HUMAN ADENOVIRUS IN MUNICIPAL SOLID WASTE LEACHATE AND QUANTITATIVE RISK ASSESSMENT OF GASTROINTESTINAL ILLNESS TO WASTE COLLECTORS

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Abstract

Leachate is a variable effluent from waste management systems generated during waste collection and on landfills. ~~This study aimed to evaluate the gastrointestinal (GI) illness risk of waste collectors exposed to human adenovirus (HAdV) contaminated leachate.~~ In this study, ~~T~~twenty-two leachate samples (1L each) from ~~a~~ waste collection trucks and a landfill were collected from March to December 2019 in the municipality of Rio de Janeiro (Brazil) ~~in a solid waste transfer station and a landfill~~ and they were analyzed for Human Adenovirus (HAdV), bacterial indicators, and various physico-chemical parameters. For viral analysis, ~~Samples~~ samples were concentrated by ultracentrifugation and processed for molecular analysis using QIAamp Fast DNA Stool mini kit® for DNA extraction followed by nested-PCR and qPCR/PMA-qPCR TaqMan® system. HAdV was detected by nested-PCR in 100% (9/9) and 83.33% (12/13) of the truck and landfill leachate samples, respectively. Viral concentrations ranged from 8.31×10^{1} to 6.68×10^{7} genomic copies per 100 ml by qPCR and PMA-qPCR. HAdV species A, B, C, and F were characterized using nucleotide sequencing. HAdV were isolated in A549 culture cells in

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100% (9/9) and 46.2% (6/13) from truck and landfill leachate samples, respectively. Regardless of the detection methods, HAdV concentration was predicted by the quantity of total suspended solids. ~~Analysis of~~A quantitative microbial risk assessment was performed to measure~~d~~ the probability of gastrointestinal (GI) illness attributable to inadvertent oral ingestion of truck leachate ~~(hand-to-mouth contact or direct splashing into the oral cavity)~~, revealing the higher probability of disease for the direct splashing ~~route exposure~~into the oral cavity (58%) than for the gloved hand-to-mouth (33%). In a scenario where waste collectors do not wear gloves as protective personal equipment, the risk increases to 67%. This is the first study ~~on~~ revealing infectious HAdV in solid waste leachate ~~that~~ and it indicates a potential health risk ~~of exposure of the~~for waste collectors ~~to GI illness~~.

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Keywords: leachate; municipal solid waste; human adenovirus; PMA-qPCR; cell culture; QMRA.

1. Introduction

Municipal solid waste (MSW) leachate is an effluent produced at different stages of waste management systems, such as landfills, collection trucks, and transfer stations. This effluent has variable ~~physical~~ chemical and biological characteristics that can be affected by MSW composition, landfill lifetime and structures, transfer stations operation mode as well as local weather conditions (Youcai 2019). MSW leachate has high values of organic matter, ammonia nitrogen, chloride, and dissolved solids (Costa et al. 2019). The ~~physical~~physico-chemical composition of MSW leachate landfill has been widely studied (Costa, Alfaia, and Campos 2019; Naveen et al. 2017), ~~however,~~but truck leachate are still unknown (Benyoucef et al. 2015). Concerning microbiological contamination, some

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studies have described the occurrence of bacteria in the leachate (Silva et al. 2011; Zhang et al. 2013), however, viruses were rarely consideredfew investigations report the virus detection, most of them related to methodologies of virus recovery from this matrix (Sobsey, Wallis, and Melnick 1974, 1975; Sobsey 1978; Costa, Alfaia, and Campos 2019). The first work that quantifiesd infectious HAdV in a landfill was described by Carducci et al. (2013), who investigated the presence of HAdV and Torque Teno Virus (TTV) in the air and on the surfaces of a landfill in Italy (Carducci, Federigi, & Verani, 2013). HAdV have been identified as potential viral indicator of human fecal contamination due to (1) stability, persistence and wide distribution in different environmental matrices; (2) detection in sewage without seasonality; (3) resistance to ultraviolet (UV) disinfection; (4) greater abundance in relation to other enteric viruses; (5) isolation in culture and molecular tools available; (6) viral specificity with the human host (Rames, Roiko, Stratton, & Macdonald, 2016). MSW handling implies exposure of waste collectors to chemical substances, dust, and microorganisms (Gorman Ng et al. 2012). An epidemiological report shows showed that MSW workers are were frequently vulnerable to health illnessissues, as respiratory and gastrointestinal (GI) diseases, especially in low-income countries (Cointreau, 2006)(WBG 2006).

Quantitative microbial risk assessment (QMRA) has been used to estimate the occupational health risk in working settings like wastewater treatment plants (Medema et al. 2004; Carducci et al. 2018; Yan, Leng, and Wu 2021; Zaneti et al. 2021) and agricultural areas that use reclaimed wastewater irrigation (Antwi-Agyei et al. 2016; Sampson et al. 2017; Kouamé et al. 2017) or fertilizer applications (Brooks et al. 2005; Brooks et al. 2012; Gerba et al. 2008; Tanner et al. 2008; Jahne et al. 2015). However, this approach is not widelyrarely used for risk assessment of MSW collection workers, with the analysis focused on bioaerosol inhalation (Akpeimeh et al. 2020). Accidental

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~~oral ingestion of microbes during waste collection is rarely addressed, except for monitoring studies on fungal and bacteria contamination on workers' hands (Madsen et al. 2016; Madsen et al. 2020).~~ To fill this gap on occupational ~~exposure~~ risk, ~~we studied~~workers ~~-~~exposure ~~scenarios from~~ through inadvertent ingestion of ~~collection~~ trucks leachate was investigated to estimate ~~and estimated~~ HAdV GI illness risk. In this study, trucks and landfill leachate samples were ~~characterized by~~analyzed for bacterial and physico~~al~~-chemical parameters ~~and bacterial findings presence~~ and ~~investigated~~ for HAdV occurrence, concentration, genetic diversity, and infectivity.

2. Materials and Methods

2.1 Study area and samples

Samples were collected in two stages from the waste management system in the municipality of Rio de Janeiro, Brazil: fresh leachate from waste collection trucks at a transfer station and raw leachate at an operational landfill. The transfer station serves 11 neighborhoods, comprehending 900 thousand inhabitants. The regional landfill serves 6.9 million inhabitants, receiving around ten thousand tons per day of MSW.

~~One~~ One-liter samples were collected from March to December 2019. Nine samples of fresh leachate were collected from collection truck basins, each sample corresponding to a pool of three to five trucks. Thirteen raw leachate samples were collected from a tank at the entrance of the landfill leachate treatment plant. All samples were collected in sterile polyethylene bottles, transported to the laboratory at 4 °C, and processed within 24 h.

