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SURVEILLANCE OF HUMAN ENTERIC AND RESPIRATORY VIRUSES AFTER A SANITARY EFFLUENT OVERFLOW AT LAGOA DA CONCEIÇÃO -FLORIANÓPOLIS/SC (BRAZIL)

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Laboratory of Applied Virology, Federal University of Santa Catarina, Department of Microbiology, Immunology and Parasitology, Santa Catarina State, Brazil. Abstract: Lagoa da Conceição, a scenic tourist spot in Santa Catarina Island, sustains local families through fishing and tourism. In January 2021, the sewage containment dam of the evapoinfiltration pond at Lagoa da Conceição's Sewage Treatment Plant (STP) ruptured causing significant sewage release into the area. Despite this evapoinfiltration lagoon's role as a storage for treated sewage, there's a risk of contamination by human feces-borne enteric and respiratory pathogens resistant to the sewage treatment processes applied in conventional STPs. This study aims to monitor human enteric and respiratory viruses in the Lagoa da Conceição after this extravasation event. The viruses considered for environmental monitoring were mastadenovirus (HAdV), enterovirus (EnV), rotavirus group A (RVA), norovirus (HNoV), hepatitis A virus (HAV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Over seven months, water samples were collected at three sites and concentrated using the organic flocculation method. Infectious EnV and HAdV were detected in 61.1% and 16.6% of samples, respectively, while other viruses weren't found. The presence of enteric viruses highlights Lagoa Conceição's contamination by viral da pathogens, even seven months after the sewage overflow. Implementing virological analysis is essential to prevent disease spread among the population. A health surveillance system with virological analysis is necessary to promptly identify contaminating pathogens and prevent disease spread among the population.

Keywords: Environmental monitoring, effluent overflow, human sewage, enteric and respiratory viruses, brackish lagoon, one health.

INTRODUCTION

Lagoa da Conceição is a hydrographic basin located in the central-eastern portion

of the Santa Catarina Island, Florianópolis municipality. It is a characteristic coastal lagoon ecosystem, separated from the ocean by portions of land and connected to the sea through a meandering 2.8 km channel known as Canal da Barra (Cabral et al., 2019; Martini et al., 2006) (Figure 1). Due to the interaction between continental fresh-water and oceanic salt water, Lagoa da Conceição stands out for its productivity and complexity, representing great environmental, social and economic importance for the municipality and providing livelihood to those who depend on the tourist and fishing activities in the region (Silva et al., 2017; Lisboa et al., 2008).

The use of its resources, however, has intensified in recent years thanks to the disorderly growth of the population (Cabral et al., 2019; Silva, 2017). A study conducted by Campanario (2007) indicated that the urban population around the lagoon grew 93.2% between 2001 and 2015, in a growth rate four times higher than the one of the state of Santa Catarina during the same period. Besides the population today exceeds the 100,000 people estimated in 2015 by Campanario, only 15% of the neighborhood is covered by the sewage treatment system (Campanario, 2007). Residences not included in the sewage network are required to have septic tanks, but unfortunately his regulation is not enforced (Cabral et al., 2019).

The Lagoa da Conceição Sewage Treatment Plant (STP) is situated on the southeast edge of the lagoon and serves approximately 16,500 residents (Figure 1). Effluents collected by the plant are directed to aeration tanks where dissolved oxygen, mechanical agitation, and microorganisms combine to form flakes. These flakes settle, forming activated sludge, which undergoes further treatment. After treatment, the effluents are deposited in an evapoinfiltration pond located 350 meters from Lagoa da Conceição (Santos, 2018; Vieira et al., 2014). Due to the close proximity of the treated sewage disposal area and the lagoon, along with the shallow water table in the region, there's a risk of contaminant transmission to both Lagoa da Conceição and groundwater (Machado, 2019; Silva, 2021).

In January 25th, 2021, the sand slope evapoinfiltration the pond supporting collapsed, resulting in the flooding of the area and the release of an immense volume of wastewater into the lagoon's interior (Odreski, 2021). Although the extravasated liquid came from treated sewage, it may contain residual pathogens that resisted treatment, in addition to very high concentrations of organic matter (Zanatta, 2014). The abrupt deposition of a large volume of effluents with the potential for the presence of biological and chemical contaminants in a lagoon that was already undergoing a eutrophication process contributes to the decline of its water quality and poses risks to the population that uses this resource (Cabral et al., 2019; SeTIC - UFSC, 2021).

Environmental contamination by enteric pathogens can have serious effects in lagoon environments such as Lagoa da Conceição. These coastal water bodies have specific environmental characteristics that increase the potential risk of exposure to pollutants, such as high turbidity, low depth, high concentration of organic matter and low dilution of contaminants (Katarzyte et al., 2018). The high turbidity and the concentration of organic matter favor the resistance of fecal coliforms (Katarzyte et al., 2018; Perkins et al., 2016). In addition, the turbidity resulting from the presence of suspended particles also favors the persistence of enteric viruses in such environments, since these pathogens tend to aggregate in solid particles to avoid inactivating factors (Hejkal et al., 1981).

