Occurrence of heavy metals and contaminants on the surface of adjacent rivers
Warita Alves de Melo, Carla Afonso da Silva Bitencourt Braga and Lilian Carla Carneiro

ABSTRACT
Water is fundamental to the survival of living beings. It registers every impact and can function as an indicator of environmental stressors. Our objective in the current study was to assess the sanitary conditions of the waters in the Açude, Maria Lucinda and Santa Rosa streams. This was done by checking pH, running quantitative analyses of heavy metals and testing for total coliforms. The effect of ultraviolet (UV) rays on samples positive for coliforms was evaluated. The average pH of the streams ranged between 4 and 7 and changed between drought and rainy season conditions. Chromium and nickel values were above those permitted by the Brazilian National Council on the Environment, CONAMA. In the dry season, zinc values were above those established by CONAMA for waterbodies of Classes 1 and 2. Thermotolerant coliforms were present in all samples collected and above permitted values. After exposing the bacteria to UV light, it was noted that UV irradiation was unable to decrease the bacteria count. Ninety residents who use stream water were interviewed: 24% of interviewees said they use the waters of the Açude and Mary Lucinda streams and along the Santa Rosa stream, 95% of inhabitants said they use the water.

Key words | analysis, bacteria, biochemistry, coliforms, pollution, water

INTRODUCTION
Water quality and the control of anthropogenic environmental impacts are very important because the pollution of water with toxic substances causes grave consequences in populations and ecosystems. The interaction of living things with dangerous waterborne pathogenic organisms can cause a wide variety of changes and maladies depending on the degree of contamination and exposure over time (Gerbersdorf et al. 2013; Vaughn et al. 2015).

Due to the growing demands of human consumption, protection of the environment and human health has enormous consequences for the quality of life of urban and rural populations, and this calls for extreme urgency regarding the sustainable use of water resources (Lehmann et al. 2015).

Urban and industrial growth has caused an increase in the pollution of ecosystems by harmful products, including heavy metals (Costa et al. 2009). These metals can be transferred, accumulated and amplified along the food chain, threatening animals, plants and human beings (Guerra-Rodríguez et al. 2006). Urban solid waste is a major problem which requires government oversight, but no current management policy fully addresses disposal of this waste. Citizens are largely uninformed and unconcerned about this, and so this waste often flows into streams (Santibañez-Aguilar et al. 2013).

The industrial use of natural resources was been critical for human development throughout the 20th century. Growing industrial demand has deepened the ecological impact of its activities, thus provoking even more demand, and it is necessary to address the problems this has caused. The fundamental concern behind this is preserving life on planet
Earth. Resolving these environmental issues also includes a socio-political dimension (Sen & Chakrabarti 2009).

Industrial production releases large amounts of heavy metals into the environment, causing major problems. The accumulation of heavy metals in the aquatic environment significantly reduces the availability of water for human use, thus requiring us to employ specialized techniques to recover fresh water (Akpor et al. 2014). Because humans and other organisms are at risk from contamination, it is important that the amount of diluted metals transported by water is monitored (Chen et al. 2015).

The amounts of chromium, cadmium, zinc, lead, copper, manganese and nickel spread throughout the environment have increased along with global industrialization and the increasing human population (Wang et al. 2011). Humans are principally exposed to these toxins by ingesting them in food and water (Lee et al. 2011). In the environment, their degradation is slow, and they remain in the soil and at the bottom of streams, lakes and reservoirs for decades (Lourenço & Landim 2005).

With exposure to air, groundwater acquires pathogenic microorganisms including Salmonella spp., Shigella spp., Vibrio spp. and Mycobacterium spp. (Cabral 2010). The bacteriological quality of water is evaluated by the presence of particular indicator organisms and their population size (Mounaouer & Abdennaceur 2015).

