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Virome in roof-harvested rainwater of a densely urbanized low-income region



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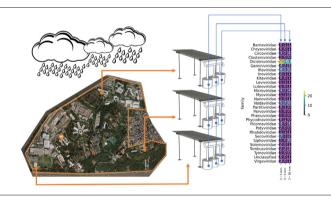
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HIGHLIGHTS

• Human viral pathogens were found in roof-harvested rainwater (RHRW).

- RNA insect viruses were the most abundant in RHRW.
- The surrounding area determined viral diversity in RHRW.

GRAPHICAL ABSTRACT



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ABSTRACT

Rainwater harvesting has been considered an affordable practice to supplement the conventional sources of water supply for potable and non-potable uses worldwide. This study characterizes the viral community found in roof-harvested rainwater (RHRW) samples obtained under different rain volumes in a densely urbanized low-income region in Rio de Janeiro, Brazil. Three pilot-scale standardized metal-sheet roofs (same catchment area, material age, and slope – 3%) were installed in the study area aiming at obtaining more reliable and representative samples. Fifty-four samples were collected from six rainfall events from January to April 2019 and concentrated by the skimmed-milk flocculation method. Pools of different rainfall volumes were submitted to high throughput sequencing using the shotgun metagenomic approach. Sequencing was performed on NextSeq platform. Genomic analysis of the virus community revealed that most are RNA non-human viruses, including two main families: *Dicistroviridae* and *Iflaviridae*, recognized for infecting arthropods. Bacteriophages were also relaively abundant, with a predominance of DNA phages belonging to *Microviridae* and *Siphoviridae* families, showing percentages from 5.3 and 3.7% of the total viral hits present in these samples, respectively. Viral genomic RNA viruses (77%) predominated over DNA viruses (23%). Concerning number of viral species identified, a higher percentage was observed for plant viruses (12 families, 58%). Hepatitis A virus and human klassevirus 1 were detected among the established human pathogens, suggesting the need for RHRW treatment before it is

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considered for human consumption. Australian bat lyssavirus was also detected, emphasizing the importance of environmental monitoring facing emerging viruses. The results corroborate the influence of the surrounding area on the rainwater quality.

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1. Introduction

In different geographical regions, especially in urban areas, water demand exceeds surface and groundwater availability, not only due to population growth but also to consumption patterns and overexploitation (Schewe et al., 2014; Gosling and Arnell, 2016; Mehran et al., 2017; Wu et al., 2017). Nowadays, more than 50% of the global population live in cities, and this share is predicted to rise up to 60% by 2030 (UNSD, 2021). In low- and middle-income countries, slums shelter fifty to 60% of the urban population. Regarding Latin American countries, the growth of informal urban developments and slums over the last 50 years has been witnessed, which means people living in precarious housing conditions with lack of sanitation, and restrict access to water (Rosenzweig et al., 2011). It is also noteworthy that people suffering from water scarcity worldwide rose from 14% to 58% in the last century (Kummu et al., 2016). Therefore, it is imperative to implement a sound integrated urban water management (IUWM), which encompasses coordinating the development and management of all water sources in all urban water cycle levels at the local scale (Jensen and Nair, 2019).

In this context, assessing alternative water sources, such as roof-harvested rainwater (RHRW), could help to fill the gap between offer and demand at the local scale (Tzanakakis et al., 2020). RHRW has been a focus of attention since it is considered a relatively cheap and affordable practice to supplement conventional sources of water supply (Kim et al., 2016; Lo and Gould, 2015). Due to some characteristics such as low hardness and sodicity, it is suitable for cooling towers, laundry, and irrigation (Sánchez et al. 2015). On the household scale, its most common uses are toilet flushing, and car and floor washing (Hamilton et al., 2019; Yannopoulos et al., 2019). The RHRW can also be used for drinking purposes when the conventional water system is not available (Lee et al., 2017). However, several factors, such as land use, roof features (covering material, age of the roof, slope), and system maintenance can affect the RHRW quality (Mendez et al., 2011; Lee et al. 2012; Hamilton et al., 2019).

