http://dx.doi.org/10.1590/1519-6984.0513 Original Article

Caffeine as an indicator of human fecal contamination in the Sinos River: a preliminary study

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Received: May 24, 2013 – Accepted: August 8, 2013 – Distributed: May 31, 2015

Abstract

The preservation of hydric resources is directly related to fecal contamination monitoring, in order to allow the development of strategies for the management of polluting sources. In the present study, twenty-five water samples from six water public supply collection sites were used for the evaluation of the presence of caffeine, total and fecal coliforms. Caffeine was detected in all samples, with concentrations ranging from 0.15 ng mL^{-1} to 16.72 ng mL^{-1}. Total coliforms were detected in all samples, with concentrations in the range of 52 NMP/100 mL to higher than 24196 NMP/100 mL, whether the concentration range for fecal coliforms was in the range of below 1 NMP/100 mL to 7800 NMP/100 mL. No significant correlation was found between total coliforms and caffeine concentrations ($rs = 0.35$, $p = 0.09$). However, a moderate correlation between fecal coliforms and caffeine concentrations was found ($rs = 0.412$, $p < 0.05$), probably indicating a human source for these bacteria. Caffeine determination in water may be a useful strategy to evaluate water contamination by human fecal waste.

Keywords: caffeine, human fecal contamination, Sinos River.

Cafeína como um indicador de contaminação fecal humana no rio dos Sinos: um estudo preliminar

Resumo

A preservação dos recursos hídricos está diretamente relacionada ao monitoramento da contaminação fecal, no intuito de possibilitar o desenvolvimento de estratégias apropriadas para a gestão das fontes poluidoras. No presente estudo, vinte e cinco amostras de água de seis pontos de captação de água para abastecimento público foram utilizadas para análise da presença de cafeína, de coliformes totais e de coliformes termotolerantes. Cafeína foi detectada em todas as amostras coletadas nos diferentes locais de amostragem, com intervalo de concentração entre 0,15 ng mL^{-1} e 16,72 ng mL^{-1}. Coliformes totais foram detectados em todas as amostras analisadas, com concentrações variando de 52 NMP/100 mL a concentrações maiores que 24196 NMP/100 mL, enquanto a faixa de variação de concentração de coliformes termotolerantes variou de menos de 1 NMP/100 mL a 7800 NMP/100 mL. Não foi verificada correlação significativa entre coliformes totais e cafeína ($rs = 0.35$, $p = 0.09$), no entanto foi observada correlação moderada entre coliformes termotolerantes e cafeína ($rs = 0.412$, $p < 0.05$), o que pode indicar uma provável fonte humana destas bactérias. A determinação de cafeína em amostras de água pode ser uma ferramenta útil para avaliar a contaminação com resíduos fecais humanos.

Palavras-chave: cafeína, contaminação fecal humana, rio dos Sinos.

1. Introduction

The population-quality of life is directly related to water availability and quality. Actually, population growth and anthropogenic activities have been major contributors to worsen environmental problems, mainly those related to the preservation of water resources.

The Sinos River watershed is located in the eastern region of the state of Rio Grande do Sul (Brazil), which provides drinking water for approximately 1.5 million inhabitants. This basin has been highly impacted by industrial and urban activities (especially in the middle and lower portions of the Sinos River watershed), as well as agricultural activities (manly upper portion of the basin). Water quality is low because of domestic and industrial sewage discharges (Spilki and Tundisi, 2010).

Water quality may be greatly affected by the presence of pathogenic microorganisms derived from fecal pollution. Thus, thermal tolerant coliforms and/or *Escherichia coli* have been commonly used to evaluate the microbiological quality and fecal pollution of water (Medeiros and Souza, 2009; Wu et al., 2011).

