the maximum allowance for each organic or inorganic chemical compound. The list varies between 18 and 90 items and depends on the intended use.

When tested in *A. cepa* L., the waters of streams Mandacaru, Maringá, Miosótis and Nazareth met the CONAMA's conditions for freshwater quality. This comprised absence of deleterious effects that affected the organisms' biological functions, such as reproduction, behavior or growth. The last item was estimated by counting the number of cell divisions in the meristem of the plant whose roots, in direct contact with the water samples during 24h, were not significantly affected.

Research on other water bodies had similar results. Caritá and Marin-Morales (2008), did not observe cytotoxic and mutagenic potential of low concentrations of effluents from a textile industry located in the city of Cajamar, São Paulo Sate when they used the test system of *A. cepa* L too. Grover and Kaur (1999) tested samples of sewage and effluents from industries in Amritsar, India. The *Allium* micronucleus test and anaphase aberrant showed that there was no difference for sewage effluents, although differences existed in industrial effluents.

These results may be explained by the influence of variables such as hydrogen ionic potential (pH), dissolved oxygen (DO), water and air temperature, transparency, turbidity, salinity and organic matter in the water and its reactions with xenobiotic substances. They may interfere with the reactions of toxic agents with biotic and abiotic components, and affect the toxicity of chemical compounds.

Table 1 shows that pH rates of water samples analyzed in current research were approximately 7.0, except for the Nazareth (5.82) and Mandacaru (5.92) stream sources which were below the limit allowed by CONAMA (7.0). In fact, the 7.0 rate, close to neutral pH, may have influenced the non-cytotoxicity of the six samples evaluated.

Table 1. Results of chemical analysis of water samples provided by the Laboratory of Sanitation of the State University of Maringá and Maringá Unity of Sanitation of Paraná (Sanepar)*.

Sites in streams	pH	Color (uH)	Turbidity (uT)	SSIC (mL Lh^{-1})	Dissolved Oxygen (mg L^{-1})	COD (mg L^{-1})	BOD_5 (mg L^{-1})	Oils and Greases (mg L^{-1})	Fecal Coliforms*	Total Coliforms*
					Mandacaru					
Source	5.92	-	-	-	-	3.0	0.3	0.30	-	-
Downstream	7.24	15	1.69	-	-	8.0	3.5	0.22	5,500	1,900
					Maringá*					
Source	7.40	12.5	7.2	0.00	7.39	7.8	2.4	1.00	9,000	8,500
Downstream	7.42	18	8.5	0.02	7.09	9.8	4.5	1.80	12,000	1,800
					Miosótis					
Source	6.82	-	-	-	-	2.0	0.6	0.25	-	-
Downstream	7.17	-	-	-	-	7.0	3.0	0.27	-	-
					Nazareth					
Source	5.82	-	-	-	-	14.0	5.0	1.50	-	-
Downstream	7.24	15	1.19	-	-	54.0	18.0	2.20	4,300	2,500

Amostrated regions of the streams: Source and Downstream of Mandacaru, Maringá, Miosótis and Nazareth. SSIC = sediment solids in IMHOFF cone; COD = chemical oxygen demand; BOD_5 = biochemical oxygen demand. = Not reported in the results analyzes water.