Many pathogenic microorganisms present in water are derived from human and animal excreta. The test most often employed to detect these kinds of pollutants examines total coliforms and thermotolerant coliforms (Tomilola et al. 2014). The Brazilian National Council on the Environment (CONAMA) states that human drinking water must not exceed a maximum acceptable concentration for thermotolerant coliforms (resolution 357, March 17, 2005).

Treatment is necessary to control waterborne diseases in the public water supply. The disinfection of water and wastewater can be accomplished with ultraviolet (UV) radiation emitted by low pressure mercury lamps. These lamps emit 85% to 90% of their radiation at a wavelength of 254 nm, which is effective in controlling microorganisms (Shetty et al. 2014).

The objective of this paper is to assess the sanitary conditions of the water in the Açude, Maria Lucinda and Santa Rosa streams, Morrinhos, Brazil. To this end, water pH at collection points was determined, and heavy metal and microbiological analyses of samples were performed. A closed questionnaire was also developed and local inhabitants along the banks were interviewed with it to investigate their knowledge of stream contaminants.

**METHODS**

The study took place along the Açude, Maria Lucinda, and Santa Rosa streams, which are located in Morrinhos, a city in the state of Goiás, Brazil. The study includes a survey of heavy metals present in the streams, and an evaluation of bacterial counts in samples and comparison with previously collected samples. The effect of exposure to UV light on bacterial counts was evaluated and the microorganisms were found to be resistant.

Along these streams are properties where sugar cane and vegetables are cultivated together with cattle and swine. Collection points were chosen near pollution sources, including a slaughterhouse, a tannery and a wastewater treatment facility, and samples were also drawn from underground wastewater.

Few factors distinguished the Açude and Maria Lucinda, so these were analyzed together. Water samples were collected at 44 points along these two streams and at six points along the Santa Rosa.

Different environment types were established to classify the sample origins. Collection points 1, 2 and 3 were in a park (Açude Stream), 4 to 6 in an urban area (Açude Stream), 7 to 18 in an urban area (Maria Lucinda Stream), 19 to 23 at a lake (Maria Lucinda Stream), 24 to 44 in another urban area (the second on Maria Lucinda Stream) and points 45 to 50 in a rural area (Santa Rosa Stream). GPS provided the coordinates for each point (data not shown).

Mann–Whitney statistical tests were performed to verify differences between the pH values and the heavy metal levels in the samples. One hundred samples were collected: 50 in the February rainy season and 50 in the July dry season. The samples were collected over the course of one month in both seasons. The water samples were collected at a depth of 50 cm and were placed in 500 mL polyethylene bottles. Their pH was measured at the collection points with
pH strips (Standard Methods 2012). Immediately after collection, the water was acidified with a nitric acid solution by adding 1 mL to each sample, resulting in a pH below 2.0. Then samples were placed in a Styrofoam box with ice and transported to a refrigerator and stored at 4 °C during analysis.

Flame atomic absorption spectroscopy was used to detect heavy metals. The heavy metals analyzed were: cadmium, lead, copper, chromium, manganese, nickel and zinc. The results were compared against the standards established by the Brazilian National Environment Council (CONAMA 2008).

Samples drawn for microbiological analysis were put in sterilized bottles. These were placed in a Styrofoam box and taken to the laboratory where they were seeded in agar for counting. Results were expressed in colony forming units (CFU)/mL (ANVISA 2013).

The positive samples were sequentially irradiated using a UV lamp (254 nm) for 5, 10, 20 and 30 minutes. The irradiation of positive samples was done only to test the UV lamp efficiency in decontamination as many food industries use this methodology to decrease microbial contamination of food. After irradiation, the samples were transferred to peptone water, incubated for 24 hours at 37 °C and then seeded in agar for counting. They were then incubated for 24 hours at 37 °C again and colonies were counted. Results were expressed in CFU/mL (Tran & Farid 2001).

A questionnaire was developed and used in interviews with residents living along the streams to collect data on water use. Ninety households along the Açude and Maria Lucinda streams were interviewed and 20 households along the Santa Rosa.

