Cd, Cu, and Mn from Uruguay River Basin in Uruguaiana, RS, Brazil, and their toxicological potential for human leukocyte

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ABSTRACT. This study assessed the limnology from the Medium Uruguay River Basin in Uruguaiana, Brazil, with a focus on the concentration of heavy metals (Cd, Cu, and Mn), to assess the toxicological potential (cytotoxicity and genotoxicity) for humans using as biological matrix of study human leukocyte cells. The conductivity, resistivity, and dissolved O₂ levels exceeded the limits recommended by the National Environmental Council (Conselho Nacional do Meio Ambiente - CONAMA). The percentage of non-viable human leukocyte cells exposed to water samples was approximately 20% higher than that of the negative control (<3%), but similar to the positive control. The DNA damage index was high for all heavy metal concentrations assayed when compared to the negative control 12±2.96, p < 0.0001, with a range of 155.66±23.89 to 194.33±23.23, but similar to the positive control (210.62±27.48). Moreover, the leukocyte degeneration index was higher in all samples containing heavy metals than in the negative control (4%), which demonstrates to be due the presence of Cu (11.8-12.5%), Cd (13-15.6%), and Mn (15.6-22.5%). Taken together, our results show that the quality from water samples analyzed is below than recommended by CONAMA and offers risk of contamination by heavy metals for the general population.

Keywords: ecotoxicology, genotoxicity, limnology, heavy metals, Uruguay River Basin.

Cd, Cu e Mn da Bacia do Rio Uruguai em Uruguaiana, RS, Brasil, e seu potencial toxicológico para leucócitos humanos

RESUMO. Este estudo avaliou a limnologia de amostras de água da bacia do rio Uruguai Médio, Brasil, focando as concentrações de metais pesados (Cd, Cu e Mn), para determinar o potencial toxicológico (citotoxicidade e genotoxicidade) utilizando como matriz biológica células leucocitárias humanas. A condutividade, resistividade e níveis de O₂ dissolvido nas amostras analisadas excederam o limite recomendado pelo Conselho Nacional de Meio Ambiente (CONAMA). O percentual de células leucocitárias humanas não viáveis expostas às amostras de água foi de aproximadamente 20% maior que o controle negativo (<3%), mas similar ao controle positivo. O índice de dano ao DNA foi maior para todas as concentrações de metais testadas quando comparadas ao controle negativo (12±2,96), p < 0.0001, com uma variação de 155.66±23.89 a 194.33±23.23, mas estatisticamente semelhante ao observado para o controle positivo (210.62±27.48). Adicionalmente, o índice de degeneração leucocitário foi maior em todas as amostras contendo metais pesados que o controle negativo (4%), pela presença de Cu (11.8-12.5%), Cd (13-15.6%) e Mn (15.6-22.5%). Analisando esses dados conjuntamente, nossos resultados demonstram que a qualidade das amostras de água analisadas encontra-se abaixo da recomendada pela CONAMA e oferece risco de contaminação por metais pesados para a população em geral.

Palavras-chave: ecotoxicologia, genotoxicidade, limnologia, metais pesados, Bacia do rio Uruguai.

Introduction

The loss of biodiversity in continental water ecosystems is primarily caused by pollution and eutrophication, which increase sedimentation in water sources and in turn compromise the equilibrium of ecosystems (Ternus, Souza-Franco, Krombauer, Mocellin, & Dal Magro, 2011). This scenario explains a decrease in water quality, making it inappropriate for human consumption (Nawab et al., 2015).

The increase in human activity such as waste disposal in rivers results in deposition of different sediments (Possamai, Viana, Schulz, Costa, & Casagrande, 2007). These sediments include heavy
metals that, isolated or as organometallic compounds, have negative effects on ecosystems and, consequently on human health through trophic transference along the food chain or by direct exposure to them (Chakarvorty et al., 2015), as is the case of water sample analyzed in this study. In Uruguaiana city there is.

Environmental contamination by heavy metals is probably associated with the intensity or frequency of anthropogenic activity industrial waste dumping. As a result, metals such as cadmium (Cd), copper (Cu), and manganese (Mn) appear as contaminants that are accumulated in particulate sediment forms (Jesus, Costa, Mendonça, & Zandonade, 2004).