Some studies also point to the occurrence of a broad spectrum of potentially pathogenic microorganisms found in RHRW that can pose hazards to human health, such as adenovirus, *Campylobacter* spp., *Campylobacter jejuni*, *Legionella pneumophila*, *Salmonella* spp., virulent *Escherichia coli* (*E. coli*), *Cryptosporidium* spp., and *Legionella* spp. (Ahmed et al., 2012a; Dobrowsky et al., 2014; Waso et al. 2016; Waso et al., 2018; Bae et al., 2019; Hamilton et al., 2019). Several strategies to reduce the microbiological contamination in RHRW, such as diverting the first millimeters of rainfall, have been described in the literature (*Gikas* and *Tsihrintzis*, 2012; Mendez et al., 2011). It is believed that diverting the first share of roof runoff is enough to reduce the microbiological concentration, helping to eliminate potential pathogens from the water used for consumption. However, few studies have evaluated the presence of viruses in RHRW (Hamilton et al., 2019).

Viruses are of particular interest to monitor the water quality since they are, in most cases, more resistant to unfavorable environmental conditions than bacteria (Adefisoye et al., 2016; Rames et al. 2016; Chidamba and Korsten, 2018). In the last years, the use of next-generation sequencing and shotgun metagenomic sequencing has revolutionized and expanded the horizon of viral studies in water and wastewater, since those viruses can be detected quickly and accurately (Fernandez-Cassi et al., 2016; Liu et al. 2021; McCall et al., 2020).

This study aimed to characterize the virome to assess the taxonomic diversity of viruses found in RHRW of a low-income region with a lack of sanitation. To our knowledge, this is the first study using a shotgun

metagenomic sequencing approach and standardized roofs to characterize the viral community in the RHRW.

2. Materials and methods

2.1. Study area

The study area is the Fiocruz *campus*, located in the Manguinhos neighborhood in northern Rio de Janeiro city, Brazil. The institutional *campus* is an 850 m² green spot with an activated sludge sewage treatment plant (STP) at an average distance of 350 m from the sampling points and surrounded by 16 slums (Fig. 1).

The neighborhood ranks among the lowest positions in the Human Development Index – 122nd of 126 districts – in Rio de Janeiro City (Magalhães et al. 2011), suffering from severe social and environmental issues such as lack of sanitation (Handam et al., 2018), thus allowing the circulation of microbiological hazards in the population.

2.2. Experimental design and sampling

Three 5 m² standardized metal-sheet roofs were allocated at different points of the *campus* to obtain more representative samples of the studied area. A 50-l sampler capable of collecting 10 mm of rainfall (5 m² \times 10 mm = 50 L) split into three chambers was installed in each sampling point. The first chamber stores water from the first three millimeters of each rainfall event (15 L). As it fills up, the collected rainwater passes through it and fills up the second chamber (15 L) with the following three millimeters (up to the sixth millimeter). Finally, the last compartment (20L) collects the final millimeters. An overflow tube discharges what exceeds ten millimeters of rainfall (Fig. 2).

The sampling process has followed an established flowchart (Fig. 3) to avoid the sediment build-up process inside the system and the consequent microbiological contamination bias via bacterial growth and contaminated sediment resuspension from the bottom of the chambers.

From January to April 2019, 54 samples were freshly collected (less than 24 h after precipitation) from six rainfall events measuring more than 10 mm. As soon as the samples arrived in the lab, they were concentrated and stored at $-80\,^{\circ}\text{C}$.

The concentrated samples were grouped into three pooled samples according to rainfall volume. The first pool was composed of 40-µl aliquots of the first chambers' samples from each roof sampler (1st to 3rd mm of each rainfall event). The second pool was composed of 40-µl from the concentrated sample of the subsequent chambers (4th to 6th mm of each rainfall event), and so on, up to the tenth millimeter.

2.3. Virus concentration

The flocculation method based on viruses' adsorption to preflocculated skimmed milk proteins, as described by Calgua et al. (2013), was applied with minor modifications to concentrate the RHRW samples. The samples were spiked with 9.2×10^{10} Genome Copies (GC)/µL of PP7 bacteriophage (ATCC TM 15692-B4) as the internal control process (Rajal et al., 2007).

