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Determination of heavy metals and genotoxicity of water from an artesian well in the city of Vazante-MG, Brazil

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The city of Vazante-MG is of great socioeconomic and environmental interest because it is the most important zinc producer district of Brazil. The mineral processing and geochemical processes may determine high concentrations of heavy metals in water intended for human consumption. Thus, the present study aimed to quantify and evaluate the heavy metal genotoxicity of artesian water in the city by Atomic absorption spectrophotometer analysis and testing with the *Allium cepa* test, respectively. This study reveals a chemical contamination in well water in the city, caused by the presence of heavy metals. Therefore, it can be considered that the high levels of heavy metals found in water samples are correlated with the genotoxic events observed in root cells of *A. cepa*.

Key words: *Allium cepa*, micronucleus, atomic absorption, chromosome aberration, mitotic index.

INTRODUCTION

The Vazante-MG region is of great socioeconomic and environmental interest, since it is the most important zinc producer district of Brazil (Hitzman et al., 2003). The inability of differentiating geogenic anomalies from those that result from processes of contamination related to human studies, suggests the need of assays to evaluate the presence of heavy metals in soil and water in order to contribute to a better evaluation of the occurrence of contamination by these metals (Borges Júnior et al., 2008). Preliminary evaluation of a suspected area of contamination is performed based on information available (CETESB, 1999, 2001). The area is considered contaminated if the concentration of elements or substances of interest are above the given threshold, which indicates the potential deleterious effect on human and animal health (Junior Borges et al., 2008a).

The effects of mineral processing together with the geochemical processes that naturally occur in reason of the soil characteristics and the entrainment of heavy particles to the aquatic system may provide high concentrations of heavy metals in water intended for domestic consumption (Yabe et al., 1998;, Guedes et al., 2005). The presence of heavy metals in groundwater may occur due to contact with rivers and lakes contaminated with sewage or by leaching by precipitation of contaminated soils (Di Natale et al., 2008).

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Abbreviations: MMS, methylmetanosulfonate; **MI,** mitotic index; **CA,** chromosome aberrations; **MN,** micronucleus.

Heavy metals are among the most common inorganic pollutants in water (Chandra et al., 2005). They are highly distributed over the earth's crust (Arambasic et al., 1995; Min et al., 2013) and represent one of the most toxic environmental pollutants (Ghosh, 2005; Sharma, 2009). Intoxication with heavy metals has been observed in many parts of the world, usually related to chronic exposure in environment through contamination of drinking water (Hang et al., 2009; Singh and Kalamdhad, 2013). Epidemiological evidence has shown that a long-term exposure is highly associated with increased risk of development of several diseases, including cancers (Zhuang et al., 2009). *In vivo* and *in vitro* assays have shown that heavy metals induce chromosomal aberrations and micronucleus in plant and animal (Rank et al., 1998; Majer et al., 2002; Rodriguez-Cea et al., 2003). Therefore it became important to carry out the environmental monitoring of water intended for human consumption.

Only with the chemical analyzes of water, it is not possible to determine the ecotoxicological risk that chemicals present in it can cause to the bodies, since such analysis alone does not indicate toxicity (Fuentes et al., 2006). Therefore, ecotoxicity tests among them those of environmental mutagenesis, have been proposed and applied to understand the genetic and physiological responses of exposed organisms (White et al., 2004; Chen et al., 2004). Bioassays with plants, such as the *Allium cepa* test, have some advantages over the tests in mammalian cells and microorganisms for environmental monitoring (Grant, 1994; Radic et al., 2014; Osakca & Silah, 2012). Plant assays are highly sensitive to many environmental pollutants, including heavy metals (Fiskesjo, 1985; Smaka-Kincl et al., 1996; Steinkellner et al., 1998; Fatima et al., 2005; Yi et al., 2007; Egito et al., 2007; Pesnya and Romanovsky, 2013). The use of the *A. cepa* bioassay is suggested because it is known that many plants are damaged by heavy metal contamination (Minissi et al., 1997; Amaral et al., 2007; Smith, 2001). This study aimed to quantify and evaluate the heavy metal genotoxicity of artesian water in the city of Vazante-MG/Brazil by atomic absorption spectrophotometer analysis and testing of the *A. cepa,* respectively.

MATERIALS AND METHODS

Samples collection

Water samples were collected at Vazante - MG/Brazil (S 17º 59'27 "W and 46º 54'04", altitude, 638 m) in May 2007, directly in wells registered by the Companhia de Saneamento de Minas Gerais (COPASA). Two collection points were determined: Sample 1 (S1): Water from the borehole, without treatment; Sample 2 (S2): water from an artesian well with simplified treatment (disinfection and fluoridation) in accordance with the rules of COPASA.