However, several recent studies on ecosystem management have focussed on the characterization of chemical markers that could indicate the presence of human fecal contaminants in water. Usually, chemical markers are more accurate and require less time for sample preparation and analysis when compared to microbiological methods. Among several proposed chemical markers of chemical anthropogenic human fecal contamination in water, the one that recently received most attention is caffeine (Buerge et al., 2003; Verenitch and Mazumder, 2008; Peeler et al., 2006; Seiler et al., 1999; Standley et al., 2000; Kurissery et al., 2012). Caffeine is especially attractive for this purpose because it is ubiquitous and almost entirely human-related, given that there are virtually no agricultural or industrial releases, and, despite the existence natural plant sources, the background levels thus generated are usually negligible and can thus be disregarded (Peeler et al., 2006). Caffeine has been reported as a stable compound under variable environmental conditions (Verenitch and Mazumder, 2008). Moreover, caffeine has high solubility and negligible volatility (Seiler et al., 1999), allowing its determination in surface, ground and wastewater effluents (Verenitch and Mazumder, 2008). Despite its application in studies performed worldwide, there are just a few reports in the application of caffeine determination to evaluate the contamination in Brazilian water sources (Ferreira, 2005; Froehner et al., 2010; Froehner et al., 2011).

2. Material and Methods

2.1. Collection of water samples

Twenty-six water samples (500 mL for caffeine analysis and 100 mL for coliforms detection) were aseptically collected at seven abstraction points for drinking water in Sinos River watershed (municipalities of Santo Antônio da Patrulha, Taquara, Parobé, Campo Bom and Nova Santa Rita). The samples were transported to the laboratory under refrigeration (4 °C). After transportation to the laboratory, coliform analysis was carried out immediately and samples to caffeine quantitation were stored at 4 °C until analyzed. The sampling occurred in 2012, on five different dates: April 25, May 23, June 20, August 22 and October 24.

2.2. Coliform analyses

The total coliforms and fecal coliforms were enumerated in the water samples through Colilert® commercial kit (Idexx Laboratories Inc., USA), according to the manufacturer's instructions.

2.3. Caffeine determination

The method for caffeine determination in water was based on Ferreira (2005), with modifications, as follows. Samples were decanted for at least 2 h at room temperature before processing. Aliquots of 500 mL of decanted water were transferred to 1-L separatory funnels, followed by pH adjustment to 9.0 through the addition of 0.1M sodium hydroxide solution. The samples were extracted using liquid-liquid extraction with three portions of 25 mL methylene chloride. The organic extracts were added and concentrated to dryness. The dried extract was recovered with 500 µL of chromatographic mobile phase and transferred to an injection vial, from which 50 µL were injected in the chromatograph. The measurement of caffeine was carried out by high performance liquid chromatography employing an Accela instrument (Thermo Scientific, Palo Alto), with diode array detection. Monitoring wavelength was 272 nm. The separation was performed in a Lichrospher RP-8 column (250 × 4.0 mm, p.d. 5 µm), obtained from Merck (Darmstadt, Germany). The mobile phase was a mixture of phosphate buffer pH 2.3 (50 mM) and acetonitrile, 90:10 (v/v) at a flow rate of 1 mL min^{-1}. Caffeine elution time was 9.0 min, with total run time of 10 min. The method was linear from 0.2 to 100 ng mL^{-1} of caffeine, with precision, measured as CV%, below 15% and accuracy between 91 and 108%. Solvents and reagents were obtained from Merck and caffeine standard was obtained from Fluka (Buchs, Switerzland).

2.4. Statistical analyses

Statistical analyses were performed using Spearman correlation analysis, together with Kurskal–Wallis and Mann–Whitney tests, both at a 5% significance level.

3. Results and Discussion

In the present study, caffeine was detected in all samples collected from the different sampling locations, suggesting the continuous inflow of human domestic waste into the river, and a tendency to higher concentrations in the more populous areas. Caffeine concentrations ranged from 0.15 ng mL^{-1}, in Santo Antônio collection site, to 16.72 ng mL^{-1} in Esteio (Table 1). Median higher caffeine concentrations were found at the collection performed in the month of June, but without statistical significant difference from other sampling dates. However, median total coliforms and fecal coliforms presented significant differences among sampling dates, as presented in Table 2.