Water sample's electrical conductivity (EC) was first adjusted to 1.5 mS by adding sea salts (Sigma–Aldrich Chemie GMBH, Steinheim, Germany). The pH was adjusted to 3.5 by adding 1 N HCl. One hundred mL of 1% (w/v) pre-flocculated skimmed milk solution (PSM), pH 3.5 (Sigma-Aldrich Chemie GMBH, Steinheim, Germany) was added to each sample. The samples were stirred for eight hours at room

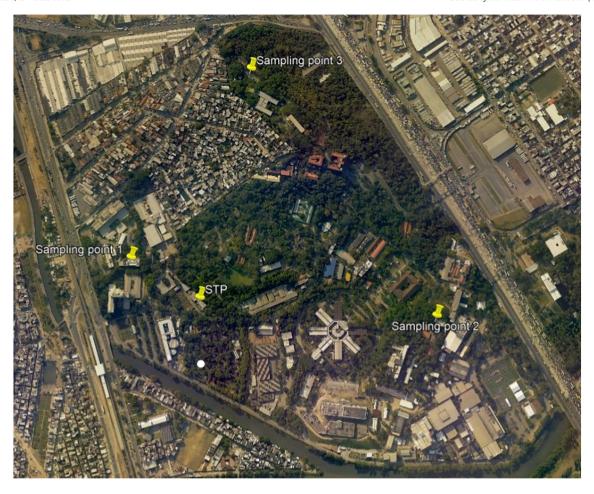


Fig. 1. Study area and respective sampling points (1–3) Source. Google Earth, modified by the authors.

temperature to allow the absorption of viruses to skimmed milk flocs. The flocs were then allowed to gravity settle overnight. The supernatant was removed using a peristaltic pump without disturbing the flocs. The remaining liquid and flocs (approximately 500 mL) were centrifuged at 7,000 xg for 30 min at 4 °C. The supernatant was carefully removed, and the pellet was resuspended in a 10 mL of phosphate buffer (1:2 v/v of Na₂HPO₄ 0.2 M) at pH 7.5. The concentrated sample was homogenized by vortexing, and an aliquot of 2 mL was prepared and stored at -80 °C for further virus analysis.

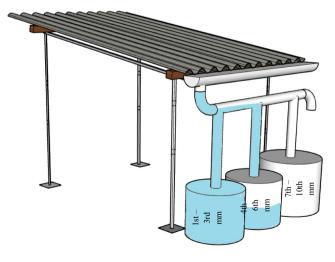


Fig. 2. Sampler functioning schematic sketching.

2.4. Nucleic acid extraction, reverse transcription, preparation of genomic libraries, and sequencing

Before the nucleic acid extraction procedure, samples were prepared according to the protocol described by Fernandez-Cassi et al. (2018) with minor modifications. Briefly, 140 µL of the viral concentrates were extracted using the QIAamp® Viral RNA Mini kit (QIAGEN, CA, USA) in a QIAcube® automated system (QIAGEN) without the addition of RNA carrier. PCR for detecting DNA and RNA genomes was performed using protocols previously described (Wang et al., 2003; Fernandez-Cassi et al., 2018). PCR products were purified and concentrated to a volume of 50 µL using the Agencourt AMPure XP PCR purification kit (Beckman Coulter, CA, USA). Negative controls (DNase/RNase free water) were included in all stages.

Amplicons purified were quantified using Qubit 2.0 and DNA libraries were generated using a Nextera XT DNA Preparation Kit (Illumina, San Diego, CA, USA). The size distribution of the libraries was evaluated using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA), and DNA High Sensitivity quantification was obtained using a Qubit 4.0 Fluorometer. Paired-end sequencing (2×150 bp) was performed on the NextSeq platform (Illumina, San Diego, CA, USA) at SENAI CETIQT's Facility (SENAI Innovation Institute for Biosynthetics, Technology Center and Textile Industry, Rio de Janeiro, RJ, Brazil).