Enteric viruses, including enterovirus (EnV), human mastadenovirus (HAdV),

human norovirus (HNoV), rotavirus A (RVA), and hepatitis A virus (HAV), are a diverse group of pathogens known for their replication in the host's gastrointestinal tract and transmission via the fecal-oral route (Fong and Lipp, 2005). These viruses are shed in high concentrations in the feces of infected individuals, with up to 10¹¹ viral particles per gram of feces (Bosch, 2008). Natural aquatic environments can host these pathogens, but human-related activities, such as sewage leaks, improper disposal, agricultural and urban runoff, and wastewater discharge from vessels, commonly introduce them into water bodies. The primary cause of environmental contamination by enteric viruses is the contact of sewage with pristine water resources through improper disposal or soil infiltration (Fong and Lipp, 2005).

The extravasation event that occurred in Lagoa da Conceição at the beginning of 2021 raises concerns about the contamination of this environment by enteric and respiratory viruses. The low infectious dose, the high rate at which they are excreted by infected individuals, the tendency to aggregate into solid particles for protection, the high resistance to variations in environmental conditions and to treatments routinely applied in STPs are characteristics of enteric viruses that make them a risk to the sanitary quality of Lagoa da Conceição.

Considering the importance of the environmental integrity of the Lagoa da Conceição watershed to the national ecological and economical scenario, the objective of this study was to monitor the presence of human enteric and respiratory viruses in the Lagoa da Conceição after the overflow of sanitary effluent occurred in January 2021. Human mastadenovirus, enterovirus, norovirus, hepatitis A virus, rotavirus A, and severe acute respiratory syndrome coronavirus 2 were investigated in three different locations over the seven months following the effluent discharge event to evaluate its possible impacts to the water quality and associated health risks in the studied coastal lagoon.

MATERIAL AND METHODS WATER SAMPLES

Water samples were sampled biweekly at three sampling sites located on the edge of Lagoa da Conceição during the months of February to August 2021. The selection of the sites was based on the activities carried out in each of the locations. The first point is in the region of Lagoa da Conceição, called Ponta das Almas and is characterized as a place for the circulation of marine vehicles, intended for both the transport of people and fishing grounds. The second point is in Osni Ortiga Avenue, a place of intense car and pedestrian traffic. Finally, the third point is in the region known as Canto da Lagoa, inside a residential condominium (Figure 1 and Table 1).

A volume of five liters (5 L) of water were sampled and stored in sterile plastic bottles. The samples were kept refrigerated between 4 and 8°C and immediately transported to the laboratory. The viral concentration of the samples was performed within 24 hours from the moment of collection and the eluates were preserved at -80°C until the realization of viability and molecular analysis assays. Table 1 presents the characteristics of the sampling sites.

WATER PHYSICOCHEMICAL ANALYSIS

Immediately upon arrival at the Laboratory of Applied Virology, the samples were submitted to analysis of physicochemical parameters. The objective of this analysis is to verify if these parameters are in accordance with the requirements of the legislation and if there is a correlation between the presence and detection of enteric pathogens

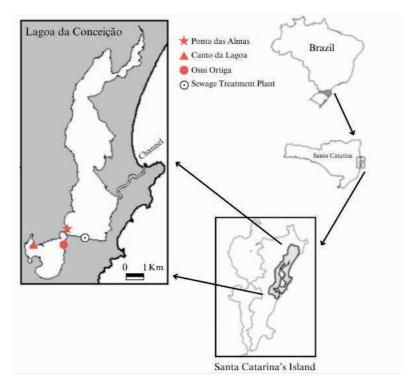


Fig. 1 Location of the Lagoa da Conceição and the sampling sites at the Santa Catarina Island (Florianópolis, Santa Catarina, Brazil)

Local	Sites (geographical coordinates)	Characteristics
Ponta das Almas	27° 35' 57" S 48° 27' 49" W	Intense circulation of boats (transport and fishing grounds)
Osni Ortiga	27° 36' 34" S 48° 27' 50" W	Intense circulation of automobiles and pedestrians
Canto da Lagoa	27° 36' 39" S 48° 28' 47" W	Residential area

Table 1 Lagoa da Conceição sampling sites characterization

and the physicochemical parameters of the water sample. The analyzed parameters were temperature, pH, dissolved oxygen, electrical conductivity, salinity, total dissolved solids and resistivity. These parameters were selected according to their importance for the analysis of water quality (Patil et al., 2012). The physicochemical parameters were evaluated using the commercial kits of Conductivity Meter Salinometer AT 215, pH meter AT315 SP Microprocessed, and Oximeter AT155 Microprocessed (Alfakit).

In addition to the parameters measured using commercial environmental analysis kits, the monthly averages of precipitation and atmospheric temperature in the Lagoa da Conceição region were also evaluated during the analyzed period. These data were obtained through the Weather Spark and Climate Data portals and were analyzed regarding their influence on viral and bacterial contamination in the sampling sites.