2.2 Virological Methods

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2.2.1 *Viral concentration*

Viral concentration was performed by ultracentrifugation at 100.000 x *g* for 1 h at 4ºC using a Sorvall® WX Ultra Centrifuge Series (Thermo Scientific, Waltham, MA, USA). Pellet was resuspended on ice for 30 min with glycine buffer and then centrifuged 12.000 x *g* for 15 min. Supernatant was centrifuged at 100.000 x *g* for 1 h at 4ºC and pellet resuspended at 500 µL PBS according to ~~a~~ previous studies~~)~~ (Pina et al. 1998, ~~(~~Lanzarini et al., 2020)~~)~~ and stored at -80 °C until nucleic acid extraction.

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2.2.2 *DNA extraction*

Nucleic acids were extracted using the QIAamp Fast DNA Stool Mini Kit® (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions and a previous study (Lanzarini et al., 2020). ~~Positive~~ A clinical fecal sample was used as a HAdV positive control. ~~and negative controls were processed~~. The UltraPure™ DNase/RNase-Free (Invitrogen, Carlsbad, CA, USA) distilled water was used as the negative control.

2.2.3 *Qualitative PCR*

A semi-nested PCR was performed, using primers previously described to obtain 245 base pairs (bp) fragments (Allard, Albinsson, and Wadell 2001), and the Platinum® Taq DNA Polymerase enzyme was used according to the manufacturer's recommendations (Invitrogen, Carlsbad, California, USA).

2.2.4 *Real-Time PCR (qPCR) and PMA-qPCR*

For PMA-qPCR, 200µl of each concentrated was inoculated with PMAxxx™ (Biotium Inc., Freemont, CA, USA) at a final concentration of 50 µM and incubated in a dark room for 10 minutes under 200 rpm at 25 °C. The samples were incubated for 15 minutes in the PMA-Lite™ LED Photolysis Device (Biotium Inc., Hayward, CA, USA) for photolysis (Fongaro et al. 2016). DNA extractions and qPCR reactions were processed simultaneously for leachate samples treated or not with PMA.

148 HAdV hexon gene was amplified using a set of primers and probe for TaqMan® system
149 protocol previously described (Hernroth et al. 2002). Each 15 µl qPCR contained 12.5 µl
150 of 2 x TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA,
151 USA), 1 µl of each 22.5 µM primer (0.9 µM final concentration each), and 0.5 µl of probe
152 (0.225 µM final concentration). Reactions were performed on ABI PRISM 7500 (Applied
153 Biosystems, Foster City, CA, USA) according to the following cycle: 2 min at 50 °C, 10
154 min at 95 °C, and 40 cycles of 15 s at 95 °C, 1 min at 60 °C. Synthetic DNA standard
155 curves were designed using specific viral gBlock Gene Fragments (Integrated DNA
156 Technologies™, Coralville, Iowa, USA) with a ten-fold serial dilution (10^7–10^0 genome
157 copies (GC)/reaction) containing the same HAdV target sequence.
158 Truck and landfill leachate were tested in duplicate using undiluted and diluted samples
159 (10^{-1} and 10^{-2}), totalizing six reactions per sample. All qPCR included non-template
160 control (NTC), positive control, and DNAse/RNAse-free water as a negative control. The
161 viral load obtained, corrected~~ing~~ for the dilution analyzed, was expressed as the number
162 of GC per 100 ml. The limit of detection for this protocol was 2.0×10^3 GC 100 ml^{-1} and
163 samples with Ct ≤ 38 were considered positive.
164 *2.2.5 Nucleotide sequencing (Sanger method)*
165 PCR product was purified from electrophoresis gel using Wizard® SV Gel and PCR
166 Clean-Up System (Promega, Madison, MI, USA). Sequences were obtained using the Big
167 Dye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3730 Genetic Analyzer
168 48 capillary sequencer (Applied Biosystems, Foster City, CA, USA) from the
169 "PDTIS/FIOCRUZ DNA Sequencing Platform", and analyzed using MEGA X® software
170 (Kumar et al. 2018) for editing, sequence analysis, and molecular characterization. The
171 sequences were compared with reference prototypes deposited at the NCBI GenBank
172 (https://www.ncbi.nlm.nih.gov/). Phylogenetic dendrograms were obtained using the

Maximum Likelihood method, employing genetic distance corrected by the Tamura 3 parameter model and Gamma Distributed (G) with 2000 bootstraps above 70% (Tamura 1992).

2.2.6 HAdV infectivity assay in A549 cells

The human lung epithelial carcinoma A549 cell line (ATCC CCL-185) was used for virus isolation and titration onto 96-well polystyrene microtiter plates. Each leachate sample was tenfold diluted (10^{-1} to 10^{-4}) in Eagle's minimum essential medium (MEM) supplemented with a 10% L-glutamine and 0.125% gentamycin. Each dilution was seeded into five wells, containing 75 µl of the sample, 75 µl of MEM supplemented with a 0.125% gentamycin, 0.1% HEPES buffer, and 50 µl of A549 cell (10^6 cells ml^{-1}). Plates were covered and incubated at 37 °C under 5% CO_2 for 5 days. Examination for cytopathic effects was performed with inverted light microscopy. The highest dilution producing a cytopathic effect in 50% of the inoculated cells was determined using the Spearman-Karber formula (Ramakrishnan 2016) and expressed as $TCID_{50}$ ml^{-1} (50% tissue culture infective dose per milliliter). The limit of detection was $10^{1.12}$ $TCID_{50}$ ml^{-1}.

2.3 Bacteriological and physico-chemical parameters~~physical-chemical findings~~

Total coliforms and *Escherichia coli* (*E. coli*) from the fresh truck and landfill leachate samples were quantified using Colilert® Quanti-Tray®/2000 (IDEXX Laboratories, Inc, Westbrook, ME, USA), and results were expressed by Most Probable Number (MPN) per 100 ml. Samples were tested using ten-fold dilutions previously standardized, truck leachate ranging from 10^{-9} to 10^{-13} and landfill from 10^{-1} to 10^{-3}.

The following physico-chemical parameters ~~physical-chemical~~ were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA 2017): pH, color, turbidity, total alkalinity, conductivity, total hardness, solids, carbon, chemical

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oxygen demand (COD), UV 254 nm, chloride, phosphorus~~phosphor~~, ammonia, and total nitrogen.

2.4 QMRA

QMRA inputs are the environmental concentration of pathogens and the amount of exposure to a matrix (dose) to estimate the associated probability (risk) of an adverse outcome (illness), using pathogen-specific functions describing the dose-response relationship. QMRA involves the description of the exposure scenario, the exposure assessment, the dose-response model, and the risk characterization (Haas, Rose, and Gerba 2014).

