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# Vegetal test-system investigation on cytotoxicity of water from urban streams located in the northeastern region of Maringá, Paraná State, Brazil

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**ABSTRACT.** The increase in consumption of water, the destruction of riparian forests and the pollution caused by humans, have severely degraded several water resources. Numerous stream crisscross the city of Maringá, Paraná state, Brazil and most of their sources are scattered through the urban region. Current analysis assesses the cytotoxic potential of water from the Corregozinho, Isalto, Morangueira and Ozório streams, located within the high-populated northeastern region of Maringá, inside the urban perimeter. Root meristematic cells of *Allium cepa* were used as test-system. The roots of onion were prepared by Feulgen's reaction and stained with Schiff's reagent. Results showed that there were no statistically significant changes evaluated by the chi-square test on the rates of cell division in cells of *Allium cepa* roots treated with the water of the streams when compared to data from controls. However, further analyses should be undertaken at different times for a periodic assessment of conditions in the streams, coupled with an awareness of the population on the environment.

**Key words:** environmental mutagenesis, water contamination, *Allium cepa*.

**RESUMO. Investigação da citotoxicidade das águas de córregos urbanos localizados na região nordeste de Maringá, Estado do Paraná, Brasil, em sistema-teste vegetal.** O aumento do consumo da água, a devastação das matas ciliares e a poluição causada pela espécie humana, fizeram com que muitos recursos hídricos fossem drasticamente degradados. A cidade de Maringá, Paraná, Brasil possui inúmeros córregos que a cortam, sendo que as nascentes da maioria deles estão espalhadas pela região urbana. Assim, este trabalho objetivou avaliar o potencial citotóxico das águas dos córregos, Corregozinho, Isalto, Morangueira e Ozório, localizados na região nordeste de Maringá, no perímetro urbano, local de grande concentração da população urbana. Foram utilizadas como sistema-teste as células meristemáticas de raiz de *Allium cepa*. As raízes de cebola foram preparadas pela reação de Feulgen e coradas com o reativo de Schiff. Os resultados obtidos indicaram que não houve alterações, estatisticamente significativas, pelo teste do qui-quadrado, nos índices de divisão celular nas células das raízes de *Allium cepa* tratadas com as águas dos córregos, em relação aos dados obtidos para os controles. Entretanto, convém que outras análises, em diferentes épocas, sejam realizadas, para uma avaliação periódica das condições apresentadas por estes córregos, e aliado a estes resultados, conscientizar a população em relação aos danos provocados ao meio ambiente e a necessidade de conservação do mesmo.

**Palavras-chave:** mutagênese ambiental, contaminação de águas, *Allium cepa*.

## Introduction

Since water has always been considered to be infinitely abundant, aquatic environments were used throughout the world for various purposes, such as water supply, energy generation, irrigation and navigation. Consequently great rivers have been degraded through the destruction of their riparian vegetation, pollution and increasing demand for water.

The concept of high water abundance is still very prevalent in Brazil, one of the few countries characterized by this feature. However, if a more detailed analysis of the Brazilian situation is made, a completely different scenario comes to the fore. In fact, the continuous use of this resource by humans is causing serious environmental problems (MIERZWA; HESPANHOL, 2005). The problem of water pollution has reached catastrophic proportions in many cities. Since there is high water consumption, several pollution sources, in the form of domestic sewage and industrial effluent, have prevailed.

All this enormous amount of water for domestic and industrial activities is not consumed, but withdrawn from nature and then returned as polluted or inefficiently treated water. Pollution implodes the receiving aquatic ecosystem by organic wastes above the decomposition organism's absorption capacity and by inorganic, non-biodegradable wastes that frequently have toxic and cumulative effects on the environment's organisms (MORAES; JORDÃO, 2002).

Human exposure to waste dumping has various health-related effects, such as headaches, nausea, skin irritations and severe neurological diseases. Evidence exists related to of genotoxic effects on health, such as cancer, birth defects and reproduction abnormalities (MORAES; JORDÃO, 2002). These results are a great concern from the environmental point of view and when related to humans since their toxicological target is human DNA.

Researching the true situation of water resources of Maringá is thus mandatory. Biological studies are being used to assess the mutagenic potential of water polluting agents from rivers and streams in urban areas. The *Allium cepa* test, using the onion roots' meristeme cells to determine the cytotoxic and mutagenic effects of various substances, is a standard procedure for rapid tests to detect toxins and pollutant levels in the environment (SMAKA-KINCL et al., 1996).