Three samples of 5 liters were collected from each point considered, being collected at intervals of 10 min and stored in sterile flasks, totaling 15 l per point. The pH of the samples was measured in the field, using a manual pH meter (Lutron pH-208).

The samples were transported in an isothermal box to the Laboratory of Chemistry and Instrumental Analytical Center of the University Center of Patos de Minas - UNIPAM.

Determination of heavy metals

For the heavy metals analyses, from each sample 100 mL was taken, 20 mL of nitric acid PA was added and then heated to evaporate until it remained 60 mL solution. After reaching room temperature, 40 mL of ultrapure water was added to obtain a final solution of 100 ml of sample for testing. The reading of the heavy metals in water was performed in triplicate and measured by the atomic absorption spectrophotometer Perkin Elmer 3300. In the present study, we analyzed the following metals: Cadmium, lead, copper and zinc. The gases used for reading were acetylene and compressed air in the flame analysis with hollow cathode lamp, a procedure performed in accordance with Santos et al. (2006).

Allium cepa test

Treatment

The experiment was conducted at the Laboratory of Plant Physiology, Centro Universitário de Patos de Minas, Minas Gerais and analyzed at the Laboratory of Herbal Medicines, Universidade Estadual Paulista, Assis-SP/ Brazil. The experimental protocol was essentially performed as described by Ma et al. (1995). Twelve (12) bulbs were exposed to water samples collected, six for samples collected in S1 and six for those collected in S2. Twelve (12) other bulbs were destined to negative control groups (NC) and positive control (PC), which were prepared using mineral water and methylmetanosulfonate (MMS) to 10 mg / L (MMS, Sigma-Aldrich®, CAS 66-27 - 3), respectively.

Exposures were performed for a fixed period of 48 h for all treatments, except for the PC group that was exposed for 6 h according to the methodology described by Fiskesjö (1988) and Majer (2003). After the exposure period of the roots, they were fixed in acetic acid and ethanol solution (1:3) for 24 h. After fixation, the roots were transferred to a solution of 70% ethanol and kept in refrigerator at an average temperature of 4°C.

Determination of mitotic index, chromosome aberration and micronucleus

For preparation of the slides, roots were hydrolyzed in 1 N HCl at 60°C for 8 min and then stained with 2% solution of carmine in 45% acetic acid. The roots were then placed on a slide and the first millimeter removed from the apex of the root, so that the meristematic region corresponding to 2 mm and F1 cells were isolated for analysis by optical microscope. 1000 cells were counted per slide in an increase of 400 times, with 5 slides per treatment and control, and mitotic division stages, aberrant anaphases and telophases, and the frequency of micronucleus were quantified. The mitotic index was calculated according to the equation:

IM [%] = the number of dividing cells (1000 per slide) / number of cells analyzed x 100

For analysis of chromosomal aberrations (aberrant anaphases and telophases) and micronucleus frequency were performed according to methods previously described by Grant (1982) and in accordance with adjustments made by Yildiz et al. (2009).

Determining the length of the root

After the period of exposure and collection of the roots, the

Table 1. Results of MI, CA (anaphase and telophase) and MN in *Allium cepa* meristematic cells and root end length after treatment with water samples.

Sampling	Mitotic index	% Aberration chromosome		Micronucleated cells (%)	Length root (mm)
		Anaphase	Telophase		
NC	12.68±0.79	0.86±0.02	0.53±0.07	0.112±0.003	48.24±2.27
S1	5.11±0.13[ab]	11.47±0.52[ab]	9.11±0.16[ab]	3.374±0.123[ab]	19.31±2.09[ab]
S2	9.14±0.57[ab]	7.94±0.37[a]	5.36±0.37[a]	2.658±0.017[a]	23.17±1,93[ab]
PC	13.46±1.17	5.06±0.46	4.23±0.16	1.680±0.038	33.42±2.46

5000 cells analyzed per treatment. Mean±S.D. [a], Significantly different from negative control ($p < 0.05$), according to Kruskal–Wallis test. [b], significantly different from positive control ($p < 0.05$), according to Kruskal–Wallis test.

measurement of the length in millimeter of the roots was performed with the help of a digital caliper (DIGIMESS®), having a total of 25 roots per treatment.

Statistical analysis

The mitotic index, frequencies of chromosomal aberrations and micronucleus obtained for each treatment during the period between exposure and the samples were compared with the controls and analyzed statistically using the Kruskal-Wallis test ($p < 0.05$), as described by Grisolia et al. (2005) and Rudder et al. (2008).

RESULTS AND DISCUSSION

The pH of samples S1 and S2 varied from a minimum of 6.55 (S1) to a maximum of 6.65 (S2), with an average of 6.60. In its resolution of CONAMA (2005), permitted range is 6.5 to 7.5, so all values remained in that range (Guedes et al., 2005).