RESULTS AND DISCUSSION

The pH values of the water from Açude, Maria Lucinda and Santa Rosa streams were measured. The results are shown in Table 1, and the pH levels are averaged separately within the dry season and rainy season.

The Lake area pH was within parameters determined by CONAMA. The other areas showed pH levels beyond CONAMA limits for waterbody classes 1, 2 and 3 which indicate normal pH values between 6 and 9. Acidification of the Açude, Maria Lucinda and Santa Rosa streams is most likely attributable to the large amount of organic matter and freshly disposed effluents from the city of Morrinhos.

The pH values between the Park area and Urban area 1 on the Açude and Maria Lucinda streams during the rainy season are significantly different (p < 0.05), demonstrating a pH variation in the environment, and also that Urban area 1 had higher pH values. This may be because the Park area contains a soil type with more clay, and because large amounts of organic matter were found at various collection points in the Park. Furthermore, in the spring these were the first collection points.

Comparing the pH values between the Lake area and Urban area 2 on the Açude and Maria Lucinda streams during the rainy season showed a significant difference (p < 0.05); the Lake area had a higher pH likely due to the large amount of illegal sewage dumping, whereas more sewage is treated in Urban area 2. Furthermore, the Lake area water may be affected by the influx of water from the Pipoca stream, causing the pH to increase.

A comparison of pH values between Urban area 2 and the Rural area of the Açude and Maria Lucinda streams, and of the Rural area of the Santa Rosa stream, during the rainy season shows the values to be significantly different (p < 0.05), demonstrating pH variation between the environments. The Rural area had higher pH likely due to the presence of underground water sources. The water in the Rural area may be burdened by the influx of water from the Cordeiro stream, causing the pH rise.

Gorayeb et al. (2010) studied the Amazon River basin pH and found that the Caeté stream basin pH values ranged

<table>
<thead>
<tr>
<th>Environment</th>
<th>Dry season</th>
<th>Rainy season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Urban area 1</td>
<td>5.43</td>
<td>5.7</td>
</tr>
<tr>
<td>Lake</td>
<td>5.17</td>
<td>5.5</td>
</tr>
<tr>
<td>Urban area 2</td>
<td>5.19</td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 | pH in different areas in the dry and rainy seasons
between 5 and 6, consistent with the results obtained in this work.

Heavy metals in collected water samples were analyzed. Table 2 shows the mean values found in this study for each metal, and the maximum reference values determined by CONAMA for Class 1, 2 and 3 waterbodies. CONAMA resolution 357 of 17 March, 2005 (CONAMA, 2008) does not set maximum values for total copper, although the maximum allowable concentrations of dissolved copper for Classes 1 and 2 is 0.009 mg.

Cadmium levels at all points listed were within CONAMA limits which establish the maximum value for Classes 1 and 2 (0.001 mg/l) and Class 3 (0.01 mg/l). The streams had values above CONAMA limits for all classes of waterbody for all other metals analyzed except zinc. Zinc levels exceeded allowed values in the rainy season for all water classes and in the dry season they exceeded those for Classes 1 and 2.

The Açude, Maria Lucinda and Santa Rosa streams contain dumped domestic sewage, municipal waste and pesticide containers and their wash water along their entire length, which explains the large amounts of heavy metals found there.

Comparing manganese levels along the Açude and Maria Lucinda streams during the rainy season, it was found that Urban area 2 had significantly more manganese than the Lake area due to the large amount of illegal sewage dumping that occurs in Urban area 2. Comparing dry season manganese levels in Urban area 2 on the Açude and Maria Lucinda streams with those of the Rural area on the Santa Rosa stream, showed such that Urban area 2 samples contained significantly ($p < 0.05$), more manganese than the Rural area. These data reflect the large amount of illegal sewage dumping in Urban area 2.