Maringá, in the northwestern region of the state of Paraná, Brazil, was built on the watershed that divides the basins of the Pirapó and Ivaí rivers. This feature provides the city with a hydrographic network full of small streams, whose sources are mostly spread throughout the urban perimeter (BORSATO; MARTONI, 2004). Consequently the streams are extremely liable to urban domestic and industrial pollution.

The experimental part of current research assesses the cytotoxicity of water from the Corregozinho, Isalto, Morangueira and Ozório streams in the northeastern urban area of Maringá, Paraná Sate, Brazil, shown in Figure 1, using the *Allium cepa*'s roots test-system.

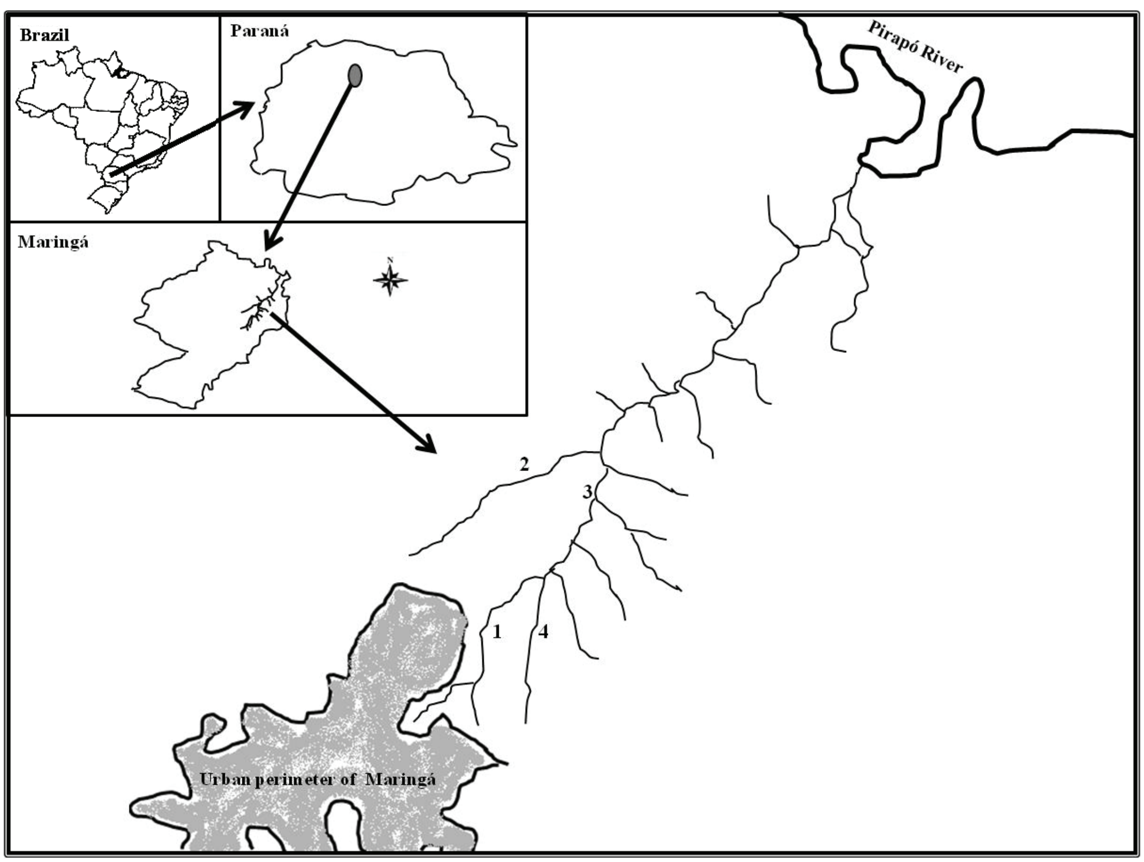


**Figure 1**. Location of streams in the Northeast urban perimeter of the city of Maringá, Paraná state, Brazil. 1 - Corregozinho; 2 - Isalto; 3 - Morangueira; 4 – Ozório.

## Material and methods

Water samples were collected from the spring source and from the confluence of the streams under analysis. Meteorological conditions on the collection day (11th August 2004) featured a clear day, with few clouds and no rainfall, with an average temperature of 19.8°C.