WATER BACTERIOLOGICAL ANALYSIS

One mL of the water sample was inoculated into plates containing selective growth medium for enterobacteria (MacConkey Agar from KASVI) and incubated at 37°C. After 24 hours, typical colonies of Escherichia coli were counted and the results were expressed in Colony Forming Units (CFU/100 mL). The isolated colonies were sent to the Instituto de Microbiologia Paulo de Góes (UFRJ) perform the MALDI-TOF-MS test. to This technique characterizes molecules by measuring the mass-to-charge ratio (m/z) of ionized species in the gas phase and can be used for the research and identification of microorganisms (Ashfaq et al., 2022).

VIRUS CONCENTRATION

Due to the properties of the analyzed water samples, which are defined as brackish due

to their average salinity (Cabral et al., 2019), which remains between 0.5 and 30 ppt, the technique of viral concentration by organic flocculation was selected, based on the methodology described by Calgua et al. (2008). This technique is based on manipulating the pH of the water, with the aim of making the viral particles present remain adhered to the milk flakes added to the sample. Murine coronavirus type 3 (MHV-3) and murine norovirus type 1 (MNV-1) were spiked to the samples to assess the effectiveness of the viral concentration method. Then, the pH of the samples was adjusted to 3.5 with Hydrochloric Acid 1M (HCl) and the solution of organic skimmed milk was added in a concentration of 1%. The samples were stirred for eight hours at room temperature. After the agitation period, the samples were decanted for eight hours and flocs in which the viruses are adsorbed precipitate. The supernatants of each sample were carefully siphoned off and their pellet centrifuged. Each pellet was resuspended in a 0.2 M phosphate buffer at pH 7.5 (1:2 v/v of Na, HPO, 0.2 M and NaH, PO, 0.2 M) and used in the detection tests. The samples were stored at -80°C until analyzed by molecular and/or cell culture techniques (Calgua et al., 2008).

After the concentration step, this eluate was subjected to molecular assays via nucleic acid extraction and real-time PCR, and assays in cell culture. All samples collected were tested for Rotavirus group A, Human Mastadenovirus, Hepatitis A virus, Enterovirus, Human Norovirus genotypes GI and GII and SARS-CoV-2. The selection of these pathogens was due to the characteristics of the sampling sites, which are close to residential centers, places with intense traffic of cars, boats and pedestrians. Therefore, viruses characterized by being biomarkers of fecal contamination were selected. The SARS-CoV-2 do not characterize as an enteric virus, but was included in the analysis due to the recent COVID-19 pandemic.

VIRAL VIABILITY BY CELL CULTURE ASSAY

For viral viability assays of environmental water samples, a continuous line of RD cells was used for the propagation of EnV (permissive cells derived from human rhabdomyosarcoma cells) and A549 continuous cell line was used for the propagation of HAdV (permissive cells derived from human lung carcinoma cells). Both cell lines were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% of fetal bovine serum in a humidified 5% CO2 atmosphere at 37° C. These cells were commercially acquired from Institute Adolfo Lutz and kindly donated by Dr. Rosina Gironès from the University of Barcelona, respectively. To evaluate the presence of infectious EnV and HAdV in the environmental samples, all samples were treated with antibiotics (10 U/ mL penicillin, 10 µg/mL streptomycin, and 0.025 µg/mL amphotericin B). For all tested samples, a non-cytotoxic dilution was then selected and inoculated (0.2 mL) in triplicate into RD and A549 cells. These cells were previously cultivated in 24-well tissue culture plates at a density of 3x10⁵ cells/well and were incubated at 37 °C in 5% CO_2 for 4 to 7 days, as described by Cromeans et al. (2008) and Rigotto et al. (2011).

EXTRACTION OF VIRAL NUCLEIC ACID AND VIRUS DETECTION BY REAL TIME QPCR

Nucleic acid extraction was performed RNA/DNA PureLink[™] Viral using the Mini Kit (Invitrogen[™]), following the instructions. manufacturer's **RNAse-free** water was used as a control to monitor the risk of cross-contamination during the procedure. Nucleic acids were eluted in 50 μ L of RNAsefree water and subjected to quantification

using the NanoVue Plus[™]. The previously selected viruses were quantified by the realtime qPCR method following protocols previously described by Hernroth et al. (2002) (HAdV), Silva et al. (2007) (NoV genogroup I), Kageyama et al. (2003) (NoV genogroup II), Jothikumar et al. (2005) (HAV), Zeng et al. (2008) (RVA), and Corman et al. (2020) (SARS-CoV-2), using the AgPath-ID[™] One-Step RT-PCR Reagents kit. All amplifications were performed using the StepOnePlus® Real-Time PCR System (Applied Biosystems). All samples were analyzed in triplicate and again free-water RNAse was used as a non template control for each assay. To control the possible inhibitors of qPCR reaction and the viral recovery rates, the water matrix was spiked with known concentrations of MHV-3 and MNV-1, and quantification was performed.