For copper, the dry season comparison between Urban area 1 and the Lake area on the Açude and Maria Lucinda streams shows that Urban area 1 had significantly ($p < 0.05$) more copper than the Lake area. Comparing the values for copper between Urban area 2 along the Açude and Maria Lucinda streams and the Rural area on the Santa Rosa stream during the dry season demonstrates that Urban area 2 had significantly ($p < 0.05$) and thus more copper than the Rural area. These results may be due to the large amount of illegal sewage dumping in Urban area 2.

Comparing the Lake area and Urban area 2 on the Açude and Maria Lucinda streams in the rainy season, a significant difference ($p < 0.05$) was found. Higher copper levels in the Lake area were found, attributable to the rain which could have added copper.

Comparing nickel levels between Urban area 2 on the Açude and Maria Lucinda streams and the Rural area located along the Santa Rosa stream in the rainy season showed that the Rural area had significantly ($p < 0.05$) more nickel than Urban area 2.

Comparing zinc levels in Urban area 1 and the Lake area located on the Açude and Maria Lucinda streams in the dry season, a significant difference ($p < 0.05$) was found. Urban area 1 had significantly more zinc than the Açude and Maria Lucinda streams with those of the Rural area on the Santa Rosa stream, showed such that Urban area 2 samples contained significantly ($p < 0.05$), more manganese than the Rural area. These data reflect the large amount of illegal sewage dumping in Urban area 2.

### Table 2

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Açude, Maria Lucinda streamlets</th>
<th>Santa Rosa streamlet</th>
<th>CONAMA maximum allowed level (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainy period</td>
<td>Dry period</td>
<td>Rainy period</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lead</td>
<td>0.14</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Copper</td>
<td>0.82</td>
<td>4.25</td>
<td>0.33</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.96</td>
<td>0.8</td>
<td>0.98</td>
</tr>
<tr>
<td>Manganese</td>
<td>43</td>
<td>55.89</td>
<td>49.33</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.72</td>
<td>0.83</td>
<td>0.87</td>
</tr>
<tr>
<td>Zinc</td>
<td>5.28</td>
<td>5.22b2.22</td>
<td>5.45</td>
</tr>
</tbody>
</table>

aStandard deviation of the mean of all the measurements made for all locations in both seasons.

bBelow the values permitted by CONAMA.
Lake area, which was explained mainly by the presence of pesticide containers and their wash water, and the large amount of sewage that is released in Urban area 1. Furthermore, Lake area water could have been diluted by the Pipoca stream which flows into it, thus decreasing the amounts of copper and zinc.

Comparing the Lake area and Urban area 2 along the Açude and Maria Lucinda streams during the dry season, Urban area 2 had significantly \((p < 0.05)\) more zinc than the Lake area due to the large amount of illegal sewage dumping in Urban area 2. In the zinc comparison between Urban area 2 and the Rural area along the Açude, Maria Lucinda and Santa Rosa streams, during the dry season, significant difference \((p < 0.05)\) was found: the Rural area had significantly, more zinc than Urban area 2.

Comparing chromium levels between the Park area and Urban area 1 along the Açude and Maria Lucinda streams during the rainy season, it was observed that Urban area 1 had significantly \((p < 0.05)\) more chromium than the Park area. These data are explained by the presence of pesticide-coated containers and a great deal of organic matter and cow dung on the stream bank. Also Urban area 2 had significantly \((p < 0.05)\) more chromium than the Lake area.

Comparing chromium levels between Urban area 2 and the Rural area along the Açude, Maria Lucinda and Santa Rosa streams in the dry season showed more chromium in the Rural area. The comparison between the Lake area and Urban area 2 along the Maria Lucinda in the rainy season showed a significant difference \((p < 0.05)\) for Urban area 2. These results are due to the large amount of illegal sewage dumping in Urban area 2 which increases the amount of these metals in the water.