2.5. Bioinformatics and data analysis

The reads obtained in the FASTQ format were generated by Illumina's BaseSpace pipeline (https://basespace.illumina.com). Filtering of low-quality reads and removal of the adapters were carried out

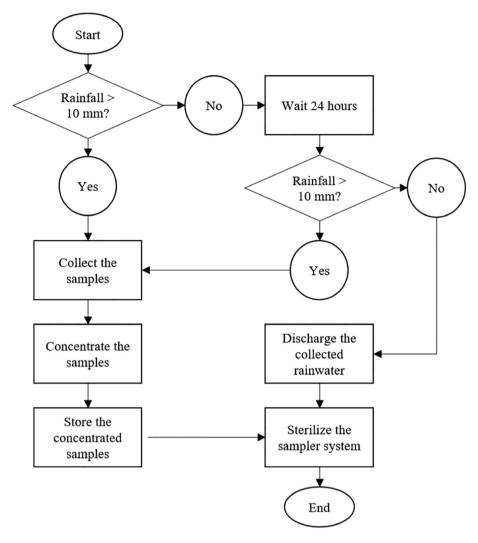


Fig. 3. Sampling process flowchart.

using PRINSEQ v. 0.20.4 (Schmieder and Edwards, 2011). Megahit v. 1.2.8 (Li et al., 2016) was used to assemble reads into contigs and viral contigs were selected using VirSorter v. 2.1 (Guo et al., 2021). DIAMOND v. 0.9.31 (Buchfink et al., 2021) was used on these filtered viral contigs to search for other similar sequences against all curated viral proteins available at UniProt (The UniProt Consortium 2021). Families were manually assigned for each species identified according to the NCBI standardized Taxonomy database. Sequences analyzed for taxonomic analysis should express an e-value of 10⁻⁵ and a minimum of 100 bp in alignment length. All raw reads are deposited at NCBI under the BioProject ID PRJNA767339.

3. Results

For virome analysis, more than 28,000,000 raw reads for each library were used for contig assembly and filtering for viral sequences (Table 1).

Fifty-seven viral species were detected, being RNA viruses (44 [77%]) predominates over DNA viruses (13 [23%]), with emphasis on RNA plant viruses (Tables S1 and S2 – Supplementary material). Fig. 4 presents the percentage of viral hosts observed in the analyzed samples, with a predominance of plant viruses, followed by insects, humans, and animals with the same percentage, and fungi viruses, respectively. One algae virus (*Paramecium bursaria Chlorella virus* 1) belonging to the *Phycodnaviridae* family was also detected (Fig. 4).

Twenty-nine viruses were observed belonging to the *Dicistroviridae* family presenting the highest number of reads, followed by *Iflaviridae*

as the second most detected viral family regardless of the studied millimeter. The percentage of viral hits represented by the *Dicistroviridae* family corresponded to 55.3% of the total viral hits found, and for *Iflaviridae*, the percentage was 10.6% in RHRW samples. The *Nodaviridae* family was the third most prevalent, representing 9.5% of the total viral hits identified (Fig. 5).

Plant viruses containing RNA genomes were the fifth most abundant family in RHRW samples, been *Secoviridae*, *Tombusviridae*, and *Virgaviridae* the most representative, with percentages varying from 2.1 to 5.3% of the total viral hits detected (Fig. 5). Regarding DNA plant viruses, the *Geminiviridae* (ssDNA circular) family was also detected in a relatively high frequency (4.7% of total viral hits). *Pepper mild mottle virus* (PMMoV), which is considered an important fecal viral indicator, was found in the first rainwater volume (Fig. 5, Table S1 – Supplementary material).

Table 1Roof-harvested rainwater samples according to rainfall heigh used to construct metagenomic libraries and an overview of the total number of contigs for each library.

Pool	Rainfall height (mm)	No. of raw reads	No. of reads after quality control	No. of viral contigsa
1	1-3	10,115,882	9,165,953	82
2	4-6	9,228,818	8,542,348	42
3	7–10	8,672,188	8,063,599	64

^a The number of contigs includes all viral contigs analyzed by Virsorter (i.e. contigs larger than 100 nt with homologies identified in the database).

