Environmental Science & Technology

Article

pubs.acs.org/est

Quantitative Headspace Analysis of Selected Odorants from Latrines in Africa and India

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Supporting Information

ABSTRACT: This analytical investigation focuses on the quantification of odorant molecules in the headspace of latrines. Hydrogen sulfide and methyl mercaptan were derivatized under a more stable *N*-ethyl maleimide conjugate. Since the amount of odorant molecules is very low in the gas phase, we developed a method that had two steps of concentration. The first step consisted of the accumulation of volatiles in buffered water by bubbling 350 L of air in a bottle. The second step consisted of loading the water on a 1 g solid-phase extraction cartridge, shipping it to our laboratories, and desorbing with Et_2O, which achieved a total concentration factor of 3.5×10^6. The acidification of the water phase gave us access to the acids, and an additional bottle containing an acidic ion-exchange resin gave us access to trimethyl amine. The limits of quantification in the gas phase were 8.7×10^{-4} μg/L air for hydrogen sulfide, 1×10^{-4} μg/L air for methyl mercaptan, 1×10^{-3} μg/L air for butyric acid, 1×10^{-4} μg/L air for *p*-cresol, 1×10^{-5} μg/L air for indole, and 1×10^{-5} μg/L air for skatole. The system was calibrated by using olfactometers, which can deliver a precisely known quantity of volatiles into the air. We were able to quantify all compounds near their odor detection thresholds (ODTs). All ODTs were measured in our laboratory with the same olfactometry method. This allowed accurate and comparable ODT values for malodorant compounds from toilets.

INTRODUCTION

A large community of scientists is now brainstorming about how to offer decent toilet systems in developing countries, not only in terms of the technical aspects of such systems, but also in terms of their cultural and economic aspects. The question is whether it is possible to create an economic model to sustain the long term implementation and maintenance of public latrines in these countries. In support of this effort, the ultimate goal of the Bill and Melinda Gates Foundation project, "Reinvent the Toilet Challenge," is to provide sustainable and friendly toilets to prevent open defecation.

The perfumery industry has been active in counteracting malodors for many decades. As is the case for most malodors, it is critical to understand which molecules are responsible for toilet malodors to improve cost efficiencies in the development of perfume compositions. During a previous part of our project, we analyzed the sludge of a pit latrine.[1] The sludge was diluted in water, loaded on a solid-phase cartridge to capture the hydrophobic molecules, and shipped back to our laboratories. The volatiles were then extracted with an organic solvent and analyzed by gas chromatography−mass spectrometry-olfaction (GC−MS-O). The volatiles of interest, that is, those most pertinent for human waste odors, were quantified by solid-phase microextraction (SPME) by using stable isotope-labeled internal standards. This was done in a sealed vial at 40 °C containing the sludge.[1] The weakness of this approach was that there was no information about the quantity of these volatiles in the toilet headspace. Mathematical models could be established if air fluxes, humidity, temperature, and the chemical composition of the sludge are determined precisely; however, each pit latrine is different. For this reason, in the present study, we decided to develop a method to quantify the key odorant volatiles in the headspace of used toilets.

The pertinent compounds to analyze, known since the 19th century,[2−4] are butyric acid, *p*-cresol,[3,4] indole, and skatole.[2] Sulfur compounds, mainly hydrogen sulfide and methyl mercaptan, are also key odorant compounds in toilet malodor,[5−7] but they are gaseous in temperate countries and not very stable. Dimethyl mono-, di-, and trisulfide, in some conditions, can also contribute to toilet malodors. In addition, ammonia and trimethyl amine are important in urinals,[8] but these compounds were not the focus of the current study because their contribution is less important in pit latrines. This may be due to the high buffering capacity of the sludge and the relatively small amount of urine in the latrines because males prefer to urinate in the open in India and Africa.

The challenge of the present work, therefore, was to develop a quantitative method that can be applied in the field with a limit of detection that is preferably lower than the odor detection threshold (ODT). For example, in the case of methyl mercaptan and skatole, the headspace concentration target corresponded to an ODT of 4×10^{-5} and 5×10^{-6} μg/L, respectively. This method permitted detection of most

Received: February 7, 2015
Revised: April 21, 2015
Accepted: April 27, 2015
Published: May 7, 2015