STATISTICAL ANALYSES

To check whether the data obtained from the analysis of physicochemical parameters, bacteriological analysis and detection of viral genomes follow a normal distribution, the Shapiro-Wilk Test was performed. After determining that the data follow a normal distribution, a one-way ANOVA was performed to assess whether the values obtained from the physicochemical analysis differed statistically depending on the sampling site, considering significance level as p < 0.05. Finally, a Pearson Correlation Test was performed to determine whether there is a linear correlation between the presence of enteric viruses and bacteria in the collected samples, as well as whether there is an association between physicochemical parameters and environmental contamination. All tests were performed using the RStudio statistical software (RStudio Team, 2023).

RESULTS AND DISCUSSION

The viral recovery rates of MHV-3 and

MNV-1, spiked to each sample before its concentration by organic flocculation, were 5.84% and 8.91%, respectively, by qPCR. In addition to losses during samples processing, inhibitory substances for qPCR presents in brackish water matrices may contributed to these low recovery rates.

Enteric viruses were detected in the three sampled locations throughout the study period. Viable EnV and HAdV were confirmed to be present in the samples through cell culture assays. HAV, HNoV, RVA and SARS-CoV-2 were not detected in any of the analyzed samples. Of the total of 36 samples analyzed, 22 had the presence of viable EnV (61.1%), with the sampling site located in Osni Ortiga having the highest frequency in the detection of EnV (9/12). The sites located in Canto da Lagoa and Ponta das Almas tested positive for the presence of viable EnV on 7 and 6 occasions, respectively.

Viable HAdV presence was observed in samples for two months following the sewage leak accident at all three sites, but real-time qPCR assays failed to detect the target viruses. Regarding EnV and HAdV, which exhibited cytopathic effects when inoculated into permissive cell cultures, their genomes may have remained undetected due to the inhibition process from water matrix components. Moreover, the volume of the sample for cell culture testing (0.2 mL) exceeded the initial volume used in qPCR (0.01 mL), potentially enhancing viral detection via cell culture alone. The data suggests that while viable HAdV was present post-accident, qPCR limitations and sample volume disparities might have affected genome detection in these cases.

As for the bacteriological analysis carried out, the presence of *Escherichia coli* was confirmed through MALDI-TOF-MS test in all sampled locations, often in concentrations that exceed the required by the CONAMA Resolution n° 274/2000 that defines the quality standard for bathing (Table 2). The sites that shown the highest averages of CFU/100 mL are those located in Ponta das Almas and Canto da Lagoa. Ponta das Almas is a region that has both intense maritime traffic and the presence of numerous residences in its surroundings, which contributes to the presence of pathogens that are indicators of environmental contamination by sanitary effluents. The site located in Canto da Lagoa is situated inside a large residential condominium. Despite being a high end neighborhood in Florianópolis, Lagoa da Conceição has 91% of its residences with irregularities in water and sanitary connections, which implies that a significant number of sanitary effluents is improperly disposed inside the lagoon (CASAN, 2021).

The correlation analysis between the detection of enteric viruses and E. coli reported an r of -0.44, suggesting that there is a negative association between the presence of the two pathogens. That is, the presence of enteric viruses in the samples is not correlated with the detection of E. coli, as can be seen in Figure 2. The detection of bacterial indicators does not correlate with the presence of enteric viruses, indicating the necessity to reevaluate the legislation that sets the criteria for assessing bathing conditions. Enteric viruses have characteristics that provide them with greater resistance to unfavorable environmental conditions when compared to bacterial indicators. While the presence of enteric viruses may be related to a long-lasting contamination event, the detection of E. coli suggests that there is periodic contamination of this environment, with frequent disposal of sanitary effluents inside the lagoon (Berthe et al., 2013).

The analysis of physicochemical parameters included the evaluation of the salinity which, together with the activities carried out at Lagoa da Conceição, classify it as a Class I brackish water lagoon, intended for recreational

Sampling date	Site	Escherichia coli	EnV detection by cell culture	HAdV detection by cel culture
02/17/21	1	Not detected	+	+
	2	Not detected	+	+
	3	Not detected	+	+
03/20/21	1	Not detected	+	+
	2	Not detected	+	+
	3	Not detected	+	+
	1	Not detected	+	Not detected
04/22/21	2	Not detected	+	Not detected
01/22/21	3	Not detected	+	Not detected
	1	230	Not detected	Not detected
04/29/21	2	270	Not detected	Not detected
	3	570	Not detected	Not detected
	1	100	Not detected	Not detected
05/20/21	2	70	+	Not detected
03/20/21	3	300	Not detected	Not detected
	1	130	+	Not detected
05/27/21	2	70	+	Not detected
05/27/21	3	530	+	Not detected
	1	Not detected	+	Not detected
0.6/10/21	2	70	+	Not detected
06/10/21	3	130	+	Not detected
0.6/00/01	1	100	+	Not detected
	2	30	+	Not detected
06/23/21	3	100	+	Not detected
	1	200	Not detected	Not detected
07/08/21	2	200	+	Not detected
07/08/21	3	100	+	Not detected
	1	2130	Not detected	Not detected
07/22/21	2	100	+	Not detected
07/22/21	3	530	Not detected	Not detected
08/05/21	1	270	Not detected	Not detected
	2	30	Not detected	Not detected
	3	770	Not detected	Not detected
	1	100	Not detected	Not detected
00/10/21	2	300	Not detected	Not detected
08/19/21	3	100	Not detected	Not detected

Table 2 Levels of bacterial and viruses indicator detected in sites in the Lagoa da Conceição (CFU/100 mL)

activities of primary contact and used for aquaculture and fishing practices. Observing the values of the physicochemical parameters established for the determination of bathing in Class I brackish waters, it is possible to determine that the dissolved oxygen content fits with that recommended by the legislation (minimum of 5 mg/L). The pH values are also in line with that established by CONAMA, which suggests a pH range between 6.5 and 8.5 as the ideal and non-toxic standard for using these waters (CONAMA, 2005).