It has been accept that heavy metals are able to oxidize several organic matrices, as different types of human cells. Moreover, heavy metals have different sites of chemical interaction or bound with proteins, enzymes, DNA, RNA, carbohydrates and lipids. The metals' characteristics interfere with the cells physiology and may lead them to several and irreversible injuries (Frederickson, Koh, & Bush, 2005; Shiwinski et al., 2009; Pereira et al., 2015).

In Uruguaiana is sort of a routine to consume fish from the Uruguay River and its tributaries, as well as to use its waters for recreation. In addition, the uptake of drinking water in the city is made directly from the Rio Uruguay, after a treatment.

Thus, the aims of this study were to analyze the limnologic parameters and to detect and quantify heavy metal concentrations (Cd, Cu, and Mn) in water samples from the Salso I Stream, a tributary of the Uruguay River, and from the river in Uruguaiana (Rio Grande do Sul, Brazil) in order to obtain the realistic water quality conditions. The study also sought to determine whether the heavy metal concentrations found could have cytotoxic and genotoxic effects on human leukocytes, which are cell models to investigate toxicity and exposures to xenobiotics, such heavy metals (Pereira et al., 2015).

**Material and methods**

Twelve sites were chosen for water sample collection. Four were from the Salso I Stream near an urban landfill which had additional exposure to human influence originating from rural and urban areas. Two sites were further downstream; the remaining six samples were collected from the Uruguay River. These included three sites upstream from the city's drinking water collection site. The three remaining sites were located downstream (Table 1).

<table>
<thead>
<tr>
<th>Table 1 Location of water sampling points: Urban Garbage Deposits - Salso I Stream, and Uruguay River, Uruguaiana Municipality, RS, Brazil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling spots</td>
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<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
</tr>
</tbody>
</table>

Collection sites were chosen on the basis of some selection criteria. Private properties were excluded, whereas publicly accessible areas and locations near the urban garbage deposit were included.

The glassware used in the laboratory was prepared for use by treating with 30% HNO₃ overnight, washing three times with Milli-Q water, and drying at 60°C.

**Limnological evaluation and heavy metal determination**

Limnological evaluation and quantification of heavy metal concentration were performed according to Resolution Number 357 of the CONAMA (2005) for classification Freshwater C-2.

The limnological assay was performed using the HI 9828 Multiparameter Water Quality Meter (Hanna Instruments; Woonsocket, RI, USA). The instrument measured pH, dissolved oxygen, conductivity, resistance, temperature, and salinity. For nitrite and ammonia content analyses the commercial Kit (Alfakit; Florianópolis, SC, Brazil) was used.

The quantitative analyses for heavy metals were performed using an atomic absorption spectrophotometer (pinAAcle500; PerkinElmer Inc., Waltham, MA, USA). Before of the metals analysis, the water samples were filtered through of membrane with 0.45 μm in order to separate the metals dissolved from metals in suspension. After that, 250 mL of each water sample was treated with concentrated HNO₃, according to the protocol established by the Associação Brasileira de Normas Técnicas (ABNT) NBR 13809:1997.

The water used was previously distilled and deionized on an ion-exchange column and purified using a Milli-Q system (Millipore®, Billerica, USA) with resistivity of 18.2 MΩ cm⁻¹. The reagent used for the decomposition of LS (HNO₃, 65%, 1.4 kg L⁻¹, Merck®, Darmstadt, Germany) was a redistilled...
analytical grade (model duoPUR distillation Subboiling Distillation System, Milestone®, Sorisole, Italy). All materials were decontaminated by immersion in an aqueous HNO₃ 20% (v/v) solution for 24 hours and subsequently rinsed with distilled/deionized water.

For each of the heavy metals Cd, Cu, and Mn, standard curves were prepared from the GFAAS Mixed Standard solution (Lot# 22-175JB, PE# N9300244; PerkinElmer Inc.) to obtain the corresponding line equation and R² values, using the following equations: Cd, \( y = 0.02004x - 0.00042 \) (R² = 0.9985); Cu, \( y = 0.00497x - 0.00366 \) (R² = 0.9986); and Mn, \( y = 0.0024x + 0.00002 \) (R² = 0.9998). All sample measurements were made three times and the average values are reported, and all reagents were PA, supra pure to avoid metal contamination. The minimum detectable limit for Cd, Cu, and Mn were 0.069 μg L⁻¹, 0.024 μg L⁻¹, and 0.083 μg L⁻¹, respectively.

