Caffeine as an environmental indicator for assessing urban aquatic ecosystems

Cafeína como indicador ambiental prospectivo para avaliar ecossistemas aquáticos urbanos

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Abstract

This study aimed to evaluate the co-occurrence of caffeine and the extent of its influence as compared to other traditional water quality parameters (microbiological and physico-chemical) in order to characterize it as an efficient indicator of anthropic pollution of urban aquatic environments. Caffeine is an ingredient in a variety of beverages (coffee, tea, and caffeinated soft drinks) and numerous food products (chocolate, pastries, and dairy desserts). Although the human body metabolizes this stimulant efficiently, between 0.5 and 10.0% is excreted, mostly in the urine. Analysis of water samples from the Leopoldina Basin and Guanabara Bay revealed a significant difference between areas not commonly affected by nutrient enrichment or sewage inputs and areas chronically influenced by sewage discharges and elevated eutrophication. Monitoring caffeine will be fundamental in stressed urban aquatic environments where frequent accidental ruptures of sewer lines and discharges of untreated effluents impede effective water quality evaluation with traditional indicators.

Aquatic Environment; Pollution; Caffeine; Toxicology

Introduction

Degradation of aquatic ecosystems is directly related to the combination of industrialization and urbanization, as well as to the political and economic development process, both of which are determinant factors in land occupation and various uses of environmental resources in States or cities.

Maintenance of the microbiological quality and safety of water systems used for drinking and recreation is imperative, since contamination of these systems can pose serious risks to human health [1]. Given the difficulties in routine isolation of pathogens from environmental samples, sanitary microbiology has used microbiological indicators of the presence of fecal material in the environment as a parameter for routine indication of contamination. Basic interpretation of marker organisms is based on the premise that their presence reflects pollution of fecal origin and thus the risk of contamination by pathogenic organisms. The density of indicators indicates the degree of pollution or contamination.

A fact that deserves special attention is the considerable alteration of the metabolic characteristics of microorganisms present in these environments, given extensive bacterial and viral contamination of surface waters. Monitoring and tracking the infiltration of domestic and industrial wastewater and untreated sewage

into aquatic ecosystems has been a critical challenge for environmental managers whose work is devoted to protecting the subtle balance between biological systems at risk and public health. In addition, the level of pharmaceutical substances in the aquatic environment is an emerging concern [2]. Pharmaceutically active compounds are produced and used in large volumes, and their use and variety are increasing yearly. The most significant route for pharmaceutical compounds to enter the aquatic environment is via release from wastewater. Due to disordered land occupation in large cities, the aquatic environment receives such effluents in large amounts, a situation aggravated by numerous clandestine disposals of effluents, thus posing a high risk of fecal microbial contamination of groundwater, gross amounts of pharmaceutical compounds, and general degradation of water quality.

To assess the infiltration by human waste and to verify the presence of pharmaceutical compounds in the study area, caffeine was selected as a marker, mainly because of its anthropic nature, distinctive origin, environmental destination, and elevated consumption. An average person consumes and excretes large amounts of caffeine, and a single person can thus generate hundreds to thousands of mg of caffeine per day. Since caffeine is largely expected to persist in the water due mainly to its high solubility (13.5g/L), low octanol-water partition coefficient (log Kow = 0.01), and insignificant volatility, it fits the profile for a good, stable, dissolved marker directly related to human activity, with no potential biogenic sources [3]. The main source of caffeine in domestic wastewater is excretion following consumption of coffee, tea, soft drinks, or medication moving through ineffective on-site wastewater treatment systems [4,5,6].

The aim of the methodological proposal in this article is to open innovative perspectives for environmental control, with caffeine as the model indicator of anthropic contamination in environmental waters, combining such lines of research as environmental toxicology, environmental sanitation, and public health.

Chemical anthropic pollution indicator versus microbial indicators

Analysis of environmental waters using marker organisms is an effort to define the public health risk of waterborne and water-contact diseases. Such indicators are used to quantify possible impacts on water from animal and sewage waste. The target organism should be native to the intestinal tract, enter the water through fecal discharge, and be found in the presence of other enteric pathogens. Such marker organisms are normally used as a substitute for more harmful pathogens. Due to cost and technical constraints, it is impossible to test for and identify all enteric pathogens potentially present in the water. Marker microorganisms are used to predict the presence of (and/or minimize the potential risk associated with) pathogenic microbes. Marker organisms are useful in that they circumvent the need to conduct assays for every pathogen potentially present in water [7,8]. Ideally, indicators are non-pathogenic, rapidly detected, and easily enumerated, have survival characteristics similar to those of the relevant pathogens, and can be strongly associated with the presence of pathogenic microorganisms.