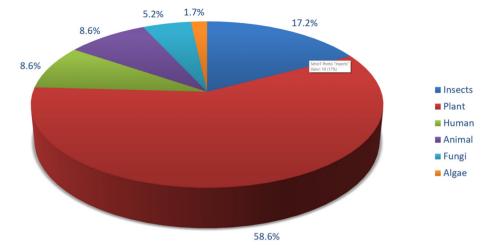


Fig. 4. Diversity of viral hosts (%) in the total RHRW samples collected.

Among animal and human viruses, the *Circoviridae* family (circular, covalently closed, single-stranded DNA (ssDNA) genomes) was the most detected DNA virus – 9.5% of the total DNA contigs analyzed – represented by human associated cyclovirus 1 (isolate *Homo sapiens/* Pakistan/PK5510/2007) present in all analyzed samples. Likewise, porcine circovirus 2 and canary circovirus species were detected in libraries 1 and 3, respectively (Table S2 – Supplementary material). The *Picornaviridae* family (RNA viruses) corresponded to 4.2% of the total viral hits detected, with the human hepatitis A virus genotype IB detected in the first library (1st to 3rd mm) and in the third one (7th to 10th mm). Theiler's murine encephalomyelitis virus (strain GDVII) was also detected in the 7–10 mm of rainwater collected. The human viruses and the number of contigs detected are indicated in Table 2.

Other human and animal viruses belonging to the *Picornaviridae* family (human klassevirus 1, human rhinovirus 3, and avian encephalomyelitis virus, strain 252 L2Z) were detected only in the first library (1st mm) analyzed. Other viral families were present in relatively low abundance (\leq 2.0%) of the total viral hits found (Fig. 5, Tables S1 and S2 – Supplementary material).

Bacteriophages belonging to the *Microviridae* (linear ssDNA), *Siphoviridae* (linear dsDNA), *Myoviridae* (dsDNA), and *Inoviridae* (ssDNA) families were the second most detected DNA viruses in RHRW samples, with percentages from 5.3, 3.7, 2.1, and 2.1% of the total viral hits present in these samples, respectively (Fig. 5, Table 3). PP7 bacteriophage (*Leviviridae* family), which was used as an internal control of concentration and detection methods used, was detected in all samples analyzed (Fig. 5).

4. Discussion

Metagenomic analysis was used in this study for a prior assessment of the viral diversity contained in RHRW collected under different rainfall volumes in a low-income area in Brazil. Results demonstrated that there is great viral diversity in RHRW from this area, which we observed some important contigs of human and animal viruses of public health concerns, such as hepatitis A virus (HAV), human klassevirus 1, human rhinovirus 3, avian encephalomyelitis virus (strain 252 L2Z), and Australian bat lyssavirus (isolate Bat/AUS/1996) found in the different libraries. Despite the difference in the number of viral contigs observed according to the different libraries representing the rainfall volumes, viruses were found up to the third pool, demonstrating that the disposal of the first millimeters of collected rainfall alone is not enough to eliminate potential viral pathogens. Detection of important viral human pathogens, such as HAV in the last sampler's compartment (7–10 mm) corroborated our previous study that detected human viral pathogens up to the tenth millimeter in RHRW (Shubo et al., 2021). The occurrence of bacteriophages that infect *Enterobacteriacea* family in almost all samples also suggests the presence of potentially pathogenic bacterial in those millimeters.

Proper maintenance, including regular cleaning of roof and gutters, and pruning of overhanging tree branches can reduce the roof-surface microbiological contamination by the roof wash-off (Ahmed et al., 2012b). However, to eliminate potential pathogenic viruses in RHRW some pre-treatment such as filtration followed by an adsorption step on granular activated carbon and UV disinfection has been suggested for human uses (Naddeo et al., 2013).

The presence of human and animal viruses in these samples may be related to the passive carrying of viruses by animals (birds, pigeons, rodents, arthropods, such as arachnids or insects) that inhabit the sampling site and the surrounding areas. Viruses adhered to sewage drops and garbage particles attached to the animals' bodies could be released onto the roof surface and dragged into the sampler by the roof runoff (Hamilton et al., 2019; Shubo et al., 2021). Similar to these findings, other studies pointed out that microorganisms detected in rainwater were assumed to be released from birds and small mammals that live around suburban areas (Ahmed et al., 2012b; Ahmed et al., 2012a; Chubaka et al., 2018). Likewise, fecal matter can also be carried with the dust and windstorms and be deposited on catchment areas, and get discharged into harvested rainwater (Chubaka et al., 2018). Airborne viruses could also contribute to viral diversity in rainwater being an interesting field of research for future studies on the subject (Whon et al., 2012; Prussin et al., 2014).