**Toxicological evaluations**

The experimental protocols used for the cytotoxicity and genotoxicity analyses, including venous blood collection, were approved by the Research Ethics Committee of the Universidade Federal de Santa Maria (UFSM), under registration number 0089.0.243.000-07.

The cytotoxicity and genotoxicity evaluations and the human leukocyte degeneration indices were performed after heavy metal concentrations were determined, as the experimental heavy metal concentrations were to be similar to those of the samples. Tests were performed on heavy metal solutions prepared in phosphate-buffered (pH 7.4).

The metals concentrations used in this study take into account the bioaccumulation concept and the data from our laboratory that show cytotoxicity, mutagenicity, and genotoxicity of heavy metals concentrations from ≤1 μg mL⁻¹ to 50 μg mL⁻¹ in ex vivo studies in human leukocytes, that is, in very low range concentration (Pereira et al., 2015; and data not shown), such as observed in results from sample water analysis in this study. Then, for these toxicological assays, solutions of metals were prepared to reflect the low, intermediate, and high sample concentrations. In this sense, it was prepared three concentration for Cd, two for Cu, and six for Mn due the major range than others.

The venous blood was collected after 12 hours overnight fasting by venipuncture using top Vacutainer® (BD diagnostics, Plymouth, UK) tubes with heparin from a single donator over 18 years old, healthy, without any medication use. The blood sample were aliquoted and then used for cytotoxic and genotoxic assays.

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The number of leukocytes was standardized (8 × 10⁶ cells mL⁻¹) for cell viability, oxidative damage of DNA (Pereira et al., 2015), and leukocyte degeneration assays (Camargo, Santos, & Zonta, 1999). All tests were performed in triplicate and always with the same group division: Negative Control composed solely of the leukocyte suspension (LS); Positive Control composed of LS and 4 mM H₂O₂; and Sample Tests, LS plus Cd, Cu, or Mn at a concentration equal to that found in a sample during the heavy metal analysis. Samples were incubated at 37°C for 1 hour, with slow and continuous mixing by inversion.

For heavy metal cytotoxicity evaluation, cell viability was determined by membrane integrity analysis using the trypan blue method (Burow et al., 1998).

A comet assay was used to evaluate genotoxicity, performed according the guidelines for comet assay use (Tice et al., 2000). Leukocytes were stained with a commercial kit (Labtest®). The analysis was performed in triplicate, using a microscope to count 100 cells per slide and classify the DNA damage level of each, from 0 (no damage) to 4 (maximum damage). The average damage level obtained for each treatment allowed calculation of the corresponding damage index. Damage indices ranged from 0 (100 cells × 0) to 400 (100 × 4).

The leukocyte degeneration index (LDI) was evaluated according to Lima, Soares, Grecco, Galizzi, & Carçado, (2001), where LDI = toxic neutrophils/total neutrophils × 100.

**Data analysis**

The data were subjected to the Kolmogorov-Smirnov test to check the normality, and showed a Gaussian distribution. Following, the data from cell viability assay, comet assay, and LDI were analyzed with a one-way analysis of variance (ANOVA) and Dunnett’s test, considered significant when p < 0.05, and expressed as average and standard deviation.
Results and discussion

Table 2 shows the results of the water limnological and heavy metals evaluations from Salsolo Stream and the Uruguay River, including pH, conductivity, resistivity, salinity, dissolved oxygen content, temperature, total ammoniacal nitrogen, and nitrate, with CONAMA recommended limits noted.

All samples showed a pH within the acceptable range (6-9), with exception of sample 10 (Table 2). There was no sample within an acceptable conductivity range, that is, less than 100 μS cm⁻¹. Almost all of them had resistivity lower than the reference values.

Acceptable freshwater salinity levels are under 0.5 ppm. Only sample 6 showed salinity higher than the established limit. Freshwater dissolved oxygen has a stipulated a limit not minor than 6 mg L⁻¹, and all the samples showed low levels of oxygen. Samples 7, 9, and 12 that presented pH between 8 and 8.5 showed ammonium contents over the permissible levels. Nitrite concentration in all samples was within the permissible range.