Infectious agents in environmental waters pose a considerable health risk to people using those waters. Human fecal waste potentially contains a wide variety of pathogenic organisms [9,10]. As the population base producing the fecal waste increases, so does the potential risk, both in terms of types and numbers of pathogens [11]. Originally, the standard indicator was fecal coliform bacteria. The *Enterococcus* and *Escherichia coli* tests were introduced. *Enterococcus* is a subgroup of fecal *streptococci,* and *E. coli* is one species of the fecal coliform group. It has been agreed, at least in temperate waters, that the presence of significant numbers of either of these two groups indicates an increased risk of waterborne and/or water-contact diseases. However, recent studies have suggested that *E. coli* may not be a reliable indicator in tropical and subtropical environments due to its ability to replicate in contaminated soils. In recent years, the usefulness of *Enterococcus* and *E. coli* as indicators of increased risk, principally in undeveloped and warm areas, has come into question. Both organisms have been shown to survive for long periods and even proliferate in the aquatic ecosystem. *Enterococci* have been used successfully as indicators of fecal pollution and are especially reliable as indicators of health risk in marine environments and recreational waters. However, it is known that environmental reservoirs of *enterococci* exist and that regrowth of these organisms may be possible once they are introduced into the environment. *Clostridium perfringens* spores can per-

sist in soil, sediments, and areas subject to human or animal fecal pollution. The soil profile contains a record of the environmental conditions in the area at the time in which the sediment was deposited. *C. perfringens* is an anaerobic, gram-positive, spore-forming rod that is an indicator of human fecal pollution. *C. perfringens* is ubiquitous in soil and streams and is present at high concentrations in feces, sewage, and polluted water. Its spores are relatively resistant to environmental stress, are consistently present in wastewater at high concentrations, and have been used as indicators of fecal pollution in water. Additionally, this microorganism is not exclusively limited to human fecal waste, but the level of spores present in water samples is much greater in water impacted by human waste, particularly in situations where prediction of the presence of viruses or remote fecal pollution is desirable [8,9].

Even as microbial indicators can be useful for predicting the possible presence of fecal contamination in water, their inadequacy as tools for risk assessment is also becoming progressively more evident [7]. Microbial technologies have improved their detection, and traditional indicator organisms are used as tools for predicting potential sources of fecal pollution as well as health risks associated with contaminated water systems.

Chemical indicators are usually by-products of human metabolism or activity. Specific chemicals can be used as tracers to indicate sources or contamination routes. Elimination rates vary depending on the individual, drug, and dosage. Until recently, pharmacologically active substances entering the environment have received little attention. Such compounds are individually and unequivocally associated with human activity, especially human waste, and interest has grown in the presence of pharmaceutical substances in the aquatic environment [10]. Such products are developed to perform a biological effect, so there is no reason to believe that they are environmentally risk-free. In addition, studies have demonstrated the presence of pharmaceutical substances in municipal sewage treatment plants.

In general, conclusive indicators should not have a natural source in the environment, since a large proportion of medication taken by patients passes through the body unaltered and travels to wastewater via urine and feces. This process attacks not only the microbial metabolism, altering its performance, but also its response characteristics, leading to the need for more reliable methods correlating with increased risk of water-related diseases.

Methods

Description of study site: Leopoldina Hydrographic Basin

Five sampling stations were selected (Figure 1), distributed in such a way as to reflect different external sources of organic matter and circulation patterns in the Leopoldina Hydrographic Basin.

Regarding the urban characteristics of the municipality of Rio de Janeiro, the Leopoldina area includes 30 housing projects and 8 slums, with a total estimated population of 1,135,000. The 11 rivers and 2 channels belonging to the Leopoldina Hydrographic Basin are responsible for discharging untreated domestic and industrial waste into Guanabara Bay, located at 22ºS, 43ºW. Guanabara Bay comprises 400km^2 of estuarine waters and is important for the piscine ecology of the southeast Brazilian coast, especially along the State of Rio de Janeiro.