The statistical analysis of the physicochemical parameters indicated that there was no significant difference between the values obtained for each parameter at each sampling site. The lack of statistical difference between the physicochemical parameters implies that variations in those parameters do not have a significant impact on the presence or absence of the pathogens under investigation. The mean, standard deviation and p-value regarding the variance analysis of the physicochemical parameters evaluated over the seven months are shown in Table 3.

addition to the physicochemical In parameters evaluated ex-situ using commercial kits, data regarding the monthly average levels of precipitation and atmospheric temperature were also collected. These parameters can influence both the presence of pathogens through urban runoff and the results of other physicochemical parameters, such as salinity, pH, and dissolved oxygen content (Silva et al., 2008). As shown in Table 4, the pattern of atmospheric temperature and precipitation characteristic pattern exhibits the of subtropical regions, with humid summers and dry winters.

In this study, the month in which the samples showed the highest bacterial load coincided with the highest monthly level of precipitation during the analyzed period. By conducting the Pearson correlation test

to assess the association between rainfall levels and E. coli detection, it was possible to demonstrate a positive correlation between the two parameters (0.9), indicating that the presence of the bacterial indicator in the samples is potentially related to monthly rainfall indices. As known, urban runoff, intensified by heavy rainfall, directs pathogens from this environment to water bodies, increasing the detection of pathogens such as coliforms. The atmospheric temperature, however, does not have an association with bacterial contamination found in Lagoa da Conceição. Through the Pearson correlation test, an average r of -0.018 was obtained, indicating that the presence of E. coli in this environment is not influenced by the average atmospheric temperature of the region (Silva et al., 2008; Mallin et al., 2009).

Enteric viruses are resistant to the conventional treatment procedures applied in STPs. Schlindwein et al. (2010) evidenced the presence and infectivity of enteric viruses in samples of sludge and treated wastewater at an STP located in Florianópolis. As a result, all sludge samples and 75% of wastewater samples had the HAdV genome present, and 91.7% of all samples were positive for EnV in cell culture. Treatments using activated sludge, oxidative tanks, activated carbon, filtration and coagulation remove only between 50 and 90% of enteric viruses present in sewage. Through the drinking water quality guide, the WHO points out that specific viral agents may be less sensitive to disinfection processes when compared to the stability of bacteria and protozoa (WHO, 2021).

Despite the suspicion that the overflow that occurred in January/2021 contributed to the viral contamination in Lagoa da Conceição, it is known that this location has already been suffering from the inadequate disposal of sanitary effluents. Florianópolis, a city located in the South region of Brazil, has emerged as

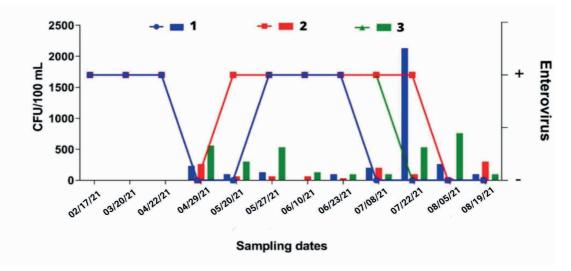


Fig. 2 A negative correlation between the presence of *E. coli* and EnV, with bars showing the number of CFU/100 mL of *E. coli* and the lines point to the sampling dates that showed positive results for EnV in cell culture

Sampling sites					
Physicochemical parameters	Ponta do Coral	Osni Ortiga	Canto da Lagoa	P-value	
	Mean ± SD	Mean ± SD	Mean ± SD		
Temperature (°C)	19.6 ± 1.8	19.35 ± 1.5	19.6 ± 1.6	0.96	
pH	7.82 ± 0.42	7.62 ± 0.46	7.65 ± 0.3	0.77	
Resistivity (Ohm.m)	32 ± 13.4	38 ± 18.6	37 ± 18.2	0.9	
Conductivity (mS/m)	$161.65 \pm 10,910.1$	133.45 ± 9,587	$104.75 \pm 9,547.8$	0.99	
Salinity (ppt)	25.35 ± 176.2	19.75 ± 106.4	19.6 ±101.6	0.75	
Total dissolved solids (ppm)	21,693 ± 38,095.5	17,096.5 ± 28,522.6	17,056 ± 53,577.2	0.85	
Dissolved oxygen (mg/L)	7.23 ± 29.3	7.76 ± 31.4	7.98 ± 29.5	0.99	