Besides the results in Table 1, results of the physical-chemical analysis and atomic absorption presented in the report analyzed by the Sanepar (only elements in the samples are mentioned) were as follows: Mandacaru stream: source - Atomic Absorption analysis: barium - 0.3 mg L^{-1} - and downstream - Physical-chemical analysis: nitrate - 5.49 mg L^{-1}; chlorine - 12.6 mg L^{-1}; total dissolved solids – 164 mg L^{-1}; sulphate - 7.6 mg L^{-1}; total alkalinity – 89 mg L^{-1}; nitrite - 0.064 mg L^{-1}; phosphate - 0.327 mg L^{-1}; ammonia - 0.33 mg L^{-1} NH_3; total nitrogen - 6.78 mg L^{-1}. Maringá stream: source - Physical-chemical analysis: nitrate - 2.14 mg L^{-1}; chlorine – 11 mg L^{-1}; total dissolved solids – 159 mg L^{-1}; sulphate - 6.9 mg L^{-1}; total alkalinity – 84 mg L^{-1}; nitrite - 0.044 mg L^{-1}; phosphate - 0.314 mg L^{-1}; ammonia - 4.98 mg L^{-1} NH_3; total nitrogen - 12.3 mg L^{-1}; Atomic Absorption analysis: aluminum - 0.6 mg L^{-1}; barium - 0.3 mg L^{-1}; total iron - 3.0 mg L^{-1}; manganese - 0.210 mg L^{-1} - and downstream - Physical-chemical analysis: nitrate - 2.53 mg L^{-1}; chlorine - 11.1 mg L^{-1}; total dissolved solids – 161 mg L^{-1}; sulphate - 8.7 mg L^{-1}; total alkalinity – 85 mg L^{-1}; phosphate - 0.026 mg L^{-1}; ammonia - 0.39 mg L^{-1} NH_3; total nitrogen - 3.2 mg L^{-1}; Atomic Absorption analysis: aluminum - 0.3 mg L^{-1}; total iron - 0.80 mg L^{-1}; manganese - 0.07 mg L^{-1}. Nazareth stream: source - Atomic Absorption analysis: barium - 0.5 mg L^{-1}; total iron - 0.1 mg L^{-1} - and downstream - Physical-Chemical analysis: nitrate - 11.03mg L^{-1}; chlorine - 24.2mg L^{-1}; total dissolved solids – 230 mg L^{-1}; sulphate - 9.0 mg L^{-1}; total alkalinity – 95 mg L^{-1}; nitrite - 0,002 mg L^{-1}; phosphate - 0.037 mg L^{-1}; ammonia - 0.39 mg L^{-1} NH_3; total nitrogen - 13.4 mg L^{-1}.

Mitteregger-Júnior et al. (2006), employing *A. cepa* L., analyzed water and sediments from three different points in the stream Arroio Estância Velha, Rio Grande do Sul State, Brazil and also found pH close to 7.0 in all samples. This fact may have decreased water toxicity. They also reported that when pH is close to neutral, water toxicity is low.

Another variable which affects results is the occurrence of rainfall, its effect on the total depth of the river and soil discharge, with modifications in the water's physical and chemical composition.

Excepting samples from the stream Maringá's source and downstream sites, the other samples in current study (source and downstream sites of streams Mandacaru, Miosótis and Nazareth) were collected during the rainy season in January, with an average rainfall of 145.7 mm per month reported by the Meteorological Station of the State University of Maringá. High average rainfall and, consequently, high dilution of various pollutants in the streams, coupled to their discharge, may result in the water's non-cytotoxicity.

According to Silva et al. (2004), the negative results obtained by an animal test system experiment with bone marrow of Wistar rats treated *in vivo* for the cytotoxic and mutagenic effects of wells and waters from streams Ficha and Minas Gerais, both in the state of Paraná, Brazil, may have been affected by the period of sample collection carried out during the rainy season. The authors concluded that after heavy rains, there is an increase in water volume which disperses harmful compounds and impairs the test from detecting pollutants.

Mitteregger-Júnior et al. (2006), carrying out experiments during winter and summer, observed that water from the stream Estância Velha, Rio Grande do Sul State, Brazil, which receives effluents from the leather-tanning industry, had the worst rates when the water's physical and chemical parameters were analyzed during the summer. This may be due to drought-caused lower stream flow, when drainage streams are flow-based, or rather, when their contribution derives exclusively from the water table.

Seasonal difference in genotoxicity was reported in municipal landfill leachate, using the *Vicia faba* root-tip cytogenetic bioassay, in February and August 2003. Due to their increasing concentrations of leachate, significant differences of mitotic index, micronucleus frequency and anaphase aberration frequency were observed between two samples. The results show that genetic damages in *Vicia faba* were more severe when the samples had been collected in the cold and dry season than in the hot and rainy season. In fact, higher temperatures would tend to increase internal landfill temperatures, accelerate the microbiological decomposition processes, and result in the predominant low molecular weight nature of the organic materials (SANG; LI, 2004).

Experiments with waters from stream Maringá were undertaken during August, with very low mean rainfall, reaching only 0.4 mm. Coincidentally, the amount of oils and greases shown on the physical and chemical reports with regard to the source site of the stream Maringá (6 mg L^{-1}) was greater but less diluted than the rates found in samples taken from the sources during the rainy season (Table 1).

According to the Minas Gerais Institute for Water Management (IGAM), greases and oils are substances derived from mineral, vegetable or animal sources, related to industrial and domestic pollution, but rarely found in natural waters. These substances, negative factors in the treatment of sewage and public water supply, reduce the dissolved oxygen and raise BO_5D and COD rates (IGAM, 2009).