Experiment

• Sample collection

Samples were collected on two different dates during July and August 2004 by submerging the open mouth of the sampling flasks 4-8 centimeters below surface in the upstream direction. All samples were placed on ice and returned to the laboratory for analysis within 6 hours of collection. In the laboratory, samples were stored at 5 ± 2°C until analyses had been completed. For each parameter to be analyzed (microbiological, physico-chemical, and caffeine content), one liter of each river sample was collected in a previously sterilized amber flask.

Analytical analyses

• Microbiological analyses

For microbiological analyses, the samples were de-chlorinated by adding 1mL of autoclave-sterilized, concentrated sodium thiosulphate solution to provide a final concentration of approximately 100mgL^{-1}. Samples were mixed by vigorous manual shaking after adding the thiosulphate and before analysis.

The target microbiological indicators of fecal pollution were: *C. perfringens*, *Enterococcus faecalis*, *E. coli*, thermotolerant coliforms, and total coliforms. Bacterial indicators were enumerated by membrane filtration (0.45µm pore size, 47mm diameter cellulose filters) and grown

on selective media. Total coliforms were incubated in a water bath at 44.5 ± 0.5°C for 24 ± 2h on mE agar (Merck). Thermotolerant coliforms and *E. coli* were incubated in a water bath at 44.5 ± 0.5°C for 24 ± 2h on ECmedium plus MUG (Merck). The nutrient indicator MUG (4-methyl-umbelliferyl-ß-d-glucoronide) is metabolized by the *E. coli* enzyme ß-glucoronidase. As *E. coli* uses ß-glucuronidase to metabolize MUG, a fluorescent (under UV light) by-product, 4-methyl-umbelliferone, is produced. To enumerate *E. faecalis*, samples were grown at 42 ± 1°C for 48 ± 2h on mE agar (Merck). Brick-red colonies that developed a black precipitate upon transfer to EIA agar (Merck) for 20min at 42°C were counted as *enterococci*. *C. perfringens* was identified and counted by anaerobic growth on mCP medium (Merck) at 45 ± 1°C for 24h. Yellow colonies that turned pink upon exposure to ammonium hydroxide fumes were counted as *C. perfringens*.

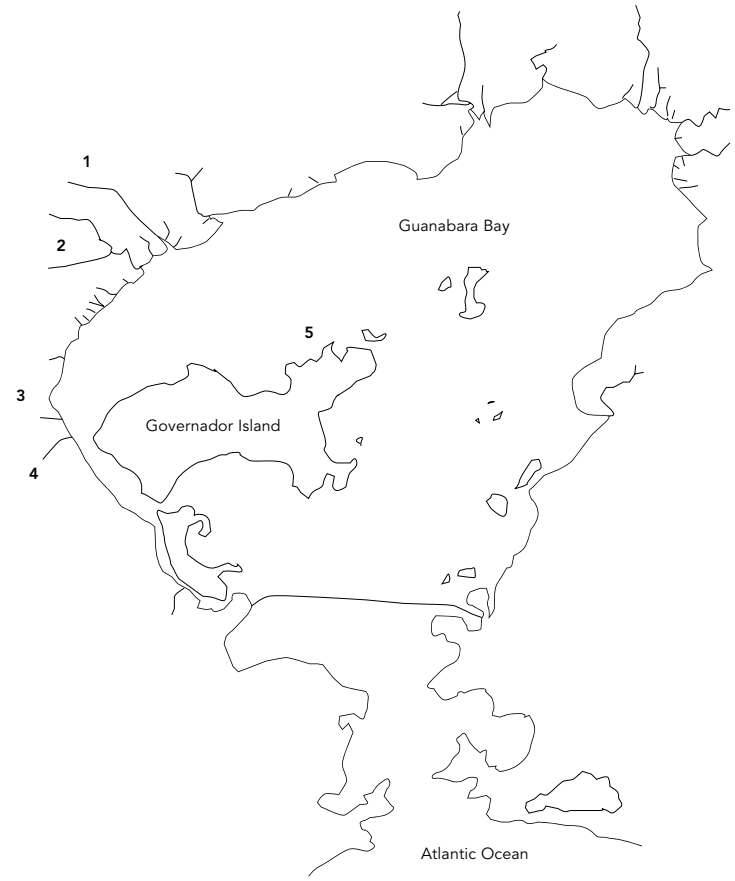
- **Physico-chemical analyses**

Physico-chemical analyses were performed according to the recommendations described by *Standard Methods for the Examination of Water and Wastewater* [12].