Figure 1 shows the concentrations of cadmium, copper, lead and zinc found in the different water samples (S1 and S2) and the maximum tolerable in the environment of each metal recommended by the WHO (1998) and according to CONAMA (2005). Both samples showed high levels of all analyzed metals which exceed the maxi-mum amount indicated and recommended by the rele-vant authorities. The sample S2 showed lower values when compared to sample S1, but all metals exceeded the maximum tolerated. The cadmium concentration in the sample S2 was 0.045 mg/L, or 4400% above recommended levels, the copper concentration was 0.086 mg/L, 855% above the indicated concentration, zinc showed 0.195 mg/L, exceeding 8% maximum tolerable concentration and lead showed 1341mg/L, being the metal with the highest value of the S2 sample, exceeding 13310% the recommended maximum. In relation to the S1 sample, it showed values for cadmium in excess of 4900% than the recommended maximum value, the value of copper exceeded 1044%, zinc exceeded 15% and lead exceeded 29410%, being the last one the highest of all metals analyzed according to the indicated maximum values (Figure 1).

According to Raskin and Ensley (2001) and Andrade et al. (2009) increased levels of heavy metals may be associated with destruction of vegetation cover in mining areas which exacerbates soil degradation, promoting water and wind erosion and leaching of contaminants into groundwater, leading to progressive degree of contamination in other areas. As reported by Rigobello et al. (1988) and Borges Júnior et al. (2008b) the region of Vazante has high levels of zinc and lead, being the largest zinc producer district of Brazil.

In recent decades, environmental contamination with heavy metals has risen dramatically. It is known that certain heavy metals can cause DNA damage and carcinogenic effects in animals and humans, and are probably, related to its mutagenic activity (Ernst, 2002; Arora et al., 2008; Megateli et al., 2009). According to Knasmüller et al. (2009), the standard tests used to detect heavy metals is currently problematic because several carcinogenic metals result in negative information on bacterial gene mutation assays and genotoxicity assays with mammalian cells, but tests performed in plant cells have become known for being a quick and useful test system in biomonitoring (Majer et al., 2005). The advantages of these tests include the similarity of the plants chromosomes organization with the human, its sensitivity to changes in environment (Grant, 1994) and the possibility of studying the effects on a wide range of environmental conditions. Repeated application of these tests to assess the genotoxic risks present in natural waters (rivers and lakes), wastewater and drinking water has affirmed its utility (Blagojevic et al., 2009).

Thus, this assay evaluated the genotoxic activity of different water samples by mean of the *A. cepa* test. Table 1 shows the results of the mitotic index (MI), chromosome aberrations (CA), micronucleus (MN) and root length of *A. cepa* after treatment with water samples. The sample S1 showed significant differences in relation to positive and negative controls in all parameters analyzed, and its average MI was the lowest (5.11) than controls, NC (12.68), PC (13.45) and the length of the roots of the sample (19.31 mm) also had decreased compared to controls: NC (48.24 mm), PC (33.42 mm). Since the percentage of MN and CA increased compared to controls, the frequency of MN of S1 was 3.374%, while the frequencies of the NC was 0.112 and 1.680% of the PC. The percentage of AC of S1 also increased, both for anaphase (11.47%) and telophase aberrant (9.11%),