2.4.3 Selection of the exposure scenario

During a working day, waste collectors ~~are~~ were engaged in manually door-to-door waste collection, filling of trucks, and driving of compactor systems, where they ~~are~~ were exposed to leachate. ~~We assume landfill~~ Landfill workers ~~as~~ were assumed as not exposed to leachate since ~~this~~ such effluent ~~flows~~ flowed into a dedicated treatment facility without human contact (Lanzarini et al. 2020). Accidental oral ingestion of truck leachate was modelled for workers that did not adopt ~~We modeled the scenario of occupational exposure to truck leachate, which can be orally ingested purely by accident. We considered the exposure scenario where the workers do not wear~~ protective masks as before the COVID-19 pandemic, but ~~they do wear~~ wearing a specific type of gloves for prevention of accidents with sharp objects, manufactured by a mixture of synthetic fibers and natural fibers (usually latex). ~~Considering~~ In a hypothetical scenario of difficulty in adhering to the use of gloves by waste collectors, ~~we also~~ the health risk without such

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protective personal equipment (PPE) was also calculated. ~~included a scenario of risk analysis when gloves are not worn~~.

2.4.4 Exposure assessment

~~To estimate the dose,~~ The dose was estimated using ~~we consider~~ the conceptual compartmental model developed by the Institute for Occupational Medicine (Cherrie et al. 2006; HSE 2007) for inadvertent ingestion of liquid chemical substances and dust in occupational settings, adapted to our specific exposure scenario. Oral exposure of waste collectors relied on two possible mechanisms for transferring HAdV to the oral cavity: contact of contaminated hands with the skin around the mouth (hand-to-mouth contact) and direct contamination of truck leachate into the mouth or the peri-oral area (splashing route). In both cases, ~~we derived~~ HAdV concentration (C_{HAdV}) was derived from the truck leachate monitoring data, considering the infective viral load results expressed in $TCID_{50}$ ml^{-1}. Since the number of samples (9) was insufficient to establish a theoretically fitted distribution, ~~we created~~ an empirical uniform distribution was drawn considering ~~based on~~ the lower and upper bound of the experimental measurements, following an approach well established in QMRA literature ~~(~~Rasheduzzaman et al. 2019).

Oral ingestion by hand-to-mouth contact. Waste collectors can contaminate gloved hands by ~~...Waste collectors can have their gloved hands contaminate by~~ touching truck leachate accumulated on the surface of the vehicles, which ~~have been~~were assumed to be heavily polluted with a leachate load of 1 drop per cm^2 ($LL_{surface}$) (Shatkin, Smith, and Moyer 2005). ~~To estimate the exposure dose, we considered~~ The input parameters used in the estimation of exposure dose were ~~T~~the contact area between the hand and the oral compartment ($SA_{hand\text{-}mouth}$) ~~was considered to estimate the exposure dose~~, the viral transfer efficiencies from surfaces-to-hands ($TE_{surface\text{-}hand}$)~~,~~ and from hand-to-mouth

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245 ($TE_{hand\text{-}mouth}$), and hourly frequency of hand-to-mouth contact ($f_{contact}$), as reported in
246 Equation 1. To understand the role of gloves on the ingested dose, ~~we used~~ different
247 values for $TE_{surface\text{-}hand}$, $TE_{hand\text{-}mouth}$, and $f_{contact}$ were used on the basis of the adoption of
248 such PPE~~based on the use of protective personal equipment (PPE)~~.

249 *Workers without gloves*. ~~We chose a~~ $TE_{surface\text{-}hand}$ was derived from a study on viral
250 transfer from hard nonporous surfaces to bare hand in a condition of high air relative
251 humidity (a Brazilian climate feature) since transfer efficiency of organisms is improved
252 under a high relative humidity of 40% to 65%, especially for viruses (Lopez et al. 2013).
253 For $TE_{hand\text{-}mouth}$, ~~we used~~ data from a study investigating viral transfer from contaminated
254 bare ~~hand~~ hand-~~to~~-to-mouth was used (34%, Rusin, Maxwell, and Gerba 2002)~~, which~~
255 ~~reported a value of 34%~~. ~~For $f_{contact}$, we adopted the~~ The $f_{contact}$~~contact frequency~~ was
256 derived from ~~of~~ workers employed in manufacturing and engineering sectors, who can
257 perform manual work~~, but~~ without wearing gloves (Zainudin 2004; Cherrie et al. 2006)

258 *Workers wearing gloves*. ~~For~~ The $TE_{surface\text{-}hand}$~~, we~~ was selected from the viral transfer
259 percentage to latex-gloved hands from a nonporous hard surface~~,~~ contaminated with a
260 "wet" inoculum, to obtain a conservative estimation of health risk (Sharps, Kotwal, and
261 Cannon 2012). In the absence of information on $TE_{hand\text{-}mouth}$ for gloved hands, ~~we adjusted~~
262 the data on bare hands to mouth (Rusin, Maxwell, and Gerba 2002) were adjusted with
263 results from studies on food production, which demonstrated that latex gloves ~~are~~ were
264 10% less efficient to transfer viruses than the bare hands~~, by a 10%~~ (Tuladhar et al. 2013;
265 Rönnqvist et al. 2014). The $f_{contact}$ was very low among workers wearing gloves, based on
266 laboratory and pesticide workers' behavior (Zainudin 2004; Cherrie et al. 2006).

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Apart from PPE adoption, exposure from each hand-to-mouth contact was considered independent of contact duration, as in other QMRA studies modeling non-dietary exposure to pathogens (Mattioli, Davis, and Boehm 2015).

$$dose_{hand\text{-}to\text{-}mouth}=C_{HAdV}*LL_{surface}*TE_{surface/hand}*SA_{hand\text{-}mouth}*TE_{hand/mouth}*f_{contact}*n_{hours} \quad \text{(eq. 1)}$$

where $dose_{hand\text{-}to\text{-}mouth}$ is the HAdV dose transferred to the oral cavity as a result of hand-to-mouth mechanisms during a working day [Number of HAdV in $TCID_{50}$], and each input variable is explained in Table S1.

Oral ingestion by splashing route. Workers can directly ingest truck leachate during the operation of waste compaction by each vehicle. The number of compacting events during a working day can vary since waste collection in densely populated city areas could imply dozens of compacting activities. Although the button to activate compactors is on the side of the truck to prevent accidents, workers are still exposed to dust and drops of truck leachate, which can splash onto the oral and peri-oral compartments of waste collectors who stand near the compactor. During a working day, ~~We assume that during a working day, the~~ a worker ~~can be~~was assumed to be exposed to 1 to 50 drops based on the professional judgment of researchers of the field and ~~after a discussion with the~~ Municipal Waste Company interview. ~~We modeled t~~Therefore, the ~~exposure~~ volume of truck leachate accidentally ingested by splashing varied from 0.01 ml to 0.5 ml ($V_{leachate}$), ~~since 10 µL droplets are considered~~because large aerosol liquid particles of 10 µL spread at a short distance from a source (Sattar, Ijaz, and Gerba 1987). The HAdV dose accidentally ingested by direct splashing in the oral cavity was computed according to Equation 2.

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$$dose_{splashing} = C_{HAdV} * V_{leachate} \quad \text{(eq. 2)}$$

where $dose_{splashing}$ is the HAdV dose transferred in the oral cavity as a result of splashing mechanisms during a working day [Number of HAdV in $TCID_{50}$] and each input variable is explained in Table S1.