In situ tests were performed for dissolved oxygen (DO) and pH parameters at each site using a Hydrolab Surveyor II multi-parameter meter. Nutrient samples (nitrite, nitrate, and nitrogen) were frozen to be analyzed at the laboratory using the AutoAnalyzer II – Technicon. Dissolved organic nitrogen and phosphorus (DON and DOP) were determined by the photo-oxidation technique. For measuring biochemical oxygen demand (BOD), a commitment of five days from initial sample collection to the end of the analysis was required. During this time, samples were initially seeded with microorganisms and supplied with a carbon nutrient source of glucose-glutamic acid. The sample was then introduced into an environment suitable for bacterial growth at reproducible temperatures, nutrient sources, and light at 20ºC incubation for oxygen to be consumed. Quality controls, standards, and dilutions were also run for accuracy and precision. Dissolved oxygen in the samples was determined through Winkler titration. The difference between initial DO readings (prior to incubation) and final DO readings (after a five-day incubation period) predicts the sample's BOD.

Figure 1

Sampling locations in the Leopoldina Basin and Guanabara Bay.



Sampling locations	Runoff (m³/s)	Latitude	Longitude
1 – Meriti River	24,0	22° 48′ 09″	43° 17′ 24″
2 – Penha Cannel	3,1	22° 49′ 10″	43° 16′ 59″
3 – Ramos River	5,2	22° 51′ 20″	43° 06′ 09″
4 – Cunha Cannel	8,9	22° 52′ 46″	43° 14′ 22″
5 – Guanabara Bay	–	22° 50′ 09″	43° 14′ 24″

- **Caffeine analyses**

(a) Sample preparation: all samples were filtered through a combusted 0.45-µm filter (Whatman) before analysis. Typically, a 1-L volume of the filtered surface water sample was transferred to a 2-L separatory funnel. After increasing the pH of the sample to 8-9 with a 0.1M sodium hydroxide solution, the samples were extracted using liquid–liquid extraction with three portions of 50mL methylene chloride. The organic extracts were concentrated to a volume of 2-5mL using a water bath and a Snyder column and further evaporated to dryness using a gentle stream of purified nitrogen. After reconstituting the samples to 500µL of 1:1 methanol/water, analytical determinations were performed [13,14].
(b) Analytical determination: quantitative determination of caffeine in the final extracts was carried out by the high performance liquid chromatography (HPLC) method. A liquid chromatograph (Waters, model 600E) with a Rheodyne injection valve (7725i) fitted with a 10µL injection loop, a variable ultraviolet detector (Waters, model 484), and an integrator (Waters, model 746) were used. Chromatographic separation was accomplished using column Nova-Pack® RPC8 (3.9 x 150mm I.D., 5µm), pre-column RPC18 (3.0 x 39mm I.D., 50µm). The mobile phase was programmed to run in a linear gradient from 30% methanol/70% water to 100% methanol at a flow-rate of 1ml/min. The total run time required for the analysis was 10min with caffeine eluting at 4.21min. To further improve the method's sensitivity, acquisition time ranges for caffeine were split from 0 to 10min.
(c) Calibration curve for HPLC: standard solutions of caffeine (10, 50, 100, 200, 300, 400, and 500µg/mL) and (10, 50, 100, 200, and 300µg/mL) were prepared using the mobile phase as solvent. The standard solutions were injected in duplicate and the peak area measured. The linearity was evaluated by linear regression, and precision and accuracy were determined by coefficient of variation. Caffeine was purchased from Merck as analytical grade.