BO_5D increase in water body is caused by discharges derived predominantly from organic origin, since an increase in COD concentration is mainly due to industrial discharges. Both tests measure the amount of oxygen required to oxidize organic matter, the first through aerobic bacteria incubated at 20°C for 5 days and the second by a chemical agent that detects even the presence of substances resistant to biological degradation (IGAM, 2009).

As verified in the chemical analysis of samples used in current research, the high rates of oils and greases are concomitant with high BO_5D and COD rates (Table 1).

Moreover, when compared to indexes in CONAMA 357, there was a high concentration of phosphate in stream water downstream Mandacaru (0.327 mg L^{-1}, normal = 0.025 mg L^{-1}), Maringá source (0.314 mg L^{-1}) and downstream (0.026 mg L^{-1}) and total nitrogen in the river downstream Nazareth (11.03 mg L^{-1}, normal = 13.3 mg L^{-1} at pH < 7.5). This fact indicated, according to Mirlean et al. (2005), that these waters were getting a high discharge of pollutants from primarily industrial.

High rates of organic matter may deplete oxygen from the water, with the extinction of fish species and other aquatic types of life.

Research undertaken by Cunico et al. (2006), who investigated the influence of urbanization on fish assemblages in streams Mandacaru, Miosótis and Nazareth, showed low species richness and a high numerical representation of only three fish species (*Poecilia reticulata*, *Hypostomus ancistroides* and *Rhamdia quelen*).

According to Cunico et al. (2006), the high representation of *Poecilia reticulata* contrasts with the low density of native species characteristic of the sites under analysis. This is due to the fact that the above-mentioned streams are highly affected by input of pollutants from urban areas of Maringá. The streams actually receive effluents from various, mainly untreated, sewage sources and leaching of impermeable surfaces, with reduced levels of oxygen due to human occupation (buildings and industries) and agricultural activities carried out in the respective microbasins.

Concerning the presence of heavy metals, the source and downstream of Maringá stream had aluminum rates (0.6 mg L^{-1} to source and 0.3 mg L^{-1} to downstream; normal = 0.2 mg L^{-1}) above those permitted by CONAMA 357. The study by Achary et al. (2008) showed that aluminium induced oxidative stress and DNA damage in root cells of *A. cepa* L.

Moreover, contrastingly to the findings of current study, the results by Fatima and Ahmad (2006), who assessed the genotoxicity of industrial wastewater samples from Aligarh and Ghaziabad in India, using the *A. cepa* L. test, showed that the samples induced various anaphase aberrations. Gana et al. (2008) studied the biotoxicity of water from industrial effluent discharge areas of the Salí river-Tucumán-Argentina, using *Allium* anaphase–telophase, and observed that all samples were phytotoxic and genotoxic for *Allium* roots.

Lemos et al. (2007), using another test system, namely the micronucleus assays in erythrocytes from peripheral blood of the fathead minnow *Pimephales promelas*, studied the quality of Caí river water, Rio Grande do Sul State, Brazil, in an area under the influence of a petrochemical complex and showed the presence of substances with clastogenic and/or aneugenic potential. And, Hajjouji et al. (2007) studied the genotoxicity of olive mill waste water generated in mills producing olive oil in

Morocco, using the *Vicia faba* micronucleus test, and showed that this was genotoxic.

Although current results fail to indicate cytotoxicity in the water samples from streams Mandacaru, Maringá, Miosótis and Nazareth, their waters contain one or more compounds which negatively affect their quality. Based on the visual inspection of the collected samples and foregrounded by results of current physical and chemical analysis, environmental awareness in the population with regard to the use, preservation and maintenance of soil and water is highly urgent.

Conclusion

The results of this experiment, undertaken with the meristemic cells of *A. cepa* L. roots, showed no statistically significant changes in the rates of cell division in water samples from source and downstream sites of streams Mandacaru, Maringá, Miosótis and Nazareth in treatments for 24 hours when compared with data obtained from their respective controls either in 0h or after 24 hours of recovery in water, or when compared to respective data from negative controls undertaken in filtered water.

Although pollution in the assessed waters failed to have any cytotoxic effect on the *A. cepa* L. test, results agree with and corroborate those from the literature. In fact, pollution degrades water quality and endangers the live of the organisms that depend on it. On the other hand, harmful effects of pollution in organisms, shown either by the test or by the evaluation methodology, depend on climatic variables, concentration of harmful substances and other environmental characteristics assessed, time and sites of the samples evaluated.

Further studies in the area should be undertaken before any use of the waters of the four streams assessed may be made. This applies not only for consumption but also for other activities such as bathing or recreation.

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