The presence of coliform bacteria alone does not specifically indicate the origin of fecal contaminants, whether it is of human or animal origin. Therefore, a comparison between coliform bacteria counts and caffeine concentrations could be useful to identify the origin of water fecal contamination. In this study, the counts of total coliforms and fecal coliforms were highly correlated (rs = 0.91), as expected, probably indicating a common source of the bacteria. Total coliform counts and caffeine concentrations presented a non-significant correlation

Table 1. Collection sites and results of microbiological analyses and caffeine determinations.

Date	Collection site	Total Coliforms (NMP/100 mL)	Fecal Coliforms (NMP/100 mL)	Caffeine (ng mL^{-1})
25/04/2012				
	Santo Antônio da Patrulha	200	< 1	0.22
	Parobé	12960	2280	0.25
	Campo Bom	5810	1680	1.04
	Esteio	15000	1730	4.17
	Nova Santa Rita	11000	630	0.77
23/05/2012				
	Santo Antônio da Patrulha	265	41	0.15
	Taquara	644	121	0.66
	Parobé	24196	5794	2.58
	Campo Bom	24196	2382	6.21
	Nova Santa Rita	> 24196	985	0.04
20/06/2012				
	Santo Antônio da Patrulha	100	100	0.41
	Taquara	610	510	0.63
	Parobé	5050	1850	4.12
	Campo Bom	4430	630	4.26
	Esteio	3290	1460	16.72
22/08/2012				
	Taquara	200	<1	0.18
	Parobé	7710	2160	0.63
	Campo Bom	4370	1580	2.64
	Esteio	> 24196	7800	0.65
	Nova Santa Rita	6400	2380	0.41
	Santo Antônio da Patrulha	100	<1	0.29
24/10/2012				
	Santo Antônio da Patrulha	203	122	0.24
	Campo Bom	324	96	0.43
	Esteio	74	10	2.29
	Nova Santa Rita	52	20	7.89

Table 2. Statistical evaluation of microbiological and caffeine analyses.

Date	N	Total coliforms Median – IQR (NMP/100 mL)	Fecal coliforms Median – IQR (NMP/100 mL)	Caffeine Median – IQR (ng mL^{-1})
25/04	5	11000^{b} (3005-13980)	1680^{b} (315,5-2005)	0.77^{a} (0.24-2.60)
23/05	5	24196^{b} (455-24598)	985^{a,b} (81-4088)	0.66^{a} (0.09-4.39)
20/06	5	3290^{b} (355-4740)	630^{b} (305-1655)	4.12^{a} (0.52-10.49)
22/08	6	5385^{a,b} (175-12032)	1870^{a,b} (1-3735)	0.52^{a} (0.26-1.15)
24/10	4	139^{a} (58-294)	58^{a} (13-116)	1.36^{a} (0.29-6.5)
Total	25	4370 (202–11980)	630 (69-2005)	0.65 (0.27-3.38)
P		0.074	0.415	0.581

IQR: interquartile range. P value as Kurskal-Wallis test (α = 0.05). The same letter (a,b) in the column does not differ in Mann-Whitney at 5% significance.

(rs = 0.35, p = 0.09), whether fecal coliforms and caffeine concentrations had a moderate significant correlation (rs = 0.412, p <0.05). Previous studies had found either significant (Glassmeyer et al., 2005; Peeler et al., 2006) or non-significant correlations (Ferreira, 2005; Kurissery et al., 2012) between coliform counts and caffeine concentrations in water sources. Recently, Kurissery et al. (2012) suggested that higher correlations between coliform bacteria and caffeine can be found in sediment samples than in water due to the possible different rates of survival of the bacteria in these sources. As mentioned before, although natural plant sources of caffeine have been described, the levels are often negligible (Peeler et al., 2006), thus the presence of caffeine in the water of Sinos River and affluents reported here may be related to the low rates of sewage treatment presently available in the basin.

4. Conclusion

Considering its high specificity to human origin, caffeine concentration in water sources is a promising indication of domestic waste contamination. This is the first study in the Sinos River and further caffeine and microbiological monitoring will allow a better comprehension of the dynamics of domestic waste into the river.

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