Statistical analysis

Statistical analyses were applied to the bacterial concentration and caffeine value data in order to improve their parametricity [15]. Multiple regressions using the stepwise selection procedure were examined to investigate relationships between microbiological indicator concentrations and caffeine under the environmental conditions on sampling days (day_1) and (day_2). Microbial concentrations were used as dependent variables (y), predicted by independent (or predictor) variables (x_i). The statistical distribution of each independent variable was examined and the variables were used to examine multivariate relationships of the form: $y = a + b_1x_1 + b_2x_2 + ... + b_ix_i \pm u$, where a is the intercept (y at $x = 0$), b is the slope (change in y per unit change in x), and u the stochastic disturbance or random error term. In the regression analysis, the tolerance value, which limits multicollinearity between independent variables, was set to exclude variables, which have an explained variance of ≥ 50% with variables already in the model. The strength of relationships was assessed using the coefficient of determination (r^2), adjusted for degrees of freedom and expressed as a percentage. Regression analysis was not applicable to rainfall data because of the high proportion of zero values. All tests were assessed at the 95% confidence level.

Results

Municipal wastewater is the aggregate of all water used and disposed of in a community. Especially in urban areas, domestic and industrial wastes can contribute with high waste loads varying in composition according to population density and type of industry. However, there is no regularity in domestic and industrial wastewater treatment prior to discharge into surface waters, corroborating the high concentrations among tested parameters but showing the efficiency of caffeine as an urban anthropic pollution indicator.

Table 1 summarizes the microbiological parameters detected in water samples collected from rivers belonging to the Leopoldina Basin and Guanabara Bay.

The pollutant agents' presence as solids, organic matter, nutrients, and microorganisms contribute significantly to river degradation. Excess nutrients like phosphorous, nitrogen, and organic matter cause water eutrophication, lowering the levels of dissolved oxygen and affecting the aquatic fauna. Table 2 shows the physico-chemical parameters detected in water samples collected from rivers in the Leopoldina Basin and Guanabara Bay.

Caffeine was detected in all the samples. Those collected from the rivers, which showed concentrations between 160 and 357µg/L, clearly express areas with water quality problems. Caffeine concentrations in water samples from

Table 1

Microbiological parameters in water samples from Leopoldina Basin rivers and Guanabara Bay, Rio de Janeiro, Brazil.

Sampling locations	Date	*Clostridium perfringens* (NMP/100mL)	*Enterococcus faecalis* (NMP/100mL)	*Escherichia coli* (NMP/100mL)	Thermotolerant coliforms (NMP/100mL)	Total coliforms (NMP/100mL)
Ramos River	day_1	2.2×10^4	1.5×105	4.5×10^4	1.6×10^6	3.4×10^6
	day_2	3.6×10^4	3.4×10^4	1.6×10^4	2.2×10^4	2.8×10^4
Meriti River	day_1	5.3×10^4	4.6×10^4	2.8×10^4	0.3×10^7	1.2×10^4
	day_2	2.1×10^5	2.2×10^4	1.2×10^3	2.2×10^4	3.6×10^4
Penha Canal	day_1	4.6×10^5	2.1×10^5	2.2×10^6	2.4×10^7	6.4×10^7
	day_2	1.6×10^4	1.8×10^6	1.1×10^4	4.2×10^6	4.0×10^5
Cunha Canal	day_1	6.3×10^6	1.6×10^5	7.2×10^5	3.0×10^6	7.2×10^6
	day_2	4.0×10^5	1.2×10^4	2.6×10^5	5.2×10^6	1.9×10^5
Guanabara Bay	day_1	1.2×10^2	2.7×10^2	0.9×10^3	1.6×10^2	3.8×10^3
	day_2	0.9×10^3	1.3×10^2	2.6×10^4	1.6×10^3	2.1×10^2

Table 2

Physico-chemical parameters in water samples from Leopoldina Basin rivers and Guanabara Bay, Rio de Janeiro, Brazil.

Sampling locations	Date	pH	BOD $mg.L^{-1}$	DO $mg.L^{-1}$	Nitrate $mg.L^{-1}$	Nitrite $mg.L^{-1}$	Nitrogen $mg.L^{-1}$	DON $mg.L^{-1}$	DOP $mg.L^{-1}$
Ramos River	day_1	6.72	40.0	0.1	0.01	0.001	1.9	28	3.00
	day_2	6.44	24.0	0.6	0.012	0.003	1.6	15	2.00
Meriti River	day_1	6.46	33.0	0.1	0.03	0.002	1.7	18	2.00
	day_2	6.89	12.0	0.2	0.01	0.019	1.8	14	1.6
Penha Canal	day_1	6.70	70.0	0.1	0.01	0.001	1.9	18	3.00
	day_2	6.63	20.0	0.1	0.01	0.004	1.8	15	1.4
Cunha Canal	day_1	6.56	30.0	0.1	0.03	0.01	1.9	14	3.00
	day_2	6.62	40.0	0.1	0.01	0.002	1.7	11	1.45
Guanabara Bay	day_1	7.4	10.5	6.4	0.03	0.009	1.2	1.4	0.5
	day_2	7.16	6.8	10.6	0.02	0.008	0.08	3.0	0.2

BOD = biochemical oxygen demand; DO = dissolved oxygen; DON = dissolved organic nitrogen; DOP = dissolved organic phosphorus.