Regarding human viruses detection, HAV genotype IB occurrence in RHRW samples reflected the circulation of these viruses in the city of Rio de Janeiro, as demonstrated by the increase in cases reported in recent years, even after the introduction of the vaccine in 2014 (De Oliveira et al., 2020). In the same region, previous studies in STPs have demonstrated a high HAV genome concentration in raw sewage samples (Villar et al., 2007; Prado et al., 2012).

The detection of emerging human klassevirus in RHRW also corroborated our previous findings on this virus' occurrence in the surrounding area. Klassevirus strains were detected in river water samples contaminated with sewage close to the campus where this experiment was conducted (Calgua et al., 2013). Klassevirus was first described by metagenomic sequencing of a novel picornavirus in pediatric stool samples in the United States been associated with gastroenteric disease (Greninger et al., 2009; Bibby et al., 2019).

Despite the importance of enteric and respiratory viruses detected, the presence of a genomic sequence suggestive of rabies virus (Australian bat lyssavirus) needs particular attention. Rabies is considered a re-emerging zoonotic disease in different countries of the world and is associated with increased rates of reservoir contact (Johnson

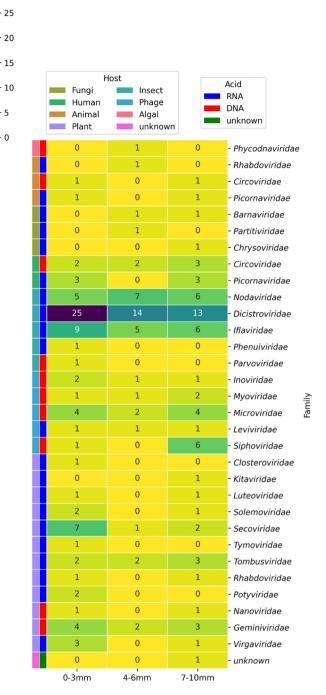


Fig. 5. Heatmap profile showing the relative abundance of viral families detected over the three different RHRW volume samples. Each cell contains the number of contigs with at least a sequence with a positive Uniprot hit that passed all the selection criteria. Data spanned from yellow (low relative abundance) to purple (high relative abundance), as illustrated by the color scale.

et al., 2014). The disease is transmitted by the bite of a rabid animal; usually dogs; although bats act as a reservoir for lyssaviruses in many regions of the world, including Brazil (Johnson et al., 2014; Uieda et al., 1995; de Almeida et al., 2011; de Almeida et al., 2020). Rabies virus is transmitted by contact with the bite or saliva of infected bats, but the presence of the virus in reused water gives rise to a warning for the correct handling and disinfection to avoid risks of transmission through this route, mainly when different scenarios for reuse are considered.

Data obtained through the shotgun metagenomic approach warn regarding rainwater-related public health risks. Such information can guide further assessments on targeted-specific pathogens by using molecular methods such as qPCR, including tests for viral viability to measure potential health risks from using this alternative water source for domestic supply.

This approach also revealed that other viruses and pathogens related to the local microbiota (plant, insect, and animal viruses) may be prevalent in RHRW, more likely to be observed than those found in sewage, such as potential indicator viruses, including human adenovirus (HAdV), JC polyomavirus (JCPyV), PMMoV and Craasphage (Bibby et al., 2019; Farkas et al., 2020). In this study, we also used a gPCR protocol (Hernroth et al., 2002) to detect HAdV in all samples collected (n = 54) with negative results, corroborating data generated by a metagenomic approach (data not shown). However, PMMoV (Virgaviridae family) was found in the first chamber (1-3 mm of rainwater volume). PMMoV has been associated with human wastewater and found in polluted surface water, groundwater, and drinking water (Rosario et al., 2009; Symonds et al., 2018; Bibby et al., 2019; Farkas et al., 2020; Bonanno Ferraro et al., 2021). The primary source of PMMoV in human excreta is through consumption of peppers (Capsicum spp.), food products containing peppers contaminated with the virus (Farkas et al., 2020), and also detected in animal fecal samples from chickens and seagulls (Rosario et al., 2009). This finding was interesting since some studies reported that PMMoV is stable under several environmental conditions, being a promising viral indicator (Rosario et al., 2009; Farkas et al., 2020; Bonanno Ferraro et al., 2021). Our data are in line with these reports, although further studies involving viral characterization in reuse water are necessary to confirm these findings.