2.4.5 Dose-response model

Dose-response models are mathematical functions obtained from clinical trials or outbreak data to describe the relationship between a specific pathogens and hosts on the basis of the transmission routes (contact, ingestion, inhalation) (**Federigi et al. 2019**).

In this study, the illness was chosen as the endpoint of the dose-response model, which was ~~We used a dose-response model that provided GI illness as the endpoint and chose~~ the hypergeometric ~~dose-response~~ equation developed by (Teunis, Schijven, and Rutjes 2016), with optimized model parameters values for oral ingestion, obtained from clinical trials on inoculation of adenovirus (AdV4, AdV7). The probability of infection (P_{inf}) was computed by the formula:

$$P_{inf}(dose; \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -dose)$$

where α and β represent infection parameters specific for HAdV ($\alpha = 5.11$, $\beta = 2.80$) and ${}_1F_1$ ~~represents~~ is the confluent hypergeometric function of the first kind.

The probability of illness (P_{ill}) ~~for each pathogen~~ was estimated by multiplying the P_{inf} and the pathogenicity, which represents the probabilities of developing illness given the infection, calculated as:

$$P_{ill|inf}(dose \mid \eta, r) = 1 - (1 + dose/\eta)^{-r}$$

where r and η are the illness parameters for HAdV ($\eta = 6.53$, $r = 0.41$).

2.4.6 Risk characterization

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A Monte Carlo analysis was run for 200 simulations, each one of 10.000 interactions (Vensim package, Ventana Systems, Inc., Harvard, MA, USA). The result of the Monte Carlo ~~can be observed as~~was the daily probability of illness ~~and~~ based on 200 measures to improve the accuracy of the health risk.

A sensitivity analysis ~~has been~~was carried out to test the relative importance of the stochastic variables on models' results: C_{HAdV}, $f_{contact}$, $T_{Esurface/hand}$ for hand-to-mouth, and C_{HAdV} and $V_{leachate}$ for splashing mechanism. To determine the effect of these variables on the final risk estimate, their value was varied, one at a time, while keeping each of the other input parameters constant or fixed at their average value of its probability distribution function.

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2.5 Statistical analysis

Microbiological data were log-transformed and analyzed using *GraphPad Prism* version 8.0.1. Samples below the limit of detection received half of this value for statistical analysis (Bucardo et al. 2011).

Fisher exact test was used to compare HAdV detection methods, and an unpaired t-test to compare HAdV, total coliforms, and *E. coli* concentrations between truck and landfill leachate. Spearman correlation and regression analysis were performed to investigate the correlation between HAdV infectivity, qPCR, and PMA-qPCR. Mann-Whitney test was used to compare the physico-chemical parameters ~~physical-chemical findings~~ between truck and landfill leachate. The results were considered significant when P values were below 0.05.

A multiple regression model was performed using each microbial variable (total coliforms, *E. coli*, and HAdV) once at a time, as the dependent variable and the physico-

chemical parameters ~~physical-chemical findings~~ as predictors (independent variables). The physico-chemical parameters ~~physical-chemical findings~~ were examined for collinearity based on Pearson's r correlation and those with low collinearity with each other (- 0.5 < r < 0.5) were selected as predictors (Zuur, Ieno, and Elphick 2010). The best model was selected with Akaike Information Criterion (AIC) (Fox and Weisberg 2011) and the analysis was carried out in R-Language using the *car* and *effects* packages (Team 2018).

The exposure dose and the probability of GI illness were described in terms of the interquartile range (IQR).

3 Results

3.1 Viral and bacteriological ~~findings~~ parameters

Commentato [IF4]: To be in line with reviewer #2 suggestion on chemical paramters

HAdV were detected in 100% (9) of truck leachate samples regardless of the detection method used (semi-nested PCR, qPCR, PMA-qPCR, cell culture) and in 92% (12) of landfill samples, with variable detection percentage according to the methodology (Table 1). No statistically significant difference was observed between cell culture detection with qPCR and PMA-qPCR in landfill leachate. Semi-nested PCR showed a significant difference when compared to cell culture (p=0.03).

Table 1. Human adenovirus (HAdV), total coliforms, and *Escherichia coli* (*E. coli*) findings from truck and landfill leachate samples according to the methodology used.

Microorganisms	Method	Leachate origin N. of positive (%)		Minimum- Maximum	
		Truck (n=9)	Landfill (n=13)	Truck	Landfill
HAdV	Semi-nested-PCR	9 (100)	12 (92)	-	-
	qPCR*	9 (100)	11 (85)	$2.07\times10^{4} – 6.68\times10^{7}$	$8.31\times10^{1} – 1.02\times10^{7}$

	PMA-qPCR*	9 (100)	10 (77)	3.25×10^{3} – 4.06×10^{7}	4.00×10^{2} – 2.53×10^{4}
	Infectivity assay** (A549 cell)	9 (100)	6 (46)	1.70×10^{3} – 6.67×10^{4}	1.70×10^{3} – 1.06×10^{4}
Total coliforms	Colilert® Quanti-Tray®***	9 (100)	12 (92)	1.00×10^{10} – 6.05×10^{14}	2.00×10^{3} – 9.00×10^{5}
E. coli	Colilert® Quanti-Tray®***	7 (78)	8 (62)	1.18×10^{11} – 1.30×10^{13}	1.00×10^{1} – 5.00×10^{4}

*GC 100 ml^{-1}
**$TCID_{50}$ 100 ml^{-1}
***MPN 100 ml^{-1}

Regarding the viral and bacteriological concentration, a higher result in samples of fresh leachate (truck) was observed when compared to landfill samples with statistically significant differences observed for HAdV qPCR (p=0.0031), PMA-qPCR (p<0.0001), total coliforms (p<0.0001), and *E. coli* (p<0.0001). No significant differences were found between ~~A549 cell~~ leachate origins based on HAdV infectivity (p=0.4854) (Figure 1).