The limnologic parameters have no direct relationship with cytotoxicity or genotoxicity. However, the limnology of waters may affect the oxidative status of the heavy metals dissolved or in suspension. This status may favor the absorption of metals in organic matrices.

The results of the heavy metal analysis are also presented in Table 3. All samples showed Cd levels over the permissible limit (1 μg L⁻¹), ranging from 2.33 μg L⁻¹ to 10.47 μg L⁻¹. Samples from sites 3, 5, 6, 8, 10 and 11 showed Cu levels over the currently permissible limits. All analyzed water samples showed Mn at levels much higher than that permitted (100 μg L⁻¹), with concentrations ranging from 164.2 μg L⁻¹ to 2,390 μg L⁻¹.

Table 2. Limnological analysis of water samples from Urban Garbage Deposits, Salsolo I Stream, and Uruguay River, Uruguayan Municipality (RS), Brazil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH (μS cm⁻¹)</th>
<th>Cu (mg L⁻¹)</th>
<th>Zn (mg L⁻¹)</th>
<th>Mn (μg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.57</td>
<td>210</td>
<td>4.76</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>8.21</td>
<td>200</td>
<td>5.00</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>8.48</td>
<td>220</td>
<td>5.54</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>8.51</td>
<td>110</td>
<td>9.09</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>8.47</td>
<td>110</td>
<td>9.09</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>8.00</td>
<td>143</td>
<td>0.69</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>8.10</td>
<td>240</td>
<td>4.16</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>7.98</td>
<td>240</td>
<td>4.16</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>8.18</td>
<td>510</td>
<td>1.96</td>
<td>0.24</td>
</tr>
<tr>
<td>10</td>
<td>9.28</td>
<td>400</td>
<td>2.50</td>
<td>0.19</td>
</tr>
<tr>
<td>11</td>
<td>8.00</td>
<td>200</td>
<td>5.00</td>
<td>0.09</td>
</tr>
<tr>
<td>12</td>
<td>8.14</td>
<td>470</td>
<td>2.12</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 3. Heavy metal analysis of water samples from Urban Garbage Deposits, Salsolo I Stream, and Uruguay River, Uruguayan Municipality (RS), Brazil.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd (μg L⁻¹)</th>
<th>Cu (mg L⁻¹)</th>
<th>Zn (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.33±0.355*</td>
<td>ND</td>
<td>205.4±12.34*</td>
</tr>
<tr>
<td>2</td>
<td>10.47±0.096*</td>
<td>ND</td>
<td>253.1±19.33*</td>
</tr>
<tr>
<td>3</td>
<td>6.32±0.669*</td>
<td>ND</td>
<td>164.2±11.25*</td>
</tr>
<tr>
<td>4</td>
<td>7.98±0.664*</td>
<td>ND</td>
<td>400±27.29*</td>
</tr>
<tr>
<td>5</td>
<td>5.38±0.342*</td>
<td>ND</td>
<td>1,796.8±58.36*</td>
</tr>
<tr>
<td>6</td>
<td>2.5±0.139*</td>
<td>ND</td>
<td>1,976.8±58.36*</td>
</tr>
<tr>
<td>7</td>
<td>5.43±0.499*</td>
<td>ND</td>
<td>267±8.36*</td>
</tr>
<tr>
<td>8</td>
<td>2.94±0.288*</td>
<td>ND</td>
<td>436.3±23.33*</td>
</tr>
<tr>
<td>9</td>
<td>3.5±0.145*</td>
<td>ND</td>
<td>2,390±67.77*</td>
</tr>
<tr>
<td>10</td>
<td>4.03±0.39*</td>
<td>ND</td>
<td>1,000±87.25*</td>
</tr>
<tr>
<td>11</td>
<td>2.26±0.197*</td>
<td>ND</td>
<td>184.55±12.14*</td>
</tr>
<tr>
<td>12</td>
<td>4.06±0.399*</td>
<td>ND</td>
<td>298.95±18.70*</td>
</tr>
</tbody>
</table>

CONAMA 1 0.069 0.024 0.083
MD = Not detected; MD = minimum detectable; *Statistically significant (p<0.05) from CONAMA values. The data are shown as mean and standard deviation from heavy metals concentration (μg L⁻¹).