Comparing lead levels between the Park area and Urban area 1 along the Açude and Maria Lucinda streams in the rainy season showed a significant difference and that the Park area had significantly more lead than Urban area 1 (both \(p < 0.05\)). Comparing the Lake and Urban area 2, during rainy season, showed that the Lake area had significantly \((p < 0.05)\) more lead than Urban area 2. This result may be due rain which may have added lead to the Lake area. The lead level comparison between Urban area 2 and the Rural area on the Açude, Maria Lucinda and Santa Rosa streams in the rainy season showed that the Rural area had more lead than Urban area 2. When comparing the Park area and Urban area 1, it was noted that the Park area had more lead in the rainy season. This may be due to the large amount of waste and organic matter found at collection points in the Park area.

The Rural area had significantly \((p < 0.05)\) more zinc and chromium than Urban area 2 in the dry season, and more lead and nickel in the rainy season. This may indicate that the water in the Rural area may be affected by adjacent farming and by the Cordeiro streams which feeds into it.

Table 3 shows total coliform values obtained in this study by analyzing water samples from the Açude, Maria Lucinda and Santa Rosa streams during the rainy and dry seasons.

Table 3 shows total coliform values obtained in this study by analyzing water samples from the Açude, Maria Lucinda and Santa Rosa streams during both the rainy and dry seasons. The values found were \(10^2\) cfu/mL up to values larger than \(10^7\), i.e. too numerous to count. Table 3 lists the smallest to highest colony counts found on the three streams.

Thermotolerant coliforms in all samples collected were verified to be present and above permitted values, indicating that these streams exceed CONAMA established limits for all water Classes. The high contamination rates of thermotolerant coliforms may be related to the illegal discharge of sewage at all collection points, and to the presence of animals near the streams that expel their excreta on the ground where rainwater can wash it into these waterbodies.

After exposing the bacteria to UV light for 5, 10, 20 and 30 minutes, it was noted that UV irradiation was unable to decrease the bacteria count. At most collection points, the results were too numerous to count, except during the

<table>
<thead>
<tr>
<th>Environment</th>
<th>Counts (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park</td>
<td>(10^6), TNC</td>
</tr>
<tr>
<td>Urban area 1</td>
<td>(10^3), (10^7), TNC</td>
</tr>
<tr>
<td>Lake</td>
<td>(10^3), (10^5), TNC</td>
</tr>
<tr>
<td>Urban area 2</td>
<td>(10^3), (10^5), TNC</td>
</tr>
<tr>
<td>Rural area</td>
<td>(10^7), TNC</td>
</tr>
</tbody>
</table>

TNC, too numerous to count.
rainy season. At point 11 in Urban area 1, 30 minutes of UV exposure resulted in the lowest growth (10⁴ CFU/mL). At point 27 in Urban area 2, 30 minutes of UV exposure resulted in the highest growth (10⁷ CFU/mL).

The dry season sample from point 17 in Urban area 1, with 30 minutes of exposure, had the highest growth (10⁷ CFU/mL). Point 26 in Urban area 2, with 20 minutes of exposure, had the lowest growth (10⁶ CFU/mL). Results were countless due to large amounts of contamination from nearby sewage disposal.

At point 46 in the Rural area during the rainy season, 20 minutes of exposure resulted in the highest bacteria growth (10⁷ CFU/mL). Point 48, with 10 minutes to exposure, showed the lowest growth value (10⁵ CFU/mL). It was noted that the form of the colonies was different. Before UV exposure, the colonies were relatively large and of a yellow color; and after exposure the colonies turned uniformly whitish such that it was impossible to distinguish them. This result suggests that with the incidence of UV light, the colonies were made resistant, or acquired a sporulated morphology, or another bacterial morphology, suggesting the loss of pathogenicity. However, further analysis to identify microscopic morphological and biochemical evidence in this organism after exposure to UV light will be necessary.

Ninety persons from the local population were questioned on the use of these waters, and the answers are 24% of inhabitants said they use the water of the Açude and Maria Lucinda streams; among the users, 86% do no treatment before consuming it. Along the Santa Rosa stream, 95% of inhabitants said they use the water; among the users, 84% do not do any kind of treatment.