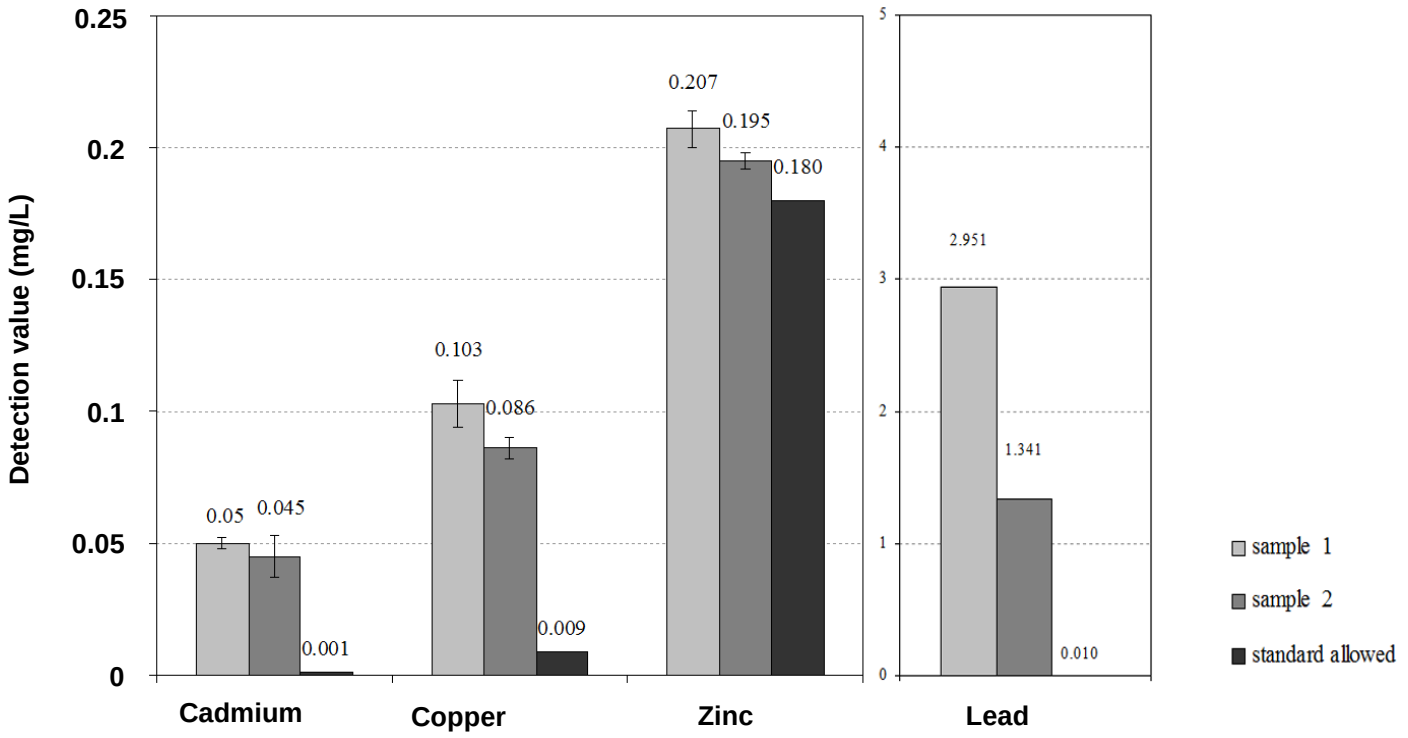

Figure 1. Concentration of heavy metals (cadmium, copper, lead and zinc) found in the water samples (S1 and S2) and maximum permitted under the Regulatory Determination CONAMA (2005) and recommended by WHO (1998).

while the negative and positive controls showed anaphase to 0.86 and 5.06% 0 and telophase, 53 and 4.23%, respectively.

The S2 differed from the positive and negative controls in relation to MI and length of roots. Both sample showed a decrease parameter, and the MI of the medium S2 is 9.14, while for the PC was 13.46 and the NC was 12.68. Values for the average length of the roots were 23.17 mm and S2 to the positive and negative controls were 33.42 and 48.24 mm, respectively. As for the parameters CA and MN percentage of the sample S2 showed no statistical difference in relation to the PC for both anaphase and telophase, but there were differences when compared to NC, and the percentages of both MN (2.658%) and AC (anaphase = 7.94% and telophase = 5.36%) increa-sed over the rate of MN, NC (0.112%) and the rate of CA of the same control (anaphase = 0.86% and telophase = 0.53%) (Table 1).

According to the results observed in this study, we consider that the high levels of heavy metals found in water samples are directly related to the genotoxic events observed in root cells of *A. cepa*. According to studies carried out by Seth et al. (2008), the high content of cadmium is associated with occurrence of chromosomal aberrations and increased frequency of micronuclei in the root of *A. cepa*. As shown by Glinska et al. (2007) and Ferraz et al. (2009), copper and its connections with macromolecules proved to be an effective cytotoxic agent and genotoxic in cell cultures and *in vivo* assays. Liu et al. (1994 and 2003) and Seregin et al. (2004) showed that the lead and cadmium inhibited root growth as a result of disruption of cell cycle and Wierzbicka (1988, 1989 and 1999), and Samardakiewicz Woz'ny (2005), Fusconi et al. (2006) showed a decrease in the mitotic cells of the root, where this value was accompanied by reduction in the number of cells in metaphase and anaphase. Furthermore, heavy metals, lead and cadmium induced c-mitosis, chromosomal adhesion and bridges, and besides that, lead also caused chromosome delay, nucleus with more condensed chromatin and inhibited cytokinesis.

Conclusion

The present study reveals a chemical contamination in artesian well water in the city of Vazante-MG caused by the presence of heavy metals. The decrease in mitotic index, reducing the average length of roots and increased frequency of chromosomal aberrations and micronucleus in root meristematic cells of *A. cepa* exposed to treatment may be correlated with the presence of certain heavy metals determined in our assay, as well as the interaction with other classes of environmental contaminants, which are probably the agents that together induced the genotoxicity observed in this assay. Taken together, these results show the importance of evaluating the genotoxicity of water wells in areas with mineral richness, especially in areas near active mining sites.

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