Although concerns regarding reclaimed water use mainly focus on human pathogens, RHRW may also be a reservoir for non-human pathogens (Rosario et al., 2009), such as demonstrated by this study, where the prevalence of non-human viruses, RNA viruses, specifically belonging to *Dicistroviridae* and *Iflaviridae* families were observed.

Dicistroviridae infects arthropod hosts, with some devastating economic consequences, such as acute bee paralysis virus in domesticated honeybees (www.ictv.global/report/dicistroviridae). The surrounding area is a green spot hosting a myriad of insects, mainly Drosophilas and bees, which may explain the large number of viral contigs observed for these viral species (Cricket paralysis virus, Drosophila C virus, and Acute bee paralysis virus). Iflaviridae also infects arthropod hosts, mainly insects, such as Ectropis obliqua picorna-like virus detected in these RHRW samples (https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/iflaviridae).

Interestingly, a higher number of viral species was obtained for plant viruses. Although slums lacking sanitary infrastructure surround the

 Table 2

 Taxonomic classification of human viruses detected in roof-harvested rainwater metagenomic libraries.

Virus	Order	Family	Genus	Library*	No. of total contigs
Human associated cyclovirus 1	Cirlivirales	Circoviridae	Cyclovirus	1, 2, 3	18
Human hepatitis A virus genotype IB (isolate HM175)	Picornavirales	Picornaviridae	Hepatovirus	3	8
Human hepatitis A virus genotype IB (Euro-African isolate MBB)	Picornavirales	Picornaviridae	Hepatovirus	1	1
Human klassevirus 1	Picornavirales	Picornaviridae	Salivirus	1	1
Human rhinovirus 3	Picornavirales	Picornaviridae	Enterovirus	1	1

Table 3Bacteriophages families and their hosts present in RHRW samples.

Microviridae	Siphoviridae	Myoviridae	Inoviridae
Bdellovibrio phage phiMH2K (1,2,3) ^a Chlamydia phage 1 (3) ^a	Escherichia phage lambda (3) ^a Enterobacteria phage 82 (3) ^a Escherichia phage N15(3) ^a	Shigella phage sf (1) ^a Burkholderia phage (2) ^a Haemophilus phage HP1(3) ^a Escherichia phage P2(2,3) ^a	Enterobacteria phage M13(1,2,3) ^a Enterobacteria phage f1(1) ^a

^a Number in parenthesis: (1) 1st to 3rd mm; (2) 4th to 6th mm; (3) 7th to 10th mm.

sampling area, the roofs were allocated inside the institutional campus, which is densely vegetated by Atlantic forest species in addition to other introduced plant species. Branches and leaves that fell on the roofs could be washed off by the roof runoff and consequently dragged the detached particles into the samplers. These facts could explain the higher diversity of plant viruses over human and animal viruses.

5. Conclusions

This study identified the dominant DNA and RNA viral types in RHRW in a low-income area, thus making a significant contribution to current microbiological data regarding reuse water for communities with a lack of potable water and basic sanitation services.

Our data indicated that RHRW can be contaminated with important viral pathogens in densely urbanized areas and future studies should be conducted to evaluate social, health, and environmental hazards when considering this alternative water supply.

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CRediT authorship contribution statement

Tatiana Prado: Formal analysis, Writing – original draft. **Tatsuo Shubo:** Methodology, Investigation, Writing – original draft. **Lucas Freitas:** Formal analysis, Writing – original draft. **Luciana Leomil:** Resources. **Adriana Gonçalves Maranhão:** Data curation. **Marize Pereira Miagostovich:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150778.

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