Householders along the Açude and Maria Lucinda streams who do treat the water use boiling, filtering or chlorination. Those interviewed along the Santa Rosa stream said they boil and filter the water. All said they use water for animals (cattle, pigs, horses, birds), garden irrigation, consumption, hygiene, fish farming and cleaning their sheds and houses.

Naime & Fagundes (2005) studied waters from the Portão stream in the state of Rio Grande do Sul and found that the dilution of pollutants depended on the amount of rainfall. Rolim et al. (2010) analyzed water springs and wells in Botucatu in the state of São Paulo, and found that 50% of surveyed owners were not performing any water treatment before use, and that 46% of sources were used for human, domestic and animal consumption.

A relative increase in hygienic care was observed on the banks of the Açude, Maria Lucinda and Santa Rosa streams. We asked whether inhabitants had noted the presence of pesticide containers floating in the streams. The answers show us that 70% of Açude and Maria Lucinda stream inhabitants interviewed and 60% of Santa Rosa stream inhabitants interviewed have observed floating pesticide containers.

Athayde Júnior et al. (2008) found that 93.1% of those interviewed said that their homes were not connected to the public sewer service, and thus the final destination of their sewage is the Sanhauá stream. Silva et al. (2008) have found more than 200 points of clandestine sewage dumping sites along the Capim Puba stream in Goiás. They also observed that along the 4 km of the Capim Puba stream, there was a large accumulation of all kinds of garbage on the stream bank.

We asked people living in houses near these streams about the disposal of waste water. Answers showed that 60% and 65% of householders interviewed from Açude and Maria Lucinda Stream and Santa Rosa Stream, respectively, had dumped garbage into these waters.

The results address subjects related to the local population’s knowledge of contaminant types and their presence, of the damage caused by them, and of environmental preservation (see below). In the study by Silva et al. (2008) on the Capim Puba stream, the residents of nearby neighborhoods reported a bad smell coming from the stream because of the garbage accumulated there.

Barbisan et al. (2007) in studying the Passo Fundo stream in the state of Rio Grande do Sul showed that the vast majority of the interviewed population had a high rate of concern for environmental matters. This concern was reported at rates above 80%, and the results of this current study are similar.

Local awareness of the pollutants put in the streams and the damage caused by these shows an understanding of the harm caused by water contamination including: disease, lack of clean water, fish kills and the increase of bacteria in the water. However, Rolim et al. (2010), in Botucatu in the state of São Paulo, Brazil, analyzed water springs and
wells and found that 75% of water consumers had little idea about water quality and 86% were unaware of waterborne diseases.

**CONCLUSIONS**

Studies done at collection points showed that several underground sewers sent waste into the streams, that dead animals were present and that cattle had free access to them. Pollution by chemicals is a major threat to living beings and aquatic ecosystems (Magdaleno et al. 2014). Contamination of waterbodies by heavy metals is a concern because the metals are difficult to remove, inhibit biodegradation activity, are bio-accumulated and have toxic effects on humans and other living organisms (Chauhan et al. 2015). For these reasons, the limits established by law may be insufficient to prevent damage to the health of the population.

Monitoring of fecal contamination by coliform bacteria in surface water signals the possible presence of pathogens and thus is used to manage the treatment of drinking water and encourage remedial measures, control and preservation (Sivaraja & Nagarajan 2014; Åström et al. 2015). Fecal coliforms, having as the main species *Escherichia coli*, are indicators of fecal contamination from warm-blooded animals and humans (Giowanella et al. 2015). *Enterococcus* spp. and fecal *Streptococcus* spp. are also commonly tested to detect water pollution (Abia et al. 2015).

The local population is aware of pollution in the water sources studied but does not take steps to reduce it. Analyzing water channels and their contamination by pathogens is very important for improving water quality. Together with taking concrete steps, this is necessary for restoring and maintaining water quality and public health.

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