Science of the Total Environment 648 (2019) 1082–1086

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Bioaerosol emissions associated with pit latrine emptying operations

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## HIGHLIGHTS

- Bioaerosols were sampled during pit latrines emptying in Blantyre, Malawi.
- *E. coli*, coliforms and enterotoxigenic *E. coli* (ETEC) were detected.
- High bioaerosols concentrations correlated with certain emptying practices
- The results highlight aerosolization of enteric pathogens during pit emptying.
- Further studies are needed to quantify exposure and health risks.

## GRAPHICAL ABSTRACT

## ARTICLE INFO

*Article history:*
Received 19 May 2018
Received in revised form 10 August 2018
Accepted 10 August 2018
Available online 12 August 2018

Editor: Frederic Coulon

*Keywords:*
Bioaerosols
Sanitation
Pit latrines
Fecal pathogens

## ABSTRACT

Pit latrines are the most common sanitation option in the developing world. They are simple to build but require periodic emptying which results in widespread dispersion of fecal pathogens in the environment. While much is known about the health risks of fecal-oral exposure, little is known about those resulting from the aerosolization of pathogens from fecal material. Bioaerosols were sampled around seven pit latrines before, after, and during emptying in Blantyre, Malawi. Bioaerosols were collected directly onto nutrient and selective medium agar plates using an impact sampler. DNA was extracted from some plates and analyzed for selected enteric pathogens. Total heterotrophic bacteria in the air during active emptying ranged from 198 to >13,000 colony forming units (CFU) per $m^3$, and generally increased above background levels during pit emptying. At about one meter from the pit latrine emptying, *E. coli* and total coliforms concentrations in air reached up to 350 and 790 CFU $m^{-3}$, respectively. Additionally, at four out of the seven pit latrines sites sampled, enterotoxigenic *E. coli* (ETEC) LT/ST was confirmed to be present in bioaerosols. This work demonstrates the potential for airborne dispersion of enteric pathogens during pit latrine emptying operations.



## 1. Introduction

Improving sanitation in the developing world has become a focus in recent decades, and is specified in the Millennium Development Goals (Organization, 2013) and now in the Sustainable Development Goals (UNDP, 2015). However, the World Health Organization estimates

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https://doi.org/10.1016/j.scitotenv.2018.08.147

that around 525,000 children die each year due to diarrheal diseases, which are often directly related to lack of proper sanitation (World Health Organization, 2014). Safely managed improved sanitation has the potential to decrease child deaths and reduce morbidity.

Currently, on-site sanitation systems, such as pit latrines, are the most common sanitation option used throughout the developing world, and it is estimated that around 1.8 billion people use pit latrines as a main means of sanitation (Berendes et al., 2017; Graham and Polizzotto, 2013; Jenkins et al., 2015). Though simple to build and use, pit latrines and other onsite sanitation systems generally require periodic emptying, transport and treatment or disposal of the fecal sludge (Chunga et al., 2016; Mbéguéré et al., 2010; Sisco et al., 2015). Safe management of fecal sludge during the emptying process can be technically and logistically challenging. Even one of the more formal and seemingly hygienic methods of waste removal, operation of a vacuum tanker truck by trained personnel, may result in contamination of the surrounding environment and risk of exposure to fecal pathogens. It is generally accepted that the most common route of transmission for enteric pathogens is via the fecal-oral route (World Health Organization, 2004). Very little is known about the airborne exposure risks associated with mechanically agitating and pumping semi-liquid human feces in the developing world.

Bioaerosols are airborne matter that originates from microbes, plants, or animals (Yoo et al., 2017). Previous studies have found bioaerosols can originate from conventional flush-style toilets (Barker and Jones, 2005; Gerba et al., 1975; D. Johnson et al., 2013; D. L. Johnson et al., 2013; Verani et al., 2014), wastewater treatment plants and operations (Dowd et al., 2000; Heinonen-Tanski et al., 2009; Karra and Katsivela, 2007; Pepper et al., 2006; Sánchez-Monedero et al., 2008; Schlosser et al., 2011; Tanner et al., 2005; Uhrbrand et al., 2011), composting operations (Kummer and Thiel, 2008; Pahari et al., 2016; Sanchez-Monedero et al., 2003), and can pose risk of person-to-person transmission of pathogens in hospital settings (King et al., 2015, 2013; Verani et al., 2014). To our knowledge, no previous work has investigated the extent and content of bioaerosols generated during mechanized pit emptying practices. This work reports the results of one week of bioaerosol sampling performed during pit emptying activities in Blantyre, Malawi, which is a moderately dense, peri-urban informal settlement. Samples were analyzed to determine the concentration of specific bioaerosols and the presence of key pathogenic organisms.