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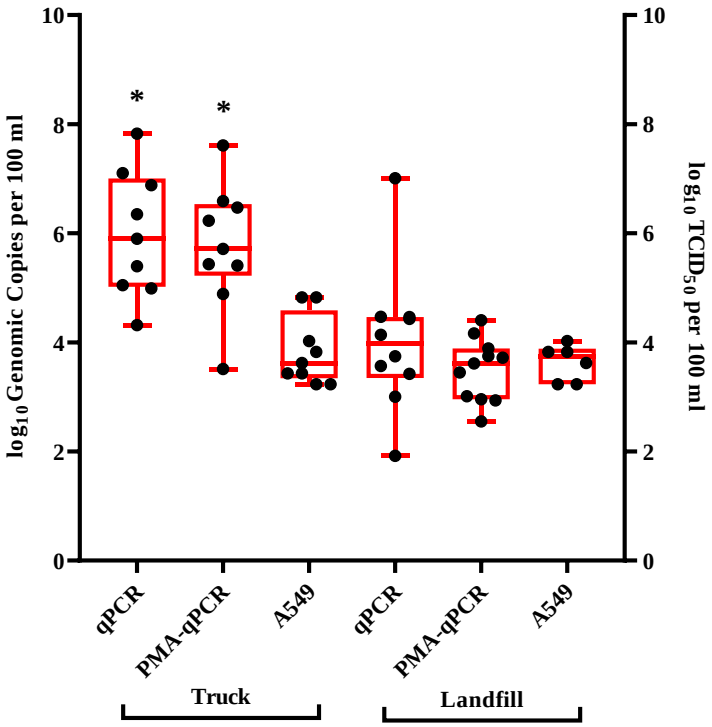
a.


log10 Genomic Copies per 100 ml
log10 TCID50 per 100 ml
10
8
6
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2
0
*
*
qPCR
PMA-qPCR
A549
qPCR
PMA-qPCR
A549
Truck
Landfill

367

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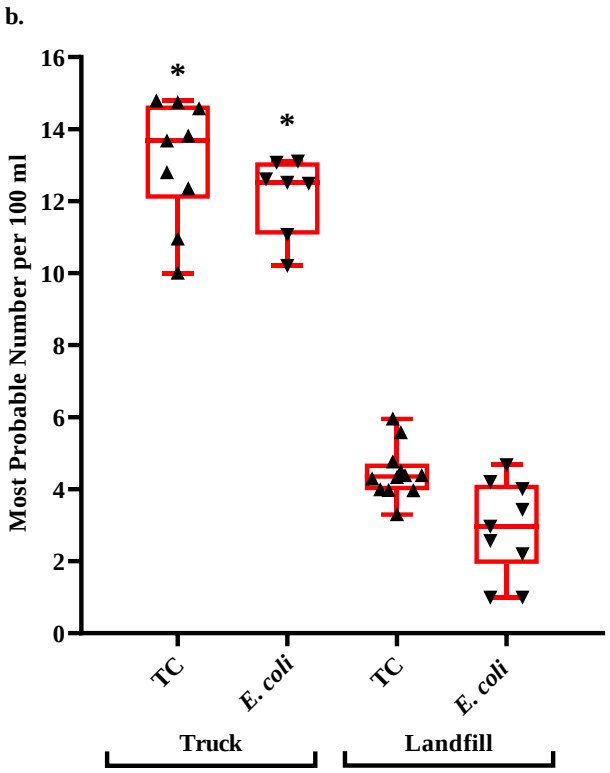
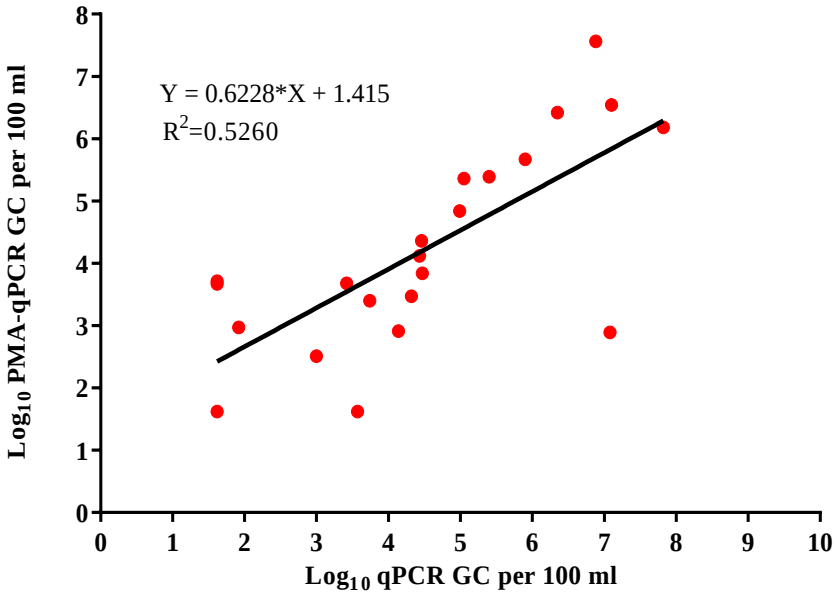


Figure 1. Quantification of human adenovirus (a), total coliforms (TC) and *Escherichia coli* (*E. coli*) (b) from truck and landfill leachate samples.

Considering HAdV quantification, a correlation between results obtained from qPCR and PMA-qPCR in truck leachate ($p < 0.05$) was observed and between infectivity titer and PMA-qPCR in landfill ($p < 0.05$). When the results obtained from the truck and the landfill samples were analyzed together, the correlation between qPCR and PMA-qPCR methods was maintained ($p < 0.001$) with a good linear correlation ($R^2 = 0.526$). (Figure 2a). The concentration values obtained by these molecular methods were also correlated with HAdV infectivity and PMA-qPCR ($R^2 = 0.543$) (Figure 2b).

a.


Y = 0.6228*X + 1.415
R2=0.5260
Log10 PMA-qPCR GC per 100 ml
Log10 qPCR GC per 100 ml
0
1
2
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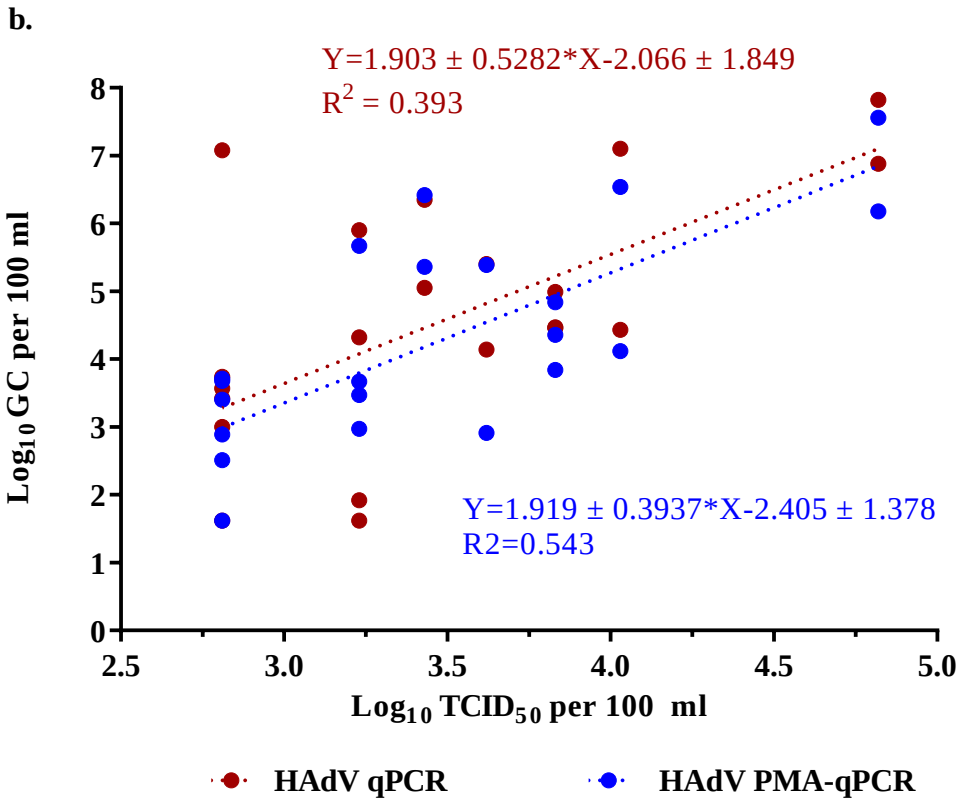

394

395 Figure 2. Regression analysis between HAdV detection methods. a) Correlation between
396 qPCR and PMA-qPCR. b.) Correlation between $TCID_{50}$ and qPCR/PMA-qPCR
397 quantification.