### Characteristics of collection sites and samples:

### Streams' source:

Corregozinho Stream: 23°24'33"S and 51°54'26"W, water featured a gray color, with patches of oil. The point of collection lies close to a crater-like hole caused by soil erosion.

Isalto Stream: 23°22'54"S and 51°54'38"W, water was collected at 30 m from a wheat-grown field. No riparian vegetation was extant; erosion was evident and a rubbish dump lay nearby.

Morangueira Stream: 23°24'31"S and 51°54'53"W, no riparian vegetation was extant at the sample collection site; erosion was evident and a domestic waste deposit lay close by. Bad smell emanated from the surrounding area.

Ozório Stream: 23°24'43"S and 51°54'0"W, water was turbid, without any bad smell. No riparian vegetation was extant; much garbage lay around, coupled to pasturing animals in the region.

### Confluence:

Corregozinho, Morangueira and Ozório streams: 23°51'50"S and 51°52'55"W, water's color was dark with oil stains, exhaling bad smell, similar to that of fuel. There was no riparian vegetation at the collection site. Eucalyptus trees had been planted in the area which seems to have been a garbage deposit, with many manure sacks.

The physical and chemical analyses of water samples were undertaken at the Sanitation Laboratory of the Department of Civil Engineering at the state University of Maringá and of the unit of Sanitation Company of Paraná (SANEPAR) in Maringá. Rates were compared to data for Class 3 of fresh water determined by the National Environment Council (CONAMA, 2005), the Brazilian official authority.

Experiment was undertaken by Feulgen's reaction and stained with Schiff's reagent. 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 (SÁEZ et al., 1966), 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 ($CO^-$).

Blade analysis consisted of a blind test, with a 40 x optical microscope. Five bulbs were used as control group and each group treated with water from each stream to assess cells with structural changes and to determine the Mitotic Index (IM-%). One thousand cells per bulb were analyzed, totaling 5,000 cells in control, treatment and recovery.

Statistical analysis was performed by the chi-square test ($\alpha = 0.05$).

## Results and discussion

Figure 2 shows that there were no statistically significant changes in the rates of cell division of the five types of water evaluated in treatments for 24h when compared to data from control at 0h. The same may be said with regard to 24h-recovery in water, or when compared with respective data from negative control, with filtered water, during respective sampling time. Results were not statistically significant when compared to those from the sources of Corregozinho, Morangueira, Ozório streams and their confluences.

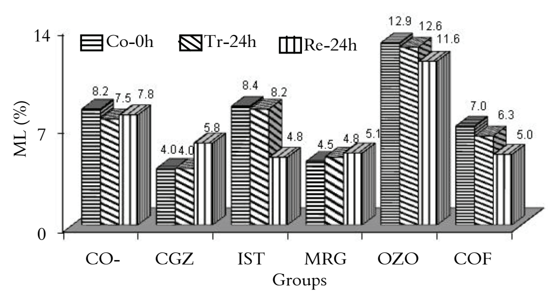


**Figure 2**. Mitotic Index (MI), mean, obtained for each control group and treated with waters from streams crossing Maringá, Paraná state, Brazil, in the meristemic cells of *Allium cepa* root. Groups: Control - $CO^-$ and Treated - Source: Corregozinho (CGZ), Isalto (IST), Morangueira (MRG), Ozório (OZO), Confluence: Corregozinho, Morangueira and Ozório (COF) Streams. 1 Time of sampling: Control (Co) = 0h, Treated (Tr) = 24h, Recovery (Re) = 24h. 2 Total number of cells examined: 5,000 per group.

Although the statistical analysis has not shown significant results on the cytotoxic potential of the waters of the tested streams, there are percentage differences between the mitotic indexes of the treatments (Tr = 24h) with water from Corregozinho, Morangueira and Ozório streams and negative control (Tr = 24h). The average percentage of the mitotic index of Corregozinho decreased 47% and of Morangueira 36% when compared to the average of the control group. This fact showed that these waters caused inhibition of cell division, probably resulting from the action of chemical co mpounds present in water on growth and development of *Allium cepa*. Ozório stream increased 68% the average percentage of mitotic index when compared to control percentage. This fact indicates that the water could contain substances with mitogenic capacity, causing an increased rate of cell division which is detrimental to cells and may lead to a disorderly cell proliferation and even the formation of tumor tissue. It is noteworthy that the cell division rates of Ozório stream were higher when compared with those from other streams. Table 1 shows that results confirm those obtained in the analysis of the physical and chemical parameters of treated water samples from all water sources. Since Ozório stream actually showed only slight changes in fecal coliform rates, it proved to be the least polluted stream.