It is well established that Cd is very mobile in aquatic environments, especially in water bodies rich in organic material, where it is present in sediments as Cd²⁺ and Cd³⁺ and is not, in this case, likely to undergo a redox reaction (Pierangeli et al., 2005). Furthermore, Cd is absorbed by aquatic organisms, and then taken up by aquatic plants and animals that may be consumed by human beings (Matsuo & Val, 2007). Increased exposure to Cd may cause several intoxications (Dias, Alleoni, Casagarande, & Camargo, 2001).

Cu is highly dependent on pH in all environments. Thus, in aquatic systems with acidic pH, this metal is usually dissolved in water (Milesi, Biasi, Restello, & Hepp, 2008). However, at a pH of about 8, Cu is mostly precipitated in the form of copper hydroxide or in stable complexes with sulfides, which exert oxidative effects and dissociation (Scheffer, Sodré, & Grassi, 2007).

The adsorption by metallic oxyhydroxides and clay minerals reduces the bioavailability of Cu (Tapia et al., 2011). However, by its ingestion, vertebrates may accumulate Cu in their livers (Bücker, Carvalho, & Alves-Gomes, 2006). The damage to humans caused by this food chain is linked to the generation of free radical species and consequently oxidative stress, which can induce degenerative diseases (Baiere et al., 2010).

Mn is a metal naturally found in the environment (Molin, Costa, Rieger, Pra, & Lobo, 2010) after being introduced in the organic systems of vegetables and animals (Silvia, Vitti, & Trevizan, 2007). The fact that all analyzed water samples had higher levels of Mn lead us to suggest that the environment is compromised, considering that leaching into streams (Ortiz, Godoi, Polakiewicz, &
Pires, 2008) and ground pollution seem to be determining factors in increased Mn contents in the analyzed samples (Agourakis, Camargo, Cotrim, & Flues, 2006).

In high pH environments, Mn is mainly present in Mn³⁺ and Mn⁴⁺ oxidation states, which are the most reactive forms for complex formation, and which contribute, indirectly, to sediment formation and the low oxygen dissolved (Who, 2011).

However, complexes formed by Mn²⁺ are prone to being weakly solvated and are readily available for its absorption by plants (Moreira, Prochnow, Kiehl, Neto, & Pauletti, 2006), especially when the environment (redox potential) provides high conductivity and low resistivity in water (Camargo et al., 1999), as in the analyzed samples.

Although there are few toxicokinetic studies of heavy metals, it has been shown in rats that the oral absorption of Mn is increased when applied in inorganic forms and at elevated doses, which are easily transported through Mn-contaminated water and food (Dorman, Struve, James, McManus, Marshall, & Wong, 2001).

In mammals, Mn is a metal that is involved in important homeostatic mechanisms, mainly in the liver, pancreas, kidneys, and adrenal glands—organs that accumulate Mn when ingested in an excessive amount (Yoon et al., 2011). On the other hand, gastrointestinal ulcers, neurotoxic effects, and hematological disturbances are attributed to Mn toxicodynamics (Yoon et al., 2011).

The heavy metals concentration performed for genotoxicity and cytotoxicity tests on human leukocytes were, for Cu, from samples 3 and 6, 10.04 μg L⁻¹ and 10.5 μg L⁻¹, respectively; for Cd, the selected concentrations were of the samples 6 (2.5 μg L⁻¹), 5 (5.58 μg L⁻¹), and 2 (10.47 μg L⁻¹), renamed Cd (6), Cd (5), and Cd (2), respectively. Finally, for Mn the selected concentrations were of the samples 3 (164.2 μg L⁻¹), 12 (298.95 μg L⁻¹), 5 (400 μg L⁻¹), 10 (1,000.8 μg L⁻¹), 6 (1,796.8 μg L⁻¹), and 9 (2,390 μg L⁻¹), which were named Mn (3), Mn (12), Mn (5), Mn (10), Mn (6), and Mn (9), respectively.

The metal concentration of Cu, Cd, and Mn found in the assayed concentrations showed higher indexes of damage than did the negative control 12±2.96, F(13.92), p < 0.0001, with a range of 155.66±23.89 to 194.33±23.23, but similar to the damage index found for the positive control (210.62±27.48).