398

399 ***3.2 Molecular characterization***

400 Nucleotide sequencing was successfully obtained from all 21 samples in which HAdV
401 was detected and ~~classified~~ the strains were classified as group F (61.9%), C (19.5%),
402 and~~,~~ A and B (9.5% each). From sequenced HAdV-F, 46.15% (6/13) were of type 40 and
403 53.84% (7/13) of type 41, associated with GI symptoms. All species were detected in both

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leachates, except HAdV group A, only detected in landfill samples (Figure 3). The nucleotide sequence data reported here received Gen Bank accession numbers (MW685861 to MW685881).

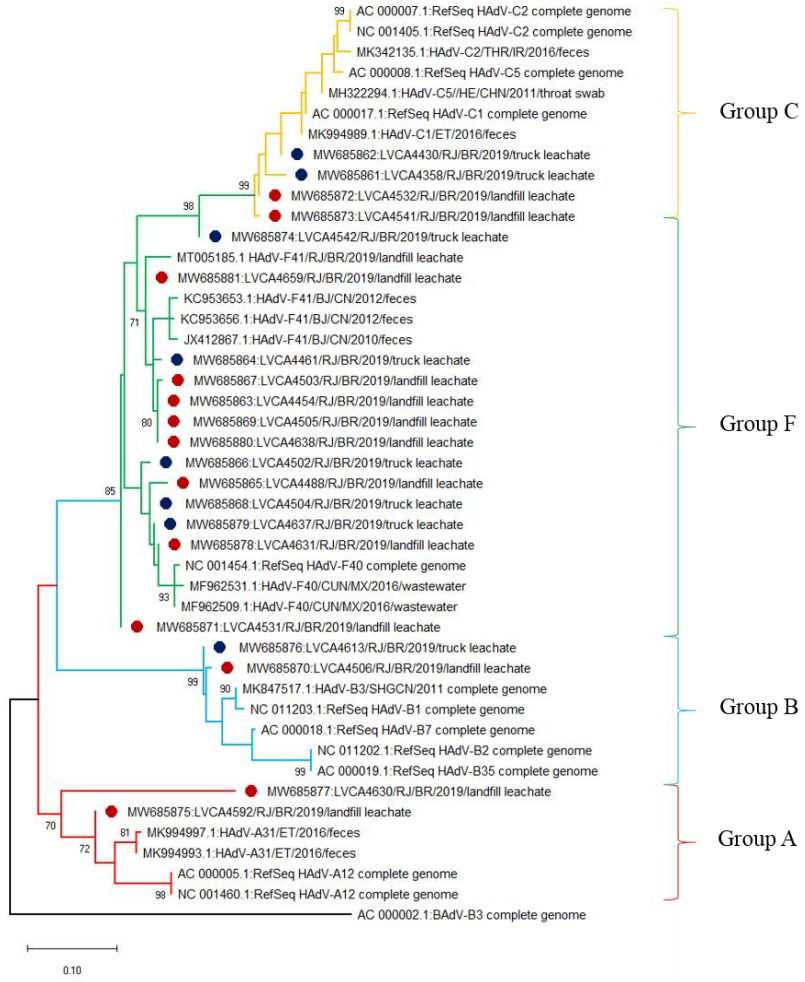

Figure 3. Dendrogram based on HAdV hexon partial nucleotide sequence (245 pb) of 21 Brazilian environmental samples from landfill (red circle) and truck leachate (blue circle). Reference strains were obtained from GenBank. Maximum Likelihood phylogenetic tree

was constructed using Mega X Software, Tamura 3 parameter model, and Gamma Distributed (G) with 2000 bootstraps above 70%.

Physico-chemical parameters ~~Physical-chemical findings~~

3.3

The apparent color, turbidity, solids, total carbon (with a large percentage of organic carbon), chemical oxygen demand (COD), and the phosphorus~~phosphor~~ ~~presented~~ were higher ~~values~~ in the truck leachate ~~when compared to~~than in landfill ~~leachate~~ one with statistically significance differences ($p<0.0001$). The values of pH, total alkalinity, total inorganic carbon, ammonia, and total nitrogen showed significantly higher values in the landfill when compared to the truck leachate ($p<0.0001$). Chloride and UV 254 nm did not show a significant difference in both leachates ($p>0,05$). ~~Physical~~Physico-chemical characterization from truck and landfill leachate samples is represented in Table S2.

The role of such variables on microbial concentrations was evaluated considering all leachate samples together in multiple regression models, to increase the sample size for statistical analysis. Based on the analysis of the correlation coefficient, the following six parameters were considered as predictors in the regression model: ~~as~~ conductivity, chloride, total hardness, UV 254 nm, total suspended solids, and turbidity ~~were considered~~. Conductivity, total suspended solids, and turbidity ~~parameters~~ were ~~correlated~~ able to explain ~~to~~ more than 50% of the bacterial concentrations' variation~~,~~ : ~~being~~ 86% of total coliforms (R^2adj = 0.8706, $p < 0.0001$) and 63% of *E. coli* (R^2adj = 0.6279, $p < 0.001$) variations ~~(R^2adj = 0.6279, $p < 0.001$)~~. Total suspended solids and conductivity were able to explain 36% and 58% of the variability of ~~correlated to~~ HAdV qPCR ~~,~~ (R^2adj = 0.3631, $p < 0.01$) and PMA-qPCR ~~variability~~ (R^2adj = 0. 5806, $p < 0.0001$), respectively, while infectious HAdV variability was explained only by total

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suspended solids (R^2adj = 0.1819, $p < 0.05$) ~~was correlated only with total suspended solids~~. Regardless of the analytical method for HAdV detection, total suspended solids represented the main predictor for viral concentration, with a positive correlation.