Besides 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) are given below:

Corregozinho:

Physical-Chemical analysis: cyanides - 0.01 mg $L^{-1}$, nitrate - 4.24 mg $L^{-1}$, surfactants - 0.10 mg $L^{-1}$, chlorine - 187.0 mg $L^{-1}$, total dissolved solids - 585.0 mg $L^{-1}$, sulphate – 179.0 mg $L^{-1}$, total alkalinity - 99.0 mg $L^{-1}$, bicarbonate - 99.0 mg $L^{-1}$, nitrite - 0.02 mg $L^{-1}$, phosphate - 0.297 mg $L^{-1}$, total nitrogen - 5.10 mg $L^{-1}$.

Atomic Absorption analysis: aluminum - 0.3 mg $L^{-1}$, barium - 1.0 mg $L^{-1}$, total iron - 0.2 mg $L^{-1}$; manganese - 0.12 mg $L^{-1}$.

Isalto:

Physical-Chemical Analysis: cyanides - 0.01 mg $L^{-1}$, nitrate - 0.40 mg $L^{-1}$, chloride - 28.0 mg $L^{-1}$, total dissolved solids - 133.0 mg $L^{-1}$; sulphate - 14.0 mg $L^{-1}$, total alkalinity - 105.0 mg $L^{-1}$, bicarbonate - 105.0 mg $L^{-1}$; free carbon dioxide - $CO_2$ - 6.0 mg $L^{-1}$, nitrite - 0.013 mg $L^{-1}$, phosphate - 0.023 mg $L^{-1}$, ammonia - 0.44 mg $L^{-1}$; nitrogen total - 2.2 mg $L^{-1}$.

Atomic Absorption Analysis: aluminum - 0.1 mg $L^{-1}$; barium - 0.9 mg $L^{-1}$, total iron - 0.6 mg $L^{-1}$; manganese - 0.86 mg $L^{-1}$.

Morangueira:

Physical-Chemical Analysis: nitrate - 4.83 mg $L^{-1}$; surfactants - 0.1 mg $L^{-1}$, chloride - 7.2 mg $L^{-1}$, total dissolved solids - 109.0 mg $L^{-1}$; sulphate - 11.9 mg $L^{-1}$, total alkalinity - 44.0 mg $L^{-1}$, bicarbonate - 44.0 mg $L^{-1}$; free carbon dioxide - $CO_2$ - 28.7 mg $L^{-1}$, phosphate - 0.004 mg $L^{-1}$, total nitrogen - 4.83 mg $L^{-1}$.

Atomic Absorption Analysis: aluminum - 2.1 mg $L^{-1}$; barium - 0.3 mg $L^{-1}$, total iron - 0.8 mg $L^{-1}$; manganese - 0.06 mg $L^{-1}$.

Ozório:

Physical-Chemical Analysis: nitrate - 6.1 mg $L^{-1}$, chloride - 14.5 mg $L^{-1}$, total dissolved solids - 130.0 mg $L^{-1}$; sulphate - 12.5 mg $L^{-1}$, total alkalinity - 38.0 mg $L^{-1}$, bicarbonate - 38.0 mg $L^{-1}$; free carbon dioxide - $CO_2$ - 29.8 mg $L^{-1}$, nitrite - 0.004 mg $L^{-1}$, phosphate - 0.027 mg $L^{-1}$, total nitrogen - 6.10 mg $L^{-1}$.

Atomic Absorption Analysis: aluminum - 0.1 mg $L^{-1}$; barium - 0.2 mg $L^{-1}$, total iron - 0.1 mg $L^{-1}$.

Results corroborate data from other researches by Grover and Kaur (1999) in which samples of sewage and effluents from industries in Amritsar, India, were tested. *Allium* micronucleus test and anaphase aberrant showed that there was no difference for sewage effluents, although differences existed in industrial effluents. In a research with fish micronucleus tests, Grisolia and Starling (2001) evaluated waste water capacity for genetic damage from two municipal sewage treatment plants discharging contents into the Paraná Lake, Brasília, Brazil, and found that there were no significant differences in the frequencies of micronucleus.