Table 3 Mean, standard deviation (SD) and P-value (one-way ANOVA) of the physicochemical parametersevaluated over seven months after the overflow of sanitary effluent

	Atmospheric temperature (°C)	Precipitation (mm)
February	25	151.2
March	24	108.5
April	22	84.2
May	18	85.6
June	17	86.3
July	17	97.2
August	18	86.1

Table 4 Mean values of monthly levels of atmospheric temperature and precipitation

a prominent tourist hub, attracting numerous visitors each year. However, this rapid influx of tourists has led to a substantial temporary population surge, surpassing the limits set by the city's Master Plans. Consequently, this uncontrolled expansion contradicts the city's capacity for sanitation and undermines efforts to prevent environmental degradation through proper planning (**Prefeitura de Florianópolis, 2021**).

Rigotto et al. (2010) and Moresco et al. (2012) demonstrated that the contamination of this lagoon environment by enteric viruses was already a reality ten years ago. Rigotto and collaborators evaluated water samples collected in Lagoa da Conceição between June/2007 and May/2008. Through cell culture assays and qPCR, it was possible to detect the presence of HAdV (75%), RVA and HAV (16.6%) in the samples. Moresco and team, similarly, evaluated water samples from Lagoa da Conceição between August/2009 and July/2010. After carrying out qPCR, it was possible to demonstrate the contamination of this environment by HAV (1x10² GC/L) and HAdV (1x105 GC/L). Contamination by enteric viruses evidenced in these works indicates that Lagoa da Conceição has been receiving improper disposal of sanitary waste periodically for at least ten years.

CONCLUSION

In January/2021 the dam that sustained the evapoinfiltration lake of the Lagoa da Conceição STP broke, releasing a huge amount of treated effluent to the interior of this lagoon. The present study compared water quality levels by monitoring enteric pathogens as parameters in three distinct sites along the Lagoa of Conceição (Florianópolis/ SC) after the sanitary effluent overflow. The results demonstrated the water contamination by viable EnV and HAdV in all sampled sites. This is of special concern, because it indicates that the conventional sewage treatment applied in STP is inefficient to completely inactivate enteric pathogens. Despite the important role of this environmental disaster in the water contamination, it is known that Lagoa da Conceição has been receiving inappropriate disposal of sanitary effluents for decades, which is proven by the detection of EnV up to six months after the extravasation event. The analyses presented illustrate how the inadequate management of waste in the water resources can have negative effects, highlighting the importance of implementing suitable policies for wastewater management and safeguarding the water resource.

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STATEMENTS & DECLARATIONS

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REFERENCES

Ashfaq, M. Y., Da'na, D. A., & Al-Ghouti, M. A. (2022). Application of MALDI-TOF MS for identification of environmental bacteria: A review. *Journal of Environmental Management*, 305, 114359. https://doi.org/10.1016/j.jenvman.2021.114359

Berthe, T., Ratajczak, M., Clermont, O., Denamur, E., & Petit, F. (2013). Evidence for Coexistence of Distinct Escherichia coli Populations in Various Aquatic Environments and Their Survival in Estuary Water. *Applied and Environmental Microbiology*, 79(15), 4684–4693. https://doi.org/10.1128/aem.00698-13

Bosch, A., Guix, S., Sano, D., & Pintó, R. M. (2008). New tools for the study and direct surveillance of viral pathogens in water. *Current Opinion in Biotechnology*, *19*(3), 295–301. https://doi.org/10.1016/j.copbio.2008.04.006

Cabral, A., Bercovich, M. V., & Fonseca, A. (2019). Implications of poor-regulated wastewater treatment systems in the water quality and nutrient fluxes of a subtropical coastal lagoon. *Regional Studies in Marine Science*, *29*, 100672. https://doi. org/10.1016/j.rsma.2019.100672

Calgua, B., Mengewein, A., Grunert, A., Bofill-Mas, S., Clemente-Casares, P., Hundesa, A., Wyn-Jones, A. P., López-Pila, J. M., & Girones, R. (2008). Development and application of a one-step low cost procedure to concentrate viruses from seawater samples. *Journal of Virological Methods*, 153(2), 79–83. https://doi.org/10.1016/j.jviromet.2008.08.003

Campanário, P. (2007). Florianópolis: dinâmica demográfica e projeção da população por sexo, grupos etários, distritos e bairros (1950-2050). *Prefeitura de Florianópolis*.