Figures 1A and Aa shows the percentage of nonviable leukocytes after exposure to different heavy metal concentrations. The samples Cu (3), Cu (6), Cd (6), Cd (5), Cd (2), Mn (3), Mn (12), Mn (5), Mn (10), Mn (6), and Mn (9), had a greater proportion of nonviable cells than did the negative control, F (2.986), p < 0.05. However, the percentage found for all the treatments (<20%) is considered adequate to give credibility to genotoxicity tests.

Figures 1B and Bb presents the DNA damage indices for human leukocytes exposed to different concentrations of heavy metals. All of the heavy metals in the assayed concentrations showed higher indexes of damage than did the negative control 12±2.96, F(13.92), p < 0.0001, with a range of 155.66±23.89 to 194.33±23.23, but similar to the damage index found for the positive control (210.62±27.48).

Figures 1C and Cc presents the degenerative index of leukocytes exposed to different Cu, Cd, and Mn concentrations. The results show the presence of cytotoxicity, as evidenced by an increase in the frequency of increased granulation in neutrophils, with all samples showing a significant increase compared to the negative control (4%), F(8.62), with p < 0.0001. Cu had a leukocyte degeneration index ranging from 11.8% to 12.5%; Cd, from 13.0% to 15.6%; and Mn, from 15.6% to 22.5%.

It is well established that heavy metals such as Cu, Cd, and Mn can cause changes in cellular physiological processes (Williams et al., 2010), including the inhibition of cellular repair mechanisms and, consequently, causing defects in cellular cycle regulation (Merzenich et al., 2002). They also induce the formation of inclusion bodies and cellular stress response (Eichler, Ransom, & Smoyer, 2005), formation of reactive oxygen species (ROS) (Lee, O’Connor, & Pfeifer, 2002), depletion of glutathione (Mikhak et al., 2008), development of cancer, premature aging (Merzenich et al., 2002), and development of degenerative diseases such as Huntington Disease (Williams et al., 2010).

Genotoxicity of Cd could be explained through mechanisms beyond ROS formation such as the inhibition of DNA repair mechanisms and the suppression of apoptotic mechanisms (Hengstler et al., 2003). On the other hand, Cu, Zn, and Mn have an important role in the conversion of ROS to H₂O₂ through the dismutation of Cu-Zn superoxide (CuZn-SOD) and Mn superoxide (Mn-SOD). A dysfunction in Mn-SOD, or even lack of it due to mitochondrial DNA depletion, leads to a reduction in its antioxidant activity, which can result in various pathological disorders such as vascular dysfunction (Wenzel et al., 2008).
Moreover, the presence of H₂O₂ and other ROS creates breaks in several strands of DNA, mainly at guanine residues (Lee, et al., 2002). Nevertheless, protein or enzyme complexes with Cd²⁺ or Cu²⁺ inhibit excision repair, a replication correction system, favoring the genetic mutations that are propagated through the cellular cycle (Merzenich et al., 2002).

It seems that Cd plays a central role among the heavy metals, acting as either inductor or inhibitor of the process of apoptosis. It can induce the activity of caspase-3 in vitro (Shimoda, Nagamine, Takagi, Mori, & Waalkes, 2001), or it can induce oxidative stress, as already described, leading to genomic instability, implying that it is essentially a carcinogen (Bal & Kasprzak, 2002).

The increased granulation in neutrophils is known as toxic granulation, which can have different etiologies (Bain, 2007). In this study, a 1-h
exposure of leukocytes to various concentrations of Cu, Cd, and Mn was sufficient to induce increased formation of abnormal granulation in the neutrophils, probably by increasing the phagocytic process of these cells.

**Conclusion**

The analyzed water samples show limnological parameters of conductivity, resistivity, dissolved O₂, and ammoniacal nitrogen, in dissonance with CONAMA Resolution 357. Cu, Cd, and Mn concentrations were also over the limit established by the legislation for nearly all tested samples. The genotoxicity and cytotoxicity assays of human leukocyte exposed to different concentrations of Cu, Cd, and Mn, equal to those found in river water samples, show elevated indexes of DNA damage and cellular degeneration.

**References**


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