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

| Streams Source | pH | Color (uH) | Turbidity (uT) | SSIC (mg $L^{-1}$) | Dissolved Oxygen (mg $L^{-1}$) | COD (mg $L^{-1}$) | $BOD_5$ (mg $L^{-1}$) | Oils and Greases (mg $L^{-1}$) | Fecal Coliforms* | Total Coliforms* |
|---|---|---|---|---|---|---|---|---|---|---|
| Corregozinho | 7.27 | 15 | 6.0 | 0.30 | 5.38 | 54.0 | 16.0 | 3.0 | 70,000 | 89,000 |
| Isalto | 7.40 | 17 | 7.5 | 0.00 | 6.25 | 39.0 | 12.0 | 2.4 | 1,800 | 7,400 |
| Morangueira | 6.82 | 10 | 4.5 | 0.01 | 7.62 | 8.8 | 2.7 | 1.4 | 3,600 | 5,800 |
| Ozório | 6.49 | 25 | 12.0 | 0.01 | 6.69 | 4.9 | 2.1 | 1.6 | 4,800 | 6,200 |
| Confluence | 7.09 | 75 | 21.0 | 0.35 | 6.79 | 29.0 | 15.0 | 2.4 | - | - |

Amostrated regions of the streams: Source and Confluence of Corregozinho, Morangueira and Ozório. SSIC = sediment solids in IMHOFF cone; COD = chemical oxygen demand; $BOD_5$ = biochemical oxygen demand.

This fact was also confirmed by Silva et al. (2004) who observed that water from wells and water from the Ficha and Minas Gerais rivers, near Ubiratã, Paraná Sate, Brazil, involving test-system of cells from the bone marrow of Wistar rats (*Rattus norvegicus*), failed to alter the rats' cell division and did not cause an increase in chromosomal aberrations. Similarly, Caritá and Marin-Morales (2008), using the *Allium cepa* test to evaluate the mutagenic potential and toxic of effluents from a textile industry, also showed no cytotoxic and mutagenic potential of low concentrations of effluent used in the test body.

Moreover, unlike the results found in current study, the results by Egito et al. (2007) in test system of *Allium cepa*, using water samples from the river Pitimbu, Natal, Brazil, were cytotoxic and mutagenic, and the water collected near an industrial area was the most toxic and genotoxic. Employing the comet with fish liver cells, Avishai et al. (2002) also showed that the Kishon river, Israel, revealed genotoxic potential. Further, Chandra et al. (2005) confirmed the genotoxic potential of solid waste leachate from a metal and dye industry using the *Allium cepa* test.

According to Cunico et al. (2006), the different regions of Maringá, Paraná State, Brazil, and consequently its streams, are differently affected by the city's urban areas. In fact, these comprise receipt of various effluents (with untreated sewage and leaching of impermeable surfaces, which establish a decrease in oxygen levels), human occupation (plots for building projects and industries) close to the streams, and agricultural activities developed in the micro-basins. Physical and chemical analyses undertaken in current study showed that the most polluted streams are those closest to downtown Maringá, or rather, Morangueira and Corregozinho streams, which possibly receive a greater load of pollutants.

Analysis of the water's physical and chemical parameters, shown in Table 1, revealed that, when compared to environmental standards for Class 3 fresh water decreed by the National Environment Council (CONAMA, 2005), all the streams indicated normal rates of pH (6-9), color (< 75 uH), turbidity (< 100 uT) and dissolved oxygen (> 4 mg $L^{-1}$). Moreover, streams were within normal rates of cyanide, nitrate, surfactant, chloride, sulfate, total alkalinity, free carbon dioxide, bicarbonate, nitrite, total nitrogen, ammonia and barium.

However, total dissolved solids (585 mg $L^{-1}$) and phosphate (0.297 mg $L^{-1}$) in Corregozinho stream were respectively higher than that allowed by Conama nº 357 (500 mg $L^{-1}$) and (0.025 mg $L^{-1}$). Moreover, Ozório stream presented a phosphate rate of 0.027 mg $L^{-1}$. Rates above those permissible for total dissolved solids may be due to the presence of organic material in suspension and higher concentration of nutrients in the water, indicating that high pollution (MARTINS; PITELLI, 2005). The high concentration of phosphate in the water of streams is an indication that they are receiving a high discharge of pollutants of industrial origin in the main, as found by Mirlean et al. (2005), who assessed the industrial impact on the chemical composition of groundwater.