~~3.3~~ 3.4 *QMRA results*

QMRA results showed that the mechanisms of oral ingestion determined different exposure doses, with different GI illness risks during a working day (Figure 4). The gloved hand-to-mouth mechanism was responsible for an average dose of 17.85 $TCID_{50}$ (IQR = 9.37 – 26.34) which corresponded to an average GI illness risk of 33% (IQR = 26% – 42%). The splashing mechanism determined a higher average exposure dose of 85.24 $TCID_{50}$ (IQR = 44.73 – 125.76) associated with a probability of illness of 58% (IQR = 52% – 67%). Considering the total daily ~~total~~ exposure dose through oral ingestion as the sum of the average dose of both mechanisms (103.10 $TCID_{50}$), the splashing route ~~is~~ was responsible for the greater contribution to the total daily dose (~83% ~~of the total daily dose~~) compared to gloved hand-to-mouth (~17%). In the case of no gloves, exposure dose greatly increased to 209.32 $TCID_{50}$ (average value), responsible for an average health risk of 67% (IQR = 63% – 74%).

Sensitivity analysis determined the impact of input variables on model output: C_{HAdV}, $f_{contact}$, and $TE_{surface/hand}$ for the inadvertent ingestion via hand-to-mouth and C_{HAdV} and $V_{leachate}$ for splashing (Figure S1). In the case of the hand-to-mouth mechanism, the most impacting parameter was the $f_{contact}$ and the C_{HAdV} in the truck leachate, followed by $TE_{surface/hand}$ (the same results have been obtained for bare hands). In the case of the splashing mechanism, the $V_{leachate}$ accidentally ingested ~~is~~ was slightly more important than the C_{HAdV}.

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~~Figure 4. Gastrointestinal (GI) illness risk (%) for waste collectors, attributable to inadvertent oral ingestion of truck leachate by different mechanisms: hand to mouth contact (bare and gloved hands) and splashing route.~~

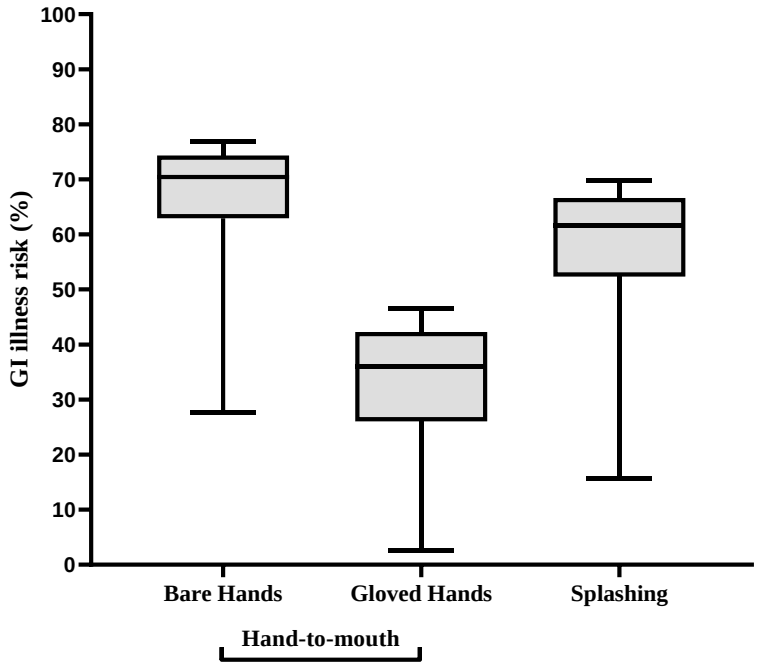

Figure 4. Gastrointestinal (GI) illness risk (%) for waste collectors, attributable to inadvertent oral ingestion of truck leachate by different mechanisms: hand to mouth contact (bare and gloved hands) and splashing route.

4 Discussion

4.1 Microbiological and physico-chemical parameters

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Microbiological and physico-chemical parameters ~~physical-chemical findings~~ demonstrated ~~a~~ significant differences based on leachate source ~~in the values and concentrations of these parameters in leachate from different sources~~ (truck and landfill). HAdV was detected in both samples, with infectious virus concentrations greater than 2.0 × 10^3 GC ml^{-1} in truck leachate.

The detected HAdV landfill concentrations ~~detected~~ were similar to those reported by a monitoring study ~~described on the air and surface of an~~in an Italian landfill~~.~~ ~~Concerning HAdV concentrations in the landfill, our results were similar to a study carried out in Italy on the air and surface of a landfill~~ (Carducci, Federigi, and Verani 2013). To our best knowledge, ~~there is~~ no study currently reported ~~reporting~~ virus occurrence in truck leachate.

The PMA, an intercalant dye that binds covalently and irreversibly to viral DNA, blocks the amplification of nucleic acids due to the release of the polymerase enzyme, and only the target genes of intact viruses were amplified by PMA-qPCR (Leifels et al., 2021). The correlation observed by different viral quantification methodologies (qPCR, PMA-qPCR, and cell culture assay) demonstrated that qPCR can be predictive of infectious HAdV in fresh leachate (truck), while PMA-qPCR in landfill samples. Differences on HAdV levels according to detection methods were similar to those obtained by (Leifels et al. 2016) on ~~In a similar study previously conducted in~~ sewage samples, who found ~~demonstrated~~ one log reduction in HAdV PMA-qPCR concentration and 2 logs in $TCID_{50}$ when compared to results obtained by qPCR, and~~.~~ ~~The correlation observed by different viral quantification methodologies (qPCR, PMA-qPCR, and cell culture assay) demonstrated that qPCR can be predictive of infectious HAdV in fresh leachate (truck), while PMA-qPCR in landfill samples. Previously, a study HAdV conducted in sewage samples demonstrated one log reduction in PMA-qPCR concentration and 2 logs in~~

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~~$TCID_{50}$ when compared to results obtained by qPCR.~~ PMA-qPCR decreased the detection rate of HAdV from 96% to 77%, similar to results obtained in cell culture assays (Leifels et al. 2016).

Fresh truck leachate is a liquid generated directly in the waste collection truck basin during the collection day and formed ~~from~~ by the initial decomposition of waste, rainwater, and the compaction process carried out in the trucks. This leachate present~~ed~~ high concentrations of ~~physical~~ chemical compounds as suspended solids and organic compounds (Benyoucef et al. 2015). Although it was~~is~~ not possible to calculate the collection time, it was~~is~~ estimated that sampling ~~they~~ do not exceed 24 hours from waste production, which would justify the high microbiological values detected in the ~~fresh~~ truck leachate, as well as the HAdV infectivity. Moreover, ~~T~~the lower concentration of bacteria and infectious HAdV in the landfill leachate samples compared to that obtained from trucks can be influenced by physico-chemical parameters. In the landfill, such parameters can ~~These~~ vary depending on the landfill lifetime and its operating methods, the interference of events before sample collection, climatic conditions, waste composition, and solids waste degradation rate. ~~The reduction of infective HAdV, as well as the lower concentration of bacteria in landfill samples, can be attributable to physical-chemical factors that depend on the landfill lifetime and operating methods, events occurrence before sample collection, climatic conditions, waste composition, and solid waste degradation rate~~ (Bulc 2006; Tchobanoglous and Kreith 2002). The physico-chemical parameters in landfill leachate samples ~~physical-chemical findings~~ (i.e., ~~as~~ pH, total alkalinity, total inorganic carbon, ammonia, and total nitrogen) ~~in landfill leachate samples are~~ were similar to those already described in other studies (Costa, Alfaia, and Campos 2019; Costa et al. 2019).