Climate Data (2021) Clima Florianópolis (Brasil). *Climate Data*. https://pt.climate-data.org/america-do-sul/brasil/santa-catarina/florianopolis-1235/. Accessed 7 Sep. 2022

Companhia Catarinense de Águas e Saneamento (2021) Trato Pela Lagoa Registra 91% de Irregularidade na Ligação de Esgoto. *CASAN.* https://www.casan.com.br/noticia/index/url/trato-pela-lagoa-registra-91-de-irregularidade-na-ligacao-de-esgoto#0. Accessed 8 Sep. 2022

ConselhoNacionaldoMeioAmbiente-CONAMA(2000)ResoluçãoCONAMANo273,de29deNovembrode2000.*MinistérioPúblico Federal.* http://portal.pmf.sc.gov.br/arquivos/pdf/17_01_2011_17.30.47.12d8482d5a7677bddba4bbc18cc3bcbb.pdf. Accessed 7 Sep. 2022

Conselho Nacional do Meio Ambiente - CONAMA (2005) Resolução CONAMA No 357, de 17 de Março de 2005 - 4ª Câmara. *Ministério Público Federal.* https://www.mpf.mp.br/atuacao-tematica/ccr4/dados-da-atuacao/projetos/qualidade-da-agua/legislacao/resolucoes/resolucao-conama-no-357-de-17-de-marco-de-2005/view. Accessed 7 Sep. 2022

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L., Mulders, D. G., Haagmans, B. L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M., ... Drosten, C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*, 25(3). https://doi.org/10.2807/1560-7917.es.2020.25.3.2000045

Cromeans, T. L., Lu, X., Erdman, D. D., Humphrey, C. D., & Hill, V. R. (2008). Development of plaque assays for adenoviruses 40 and 41. *Journal of Virological Methods*, *151*(1), 140–145. https://doi.org/10.1016/j.jviromet.2008.03.007

Fong, T.-T., & Lipp, E. K. (2005). Enteric viruses of humans and animals in aquatic environments: Health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews*, 69(2), 357–371. https://doi.org/10.1128/mmbr.69.2.357-371.2005

Hejkal, T. W., Wellings, F. M., Lewis, A. L., & LaRock, P. A. (1981). Distribution of viruses associated with particles in waste water. *Applied and Environmental Microbiology*, 41(3), 628–634. https://doi.org/10.1128/aem.41.3.628-634.1981

Hernroth, B. E., Conden-Hansson, A.-C., Rehnstam-Holm, A.-S., Girones, R., & Allard, A. K. (2002). Environmental Factors Influencing Human Viral Pathogens and Their Potential Indicator Organisms in the Blue Mussel, Mytilus edulis : The First Scandinavian Report. *Applied and Environmental Microbiology*, *68*(9), 4523–4533. https://doi.org/10.1128/aem.68.9.4523-4533.2002

Jothikumar, N., Cromeans, T. L., Sobsey, M. D., & Robertson, B. H. (2005). Development and evaluation of a broadly reactive taqman assay for rapid detection of hepatitis A virus. *Applied and Environmental Microbiology*, 71(6), 3359–3363. https://doi. org/10.1128/aem.71.6.3359-3363.2005

Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F. B., Takeda, N., & Katayama, K. (2003). Broadly reactive and highly sensitive assay for norwalk-like viruses based on real-time quantitative reverse transcription-pcr. *Journal of Clinical Microbiology*, *41*(4), 1548–1557. https://doi.org/10.1128/jcm.41.4.1548-1557.2003

Kataržytė, M., Mėžinė, J., Vaičiūtė, D., Liaugaudaitė, S., Mukauskaitė, K., Umgiesser, G., & Schernewski, G. (2018). Fecal contamination in shallow temperate estuarine lagoon: Source of the pollution and environmental factors. *Marine Pollution Bulletin*, *133*, 762–772. https://doi.org/10.1016/j.marpolbul.2018.06.022

Lisboa, L. K. (2008). Lagoa da Conceição: Uma revisão da disponibilidade de dados ecológicos visando o direcionamento de novas pesquisas no ecossistema. *Biotemas*, 21(1), 139-146

Machado MA (2019) Avaliação Da Influência Do Crescimento Populacional Na Balneabilidade Da Lagoa Da Conceição, Florianópolis - SC. Federal University of Santa Catarina

Mallin, M. A., Johnson, V. L., & Ensign, S. H. (2009). Comparative impacts of stormwater runoff on water quality of an urban, a suburban, and a rural stream. *Environmental Monitoring and Assessment*, *159*(1–4), 475–491. https://doi.org/10.1007/s10661-008-0644-4

Martini, L. C. P., Mattos, D. S., Barbosa, D. F. P., & Rech, A. I. B. (2006). Uso de sensoriamento remoto orbital para avaliação da distribuição espacial de Clorofila_a na Lagoa da Conceição - Florianópolis, SC. *Engenharia Sanitaria e Ambiental*, *11*(4), 318–324. https://doi.org/10.1590/s1413-41522006000400004

Moresco, V., Viancelli, A., Nascimento, M. A., Souza, D. S. M., Ramos, A. P. D., Garcia, L. A. T., Simões, C. M. O., & Barardi, C. R. M. (2012). Microbiological and physicochemical analysis of the coastal waters of southern Brazil. *Marine Pollution Bulletin*, *64*(1), 40–48. https://doi.org/10.1016/j.marpolbul.2011.10.026

Odreski, F. (2021). Estudo técnico para suporte às ações de promoção e recuperação ambiental da lagoa da conceição. Associação comercial e industrial de florianópolis. https://www.pmf.sc.gov.br/arquivos/arquivos/pdf/18_05_2021_17.08.49. b2387d39341b32f6e9d5f201159c081f.pdf