Although Conama nº 357 requires that oil and grease should be practically absent, water samples from all streams exhibited oil patches, possibly derived from domestic pollution, and oil/fuel coming from the urban areas. The presence of this oil may cause disruption of the interface air/water, stop the flow of gas and have toxic effects on organisms. In their analysis of chromosomal aberration in *Allium cepa* in the water of the Guaeca river, São Paulo State, Brazil, affected by oil spill, Leme et al. (2008) revealed clastogenic and aneugenic effects.

$DBO_5$ rates in Corregozinho (16 mg $L^{-1}$) and Isalto (12 mg $L^{-1}$) streams and confluence of Morangueira, Ozório and Corregozinho (15 mg $L^{-1}$) streams were above the allowable limit (10 mg $L^{-1}$). Moreover, the concentration of total coliform in Corregozinho stream (89,000) was above that permitted (20,000); fecal coliform in Corregozinho (70,000) and Ozório (4,800) streams was above that permitted (4,000). These results show that there is a high load of organic compounds in the streams and, consequently, eutrophication.

Concerning the presence of heavy metals, Morangueira stream had rates above those permitted by Conama nº. 357, or rather, aluminum (2.1 mg $L^{-1}$; normal 0.2 mg $L^{-1}$) and total iron (0.8 mg $L^{-1}$; normal 0.3 mg $L^{-1}$); aluminum in Corregozinho stream reached 0.3 mg $L^{-1}$ and manganese in Isalto stream reached 0.860 mg $L^{-1}$ when the latter's normal rate should be 0.5 mg $L^{-1}$ and iron reached 0.6 mg $L^{-1}$ in Isalto stream. In spite of such high levels, results in current study showed no cytotoxic potential in the analyzed streams. However, Roy et al. (1989) identified an increase in the frequency of anaphase and telophase and abnormal cells in tests with *Allium sativum* with regard to aluminum, and Achary et al. (2008) showed that aluminum induced oxidative stress and DNA damage in root cells of *Allium cepa*. Similarly, but using other test-systems, Pascal and Tessier (2004) identified cytotoxicity in epithelial cells and Lima et al. (2008a) identified the cytotoxicity of manganese in cell culture of lymphocytes, whereas Dayeh et al. (2005) identified the cytotoxicity of manganese in fish cells and in the protozoan *Tetrahymena thermophila*. Lima et al. (2008b) showed that iron has mutagenic and cytotoxic potential in cell culture of lymphocytes.

The above data show that while the results indicate that water from streams was not cytotoxic and did not cause chromosomal changes in cells, one or more components in the water may have affected its quality. The impact of foreign substances on water from rivers depends on the concentrations of agents released, their type and duration, the ecosystem's capacity to resist these changes, the required time for the return to the state of equilibrium after the compound's removal, and the site of the ecosystem in its relationship to the place of compound's disposal (SILVA et al., 2003).

Although the data obtained in this study showed no cytotoxic effects, the physical-chemical analysis of water from streams showed values different from Conama standards for Class 3. This is worrying because, despite the results of the analysis, the waters of these streams, classified as Class 3, may be taken by animals and for human consumption after conventional or advanced treatment.

Further studies should be undertaken in the region analyzed at different times to make a periodic assessment of the conditions presented in these streams, coupled to a greater environmental awareness of the population.

## Conclusion

The results of current experiment, undertaken with the meristemic cells of *Allium cepa* root, evaluating the effect of water collected at the sources of the Corregozinho, Isalto, Morangueira and Ozório streams, and their confluence, within the urban region of Maringá, Paraná State, Brazil, showed no cytotoxicity of the streams under analysis. This fact does not guarantee that the water from these streams is fit for consumption, bathing and the practice of sports.

It is worth noting that there is a need for the deployment of an effective system of collection and treatment of household and industrial sewage so that, after being used, clean water may be returned to nature. Moreover, it is important that water is effectively treated in treatment plants so that it may be proper for consumption within several modalities.

Although research in diagnosing and treating degraded aquatic environments has greatly increased in the last decades, no magical or immediate solutions may solve the problems of environmental degradation. Much more than punitive measures contemplated by law, higher environmental awareness is still the most effective means for preventing a great crisis in water supply.

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