Commentato [IF8]: Could this part be removed, since the meaning is included in the above sentence?

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Multivariate regression analysis, analyzing considering leachate from both sources, and correlating physical-chemical factors with detection of HAdV revealed that the concentration of suspended solids is was a predictor of the HAdV occurrence of these viruses detected, regardless of the method used. Such result can be attributable to the Adsorption adsorption of viral particles to suspended solids, that – prolongs viral infectivity in the environmentleachate, protecting from enzyme degradation and UV inactivation (Fong and Lipp 2005). This Such result finding suggests can be used for further investigations on the adoption of physico-chemical parameters physical-chemical findings as a proxy for viral contamination, since viral measurements are time-consuming and expensive, and cannot be used for real-time monitoring in the perspective of timely risk management (Hess, Niessner, and Seidel 2021).

Regarding HAdV diversity, our results corroborated the role of disposable diapers, toilet paper, septic tank sludge, and secretions in MSW microbial contamination (Gerba et al. 2011), since the excretion of all types of HAdV in feces is well known (Bonot et al. 2014). HAdV characteristics as DNA capsid virus, of high stability, resistance to adverse environmental conditions (Rames et al. 2016) contribute to the occurrence in the leachate. The high concentration of total coliforms and *E. coli* from waste collection truck leachate found in this study was similar to another Brazilian investigation conducted in Brazil that demonstrated no differences between bacteriological contamination from household and health services trucks (Silva et al. 2011).

4.2 Risk analysis for truck worker

Two The modelled exposures scenarios were modeled, assessingrepresented the worst-case scenarios of workers involved in daily waste-related activities exposed toin the context of heavily polluted truck surfacesenvironment. Although such assumptions lead

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to an overestimation of the risk, an exposure assessment study carried out with Danish waste collectors showed high bacteria and fungi levels on truck surfaces during the collection of household waste by compactor trucks (Madsen et al. 2020). Moreover, ~~We~~ we made the conservative assumption that all infective particles were enteropathogenic, since ~~a~~ the higher prevalence of HAdV-F, a virus associated with GI illness (Reis et al. 2016; Arcangeletti et al. 2014) ~~was detected~~, although Group B and C serotypes responsible for respiratory symptoms were also detected.

The lack of surveillance data on GI illness among Brazilian waste collectors ~~does~~ did not allow any inference between model outputs and epidemiological data. Nevertheless, previous studies revealed an increased incidence of GI symptoms among waste collectors in other countries (Poulsen et al. 1995; Thorn, Beijer, and Rylander 1998; Ivens et al. 1999).

The QMRA results showed the protective role of gloves for GI illness attributable to the hand-to-mouth contact. In a real-world scenario, the hands could be also contaminated from accidental self-inoculation during glove removal or penetration of the microbes through the glove material, therefore hand hygiene procedures when removing gloves should be performed. ~~Considering QMRA results from both mechanisms, the most relevant oral exposure mechanism was represented by the splashing route, so wearing PPE could reduce these GI illness risk.~~ Moreover, the exposure mechanism through gloved hand was less relevant than splashing and such result highlighted the importance of wearing PPE (i.e., face shields) for reducing GI illness risk. In the present study, we focused on the exposure through accidental leachate ingestion, because oral pathway is the main route for enteric disease. However, the inhalation of HAdV-laden aerosol could be an additional exposure pathway, since aerosol particles with an aerodynamic diameter larger than 10 µm remain entrapped on the surface of the respiratory system and can be

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swallowed. Some occupational QMRA studies quantified such ingestion rate between 10% and 50% of the inhaled microorganisms (**Brooks et al., 2005**; **Medema et al., 2004**; **Akpeimeh et al. 2020**). The additional GI risk attributable to inhalation needs direct measurements of HAdV in air sample, but such investigations are beyond the scope of this study.

~~Monitoring of HAdV load on workers' gloves is important to avoid assumptions on truck surface contamination and pathogens transfer efficiency from surface to hand, but it needs viral elution process from gloves which can reduce HAdV detection sensibility.~~

4.2.1 Limitation of the risk assessment analysis

~~To investigate the inadvertent ingestion exposure by hands, we started from HAdV load measured in truck leachate and used input parameters derived from literature, such as the transfer efficiency from surface-to-hand and hand-to-mouth.~~ The estimated health risk could be affected by uncertainties in the input parameters, especially on the HAdV concentration, that has been modelled as uniform distribution owing to the little sample size of monitoring data. Regarding inadvertent ingestion exposure by hands, transfer efficiency from surface-to-hand and hand-to-mouth were derived from literature. Transfer efficiency of biological agents is currently little investigated~~,~~ and studies are focused on bare hands as reviewed by (Gorman Ng et al. 2012). The role of gloves is considered only in laboratory studies on food production, owing to their importance in the transmission of viral food-borne gastroenteritis (Rönnqvist et al. 2014; Sharps, Kotwal, and Cannon 2012; Stals et al. 2013). Therefore, we used transfer efficiency from surface to gloved hand derived from such experimental studies, but the latex gloves of food-handling personnel could differ from waste collectors´ gloves, thus determining a difference in viral acquisition from surfaces and/or in releasing to the oral compartment.

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5 Conclusions and future perspective

MSW leachate is a source of HAdV, with a higher prevalence of group F HAdV, and potential risk of GI illnesses for waste collector's workers. HAdV detection and quantification were higher in fresh truck leachate than in landfill samples, with 100% of infectivity. In the exposure scenario of inadvertent ingestion of truck leachate, bare hand-to-mouth exposure was ~~is~~ responsible for the highest GI risk, followed by splashing exposure and gloved hand-to-mouth. This reinforces the need for risk analysis studies and the adoption of PPE by waste collectors, especially on the evaluation of the protective role of wearing masks, whose adoption is progressively increasing as a result of changes in infective risk perception during COVID-19 pandemic. Since some HAdV serotypes can be responsible for conjunctivitis (Gopalkrishna, Ganorkar, and Patil 2016), the role of truck leachate in increasing the conjunctivitis risk should be further explored, as data on ocular exposure are missing.

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Authorship contribution statement

Conceptualization: JCM, CFM, MPM; Formal analysis: NML, IF, MV, AC, MPM; Funding acquisition: EMS, CFM, MPM; Field work: NML, RFM, CFM; Methodology: NML, RFM, MDNB, IF, MV, AC, MPM; Software: LC, IF, NML; Supervision: AC, MPM, CFM, JCM; Roles/Writing - original draft: NML, IF, MV, AC, MPM; Writing - review & editing: NML, IF, LC, MV, CFM, AC, MPM.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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