Guanabara Bay ranged from 134 to 147ng/L (Table 3).

The retention time (rt) of caffeine was 4.21min with detection at 285nm (0.05 AUFS). No significant differences in concentrations were observed between the dates of collections. The regression equations (y = ax + b) and their correlation coefficients (r) were calculated, obtaining for caffeine y = 0.0461x + 238.17 (r = 0.99995), and for microbiological indicator and caffeine data were obtained y = -0.1362x + 5.3738 (r = 0.69817). The recovery of caffeine varied from 95.4 – 100.1% (Figure 2).

Discussion

The results of the present article show the quantification of unmetabolized caffeine in surface water samples in a simple and sensitive way. These results indicate the complexity of water quality but support the hypothesis that independently of the high concentration of microbiological indicators detected and physico-chemical parameters altered due to anthropic pollution, caffeine is an excellent indicator of urban pollution. Additionally, the results show both the occurrence and extent of caffeine wastewater contamination and the potential

environmental health effects on urban ecosystems and public health. The relevance of these findings is that if caffeine is present in water, numerous pathogens, biologically active pharmaceuticals, and personal care products are certain to be present as well. Based on these results and future research, we intend to assist public mangers by defining critical values for use as environmental parameters in the prevention of public health risks.

Table 3

Caffeine concentrations in the Leopoldina Basin and Guanabara Bay, Rio de Janeiro, Brazil.

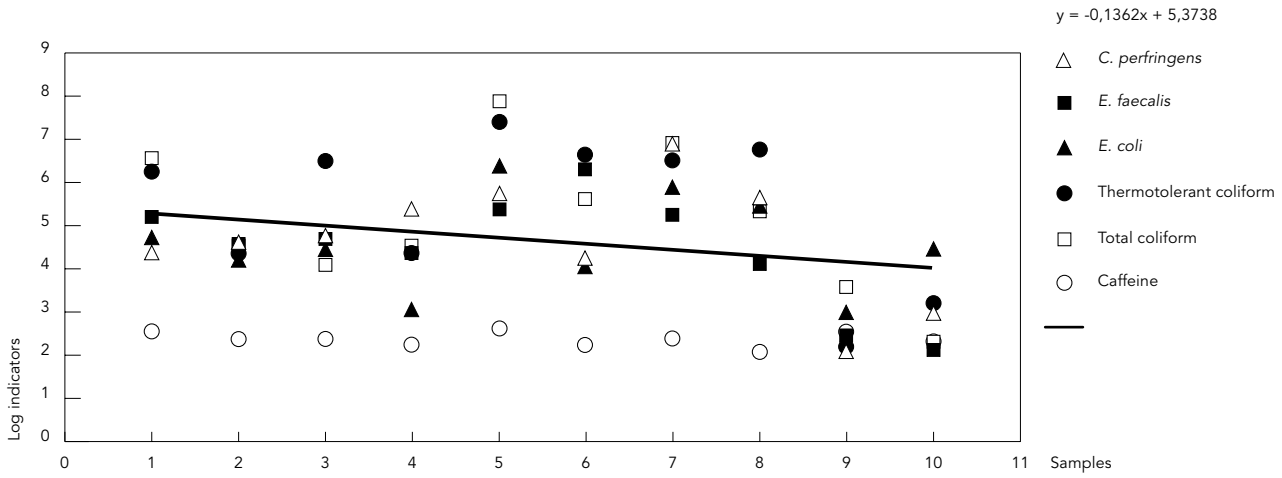
Date	Caffeine (µg/L)	n = 3 (mean)
Sampling locations		
Ramos River	day_1	357.0
	day_2	296.0
Meriti River	day_1	196.0
	day_2	201.0
Penha Canal	day_1	208.0
	day_2	225.0
Cunha Canal	day_1	160.0
	day_2	267.0
Sampling location		
Guanabara Bay	day_1	134.0
	day_2	147.0

The relationship between water pollution and public health is an increasing concern and motivates the increased efforts at pollution control [5]. Numerous pathogens can cause waterborne or water-contact diseases in humans, and their combined effect is highly significant worldwide in terms of both morbidity and mortality [7]. This concept is based on the idea that fecal-oral transmission is the most important route for human waterborne and water-contact diseases.