## 2. Materials and methods

In December 2016, we shadowed a pit latrine emptying crew in Blantyre, Malawi. Our investigations were conducted in conjunction with a study of novel equipment designed to reject trash from pit latrines during emptying. Pit emptiers used a standard vacuum tank system to empty pit latrines using a prototype (Sisco et al., 2015) that included a trash rejecting system. The pits' contents were first "fluidized" by pumping water under the sludge surface with a pressure washer and then mixing by hand with a spiked rod. These are common practices used by pit emptiers. Once the sludge was deemed fluid enough for pumping, the trash rejecting hose was placed in the pit to begin sludge removal. If needed, the fluidization step was repeated. Any trash stuck to the spiked rod was removed by hand and set in a corner of the latrine.

### 2.1. Agar preparation

Nutrient and MI agars (Difco™) were prepared per manufacturer's instructions with the following field-based modifications due to lack of access to an autoclave: an electric tea kettle was used to boil distilled water for 5 min, then MI or nutrient agar powder was added directly to the kettle, mixed by hand using a metal spatula, and boiled for 5 min. The agar was cooled to 55–60 °C and poured into sterile plates. Plates from each batch were reserved for use as negative controls which showed absence of contamination.

### 2.2. Bioaerosol sampling

Air near target pit latrines was sampled using an MAS-100 ECO microbial air sampler (MBV AG, EMD Chemicals Inc. Gibbstown, NJ). The portable sampler relies on impaction directly onto agar plates and operates at a flowrate of 100 L/min. High volume (0.5–1.0 $m^{-3}$ of air) and low volume (0.10–0.20 $m^{-3}$ of air) samples were attempted before, during, and after emptying at 7 pit latrines. Temperatures were recorded during sampling and ranged from 30 to 33 °C. Wind direction was recorded on individual site drawings, but wind speed and relative humidity levels were not recorded. Non-specific nutrient agar was used to enumerate total heterotrophic bacteria and MI agar was used to enumerate *Escherichia coli* (*E. coli*) and total coliforms. DNA extraction was performed on bacterial growth recovered from agar plates. Not all combinations of sampling volumes and agars were taken at every pit due to logistical issues with the emptying procedures, stoppages, or emptying equipment adjustments occurring during sampling (see SI for listing of all samples taken). The locations of samples were selected to be as similar as possible between sites, but variations were required due to drastic differences between pit latrines design and location. The distance from the pit opening to the pit sample location therefore varied between 1 and 5 m. Background, pit emptying, pit cleaning, and post cleaning samples were taken at the same location during the operation. When pit emptying facilitated it, some locations could be sampled upwind and/or downwind as well. Samples related to the vacuum truck, such as the truck vent, were taken in the same location relative to the truck when possible. The location of the truck relative to the pit latrine varied from latrine to latrine depending on how the workers could position the vacuum truck near the latrine. In some cases, the truck was positioned behind a compound wall which physically separated the truck vent from the work space and workers (see site drawings and pictures in Supplementary Data). Locations with street access had the workers within sight of the truck. Post-emptying samples were at some pit latrines 30 to 60 min after emptying was completed. To measure passive deposit of bioaerosols on surrounding surfaces, nutrient and MI agar plates were set out in selected locations near the pit latrines. Locations were chosen based on an ad hoc basis depending on what nearby surfaces were available for placement of petri dishes (see SI – "Flat" samples). Locations chosen included a front porch, near a clothesline, and near an open kitchen window. See SI for a full list of sample locations and site drawings.

### 2.3. Bacterial culture methods

After sampling, agar plates were stored in a cooler without ice and transported to the lab within 8 h of collection. Plates were incubated at 35 °C overnight and colony forming units (CFUs) were counted the following morning. Since the ambient temperature after sampling and during transportation was close to the incubation temperature, total time for colony growth was 18 to 22 h. Final bacterial concentrations were calculated using the most probable number method, per instructions of the MAS-100 ECO sampler.

### 2.4. Sludge sampling

At 4 out of 7 of the pits (pits 2, 5, 6, 7), sludge samples were collected following fluidization. 1 mL of sludge was mixed with 100 mL of UV sterilized distilled water, and 2 mL of the resulting suspension was diluted 1:1 with UNEX buffer (Microbiologics, St. Cloud, MN), vortexed, and frozen for future molecular analysis. As will be discussed in the Results section, this turned out to be excessive dilution.