Patil, P. N. (2012, January 1). *Physico-chemical parameters for testing of water -A review*. Integrated Publishing Association. https://www.researchgate.net/publication/344323551_Physico-chemical_parameters_for_testing_of_water_-A_review

Perkins, T. L., Perrow, K., Rajko-Nenow, P., Jago, C. F., Jones, D. L., Malham, S. K., & McDonald, J. E. (2016). Decay rates of faecal indicator bacteria from sewage and ovine faeces in brackish and freshwater microcosms with contrasting suspended particulate matter concentrations. *Science of The Total Environment*, 572, 1645–1652. https://doi.org/10.1016/j.scitotenv.2016.03.076

Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C. K., Corrêa, A. A., Souza, D. S. M., Miagostovich, M. P., Simões, C. M. O., & Barardi, C. R. M. (2010). Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. *Journal of Applied Microbiology*, *109*(6), 1979–1987. https://doi.org/10.1111/j.1365-2672.2010.04827.x

Rigotto, C., Hanley, K., Rochelle, P. A., De Leon, R., Barardi, C. R. M., & Yates, M. V. (2011). Survival of adenovirus types 2 and 41 in surface and ground waters measured by a plaque assay. *Environmental Science & amp; Technology*, 45(9), 4145–4150. https://doi.org/10.1021/es103922r

Santos V (2018) *Disposição de Efluentes Tratados Em Uma Lagoa de Evapoinfiltração*. Dissertation, Federal University of Santa Catarina

Schlindwein, A. D., Rigotto, C., Simões, C. M. O., & Barardi, C. R. M. (2010). Detection of enteric viruses in sewage sludge and treated wastewater effluent. *Water Science and Technology*, *61*(2), 537–544. https://doi.org/10.2166/wst.2010.845

Prefeitura de Florianópolis. PLANO MUNICIPAL DE SANEAMENTO BÁSICO - Versão Final, (2021). https://www.pmf. sc.gov.br/arquivos/arquivos/pdf/05_04_2022_11.50.56.a6d0cb8eb0ca6e77f9eb77a9dd8cbe40.pdf

SeTIC - UFSC (2021) Nota Técnica Analisa Mortandade de Organismos e Cheiro de Água Podre Na Lagoa Da Conceição. *Notícias Da UFSC*. https://noticias.ufsc.br/2021/02/nota-tecnica-analisa-mortandade-de-organismos-e-cheiro-de-agua-podre-na-lagoa-da-conceicao/. Accessed 9 Sep. 2022

Silva, A. K., Le Saux, J.-C., Parnaudeau, S., Pommepuy, M., Elimelech, M., & Le Guyader, F. S. (2007). Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-pcr: Different behaviors of genogroups I and II. *Applied and Environmental Microbiology*, *73*(24), 7891–7897. https://doi.org/10.1128/aem.01428-07

Silva, A. E. P., Angelis, C. F., Machado, L. A. T., & Waichaman, A. V. (2008). Influência da precipitação na qualidade da água do Rio Purus. *Acta Amazonica*, *38*(4), 733–742. https://doi.org/10.1590/s0044-59672008000400017

Silva, V. E. C., Franco, D., Fonseca, A. L., Fontes, M. L., & Donnangelo, A. R. (2017). Space time evolution of the trophic state of a subtropical lagoon: Lagoa da Conceição, Florianópolis Island of Santa Catarina, Brazil. *RBRH*, 22(0). https://doi. org/10.1590/2318-0331.011716027

Silva VEC (2021) Análise Hidrodinâmica e Biogeoquímica de Uma Laguna Costeira Subtropical Em Bacia Hidrográfica Urbanizada: Lagoa Da Conceição, Florianópolis, Santa Catarina. Thesis, Federal University of Santa Catarina

Vieira, J. S., & Henkes, J. A. (2014). Uma analise nos impactos ambientais causados na lagoa da conceição pelo despejo de efluentes. *Gestão e Sustentabilidade Ambiental*, 2(2), 309–337.

Weather Spark (2021) Clima e Condições Meteorológicas Médias Em Florianópolis No Ano Todo. Cedar Lake Ventures. https://pt.weatherspark.com/y/30020/Clima-caracter%C3%ADstico-em-Florian%C3%B3polis-Brasil-durante-o-ano. Accessed Sep. 2022

World Health Organization (WHO) (2021) Guidelines on Recreational Water Quality. Volume 1: Coastal and Fresh Waters. World Health Organization

Zanatta, L. C., & Ramage, L. (2014). AVALIAÇÃO DA EFICIÊNCIA DA INFILTRAÇÃO DE EFLUENTES NAS DUNAS DO AQUÍFERO CAMPECHE, FLORIANÓPOLIS, S.C. Águas Subterrâneas

Zeng, S.-Q., Halkosalo, A., Salminen, M., Szakal, E. D., Puustinen, L., & Vesikari, T. (2008). One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. *Journal of Virological Methods*, 153(2), 238–240. https://doi.org/10.1016/j. jviromet.2008.08.004