Systematic utilization of microbial indicators as risk assessment tools has been questioned worldwide due to the reiterated need to develop methods and analytical techniques that can define specific sources of such organisms. Determination of some pathogens is both difficult and expensive. It is not technologically feasible to analyze some pathogens in environmental water samples. Even when single pathogens are readily and inexpensively determined, there are so many that it would be too expensive to test for all of them, or even the most probable. Viral agents in the environment are a case in point, since they pose the greatest difficulties in detection. More than 150 types of enteric viruses may be present in raw sewage, depending on those circulating in the community [8]. Although many enteric viral infections are asymptomatic, some can cause gastroenteritis, hepatitis, respiratory infections, eye infections, meningitis, carditis, muscular disease, and rashes. In many cases, infected individuals

Figure 2

Analyses of caffeine concentrations and correlation with microbiological indicators.



excrete enteric viruses in high numbers. Norwalk and related viruses, together with parvovirus, may cause up to 60-70% of all cases of human gastroenteritis. However, little is known about virus distribution in the environment, particularly in the water supply, or the effects of water treatment processes on these viruses. Since so many different pathogens are potentially present in recreational water, it is not practical to test for all of them.

Because of its substantial consumption, caffeine has been detected in wastewater and surface, ground, and marine waters worldwide. Caffeine has been demonstrated to be chemically stable [3], and its level is not significantly reduced through traditional wastewater treatment, while its removal by more sophisticated treatment options is largely unknown. Failure of wastewater treatment plants and the release of persistent amounts of untreated wastewater confirm caffeine in environmental samples. This reinforces the suggested use of caffeine as a chemical indicator of treatment plant efficiency and contamination of natural aquatic environments due to anthropic activity [4].

Conclusion

Science as a whole is seeking to rediscover environmental parameters, and in this context caffeine research has been based on toxicological concepts [16,17,18], which are becoming increasingly important for environmental decision-making. Environmental toxicology deals with the effects of a category of poison (toxic chemicals) on different ecosystems and the basic scientific principles which allow discovery and measurement of environmental pollution effects. The predominant pharmacological aspects related to environmental hazards are becoming fundamental components in the prevention of public health risks [19]. The field of toxicology was developed mainly in order to develop better drugs, but it is also necessary to understand the environmental reactions to chemical substances, mainly due to the harmful effects of pollution on the environment caused by the extensive consumption of pharmaceuticals, an issue directly related to the shortcomings of microbiological indicators. This fact also supports the use of chemical indicators of anthropic pollution, and caffeine thus emerges as a key marker for urban aquatic ecosystems.

Resumo

Este estudo visou avaliar a co-ocorrência de cafeína e a extensão de sua influência frente a outros parâmetros tradicionais de qualidade de água (microbiológicos e físico-químicos), de modo a caracterizá-la como um eficiente indicador de poluição de origem antrópica em ambientes aquáticos urbanos. Cafeína é um componente de uma variedade de bebidas (café, chá e bebidas cafeinadas) e de numerosos produtos alimentícios (chocolate, massas e sobremesas). Embora o corpo humano seja eficiente na metabolização deste estimulante, entre 0,5-10,0% são excretados, principalmente na urina. A análise de amostras da Bacia Hidrográfica da Leopoldina e Baía de Guanabara, Rio de Janeiro, Brasil, revelou uma significante diferença entre áreas não comumente afetadas por enriquecimento de nutrientes ou esgoto, contra áreas cronicamente influenciadas por descargas de esgoto e eutrofização elevada. O monitoramento de cafeína será fundamental em ambientes aquáticos urbanos estressados, onde freqüentes rupturas acidentais de linhas de esgoto e descargas de efluentes não-tratados impedem a efetividade de avaliação da qualidade hídrica com os indicadores recomendados.

Ambiente Aquático; Poluição; Cafeína; Toxicologia

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