DOI: 10.1021/acs.est.5b00692
Environ. Sci. Technol. 2015, 49, 6134−6140

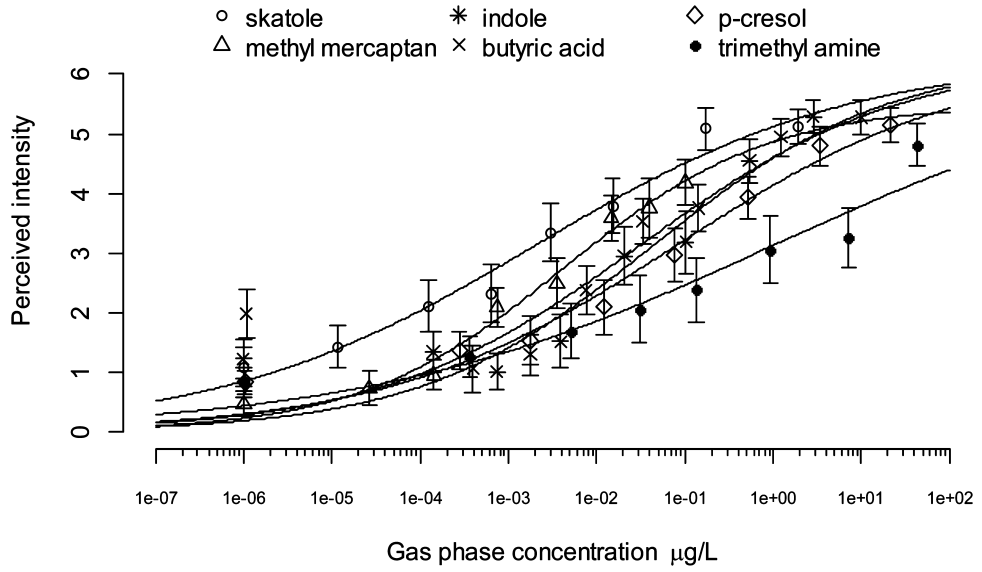

Figure 1. Mean ±95% confidence interval (CI) of the perceived intensity as a function of the gas phase concentration of the different compounds delivered by the olfactometer. The intensity was rated on a scale from 0−6.25. Curves were obtained as described in 14 and show the relationship between the intensity and the gas-phase concentration for each compound, allowing interpolation to predict the intensity from a gas-phase concentration.

compounds just above their odor threshold.[9] Quantifying compounds by GC−MS in single ion monitoring (SIM) mode requires a minimum injection quantity of about 0.1 ng. To reach this objective, we needed to concentrate the headspace to quantify the malodorant volatile compounds under the constraints of being in crowded slums and in following airline safety regulations for luggage. Convenient existing technologies for travelers, such as SPME, are not quantitative in an open space.[10−13]

This article describes the ODTs in air and the dose−response curves for the key malodorant compounds from toilets such as methyl mercaptan, butyric acid, *p*-cresol, indole, and skatole, as well as trimethyl amine. These values in air can be compared because they were measured by using the same validated olfactometry method (Figure 1).[14] The article also describes how it was possible to deliver a precise concentration of a volatile in the headspace and to precisely quantify, in the range of their odor thresholds, selected key odorants. This method was then used in Ahmadabad, India; Nairobi, Kenya; and Durban, South Africa.

EXPERIMENTAL SECTION

Materials and Chemicals. Butyric acid, *p*-cresol, indole, skatole, and CH_3SH in triethyl citrate (5%) were in-house ingredients (Firmenich S.A., Geneva, Switzerland). Other reagents, including the internal standard (IS) methyl octanoate, salts for buffers, trimethyl amine, 1H-pyrrole-2,5-dione, 1-ethyl- (*N*-ethyl maleimide) (NEM) **1**, Amberlite IR 120 H^+, and CH_3SH (liquefied gas, purum), were purchased from Sigma-Aldrich (Buchs, Switzerland). OASIS HLB cartridges (1 g) were purchased from Waters (Montreux-Chailly, Switzerland). We used hydrogen sulfide in a pressurized cylinder containing 52.5 $\mu L/m^3$ and 15.4 × 10^3 $\mu L/m^3$ in N_2 (Carbagas, Carouge, Switzerland). Pure hydrogen sulfide (99.5%, 227 g cylinder) was purchased from Aldrich (Steinheim, Germany). Trimethylamine DCl (D10, 98%) was purchased from Cambridge Isotope Laboratories (Burgdorf, Switzerland). Two-centimeter custom SPME fibers (55/30 μm, DVB/CAR-PDMS) were purchased from Supelco (Bellefonte, PA, USA). Clear headspace vials (20 mL) with screw caps were purchased from Thermo Scientific (Langerwehe, Germany).

The preparation of the authentic samples (+-)-3,3′-thiobis(1-ethyl-2,5-pyrrolidinedione), NEM_2S; (+-)-1-ethyl-3-(methylthio)-2,5-pyrrolidinedione, **2**; and (+-)-1-ethyl-3-(ethylthio)-2,5-pyrrolidinedione, **3** (Figure 2)[15,16] is available in the Supporting Information.

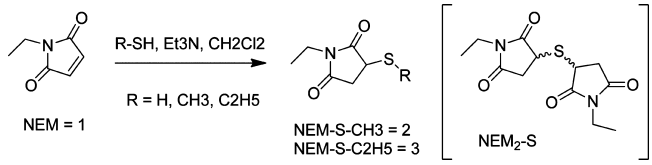

Figure 2. Preparation of NEM derivatives used as external standards to calculate the response factor, kinetics, and stabilities on SPE.

Gas Chromatography−Mass Spectrometry (GC−MS). Compound identifications were performed on a GC 6890 N (Agilent, Palo Alto, CA, USA) equipped with a fused silica SPB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and an SPBwax GC column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA). The initial oven temperature was held at 50 °C for 5 min and then increased at 5 °C/min to 250 °C, split mode 1/5. The carrier gas was He (52 kPa), and the injector temperature was 250 °C. For trimethylamine, the initial temperature was held at 40 °C for 5 min and then increased at 5 °C/min to 250 °C. The column was coupled to a MS 5975B Inert XL MSP from Agilent. The mass spectra in the electron impact mode were measured at 70 eV in a scan range from 30−300. MS interpretation was based on authentic samples from the Firmenich data bank or Wiley/NIST libraries. The GC−MS was equipped with an auto sampler Combi-PAL (Zwingen, Switzerland).

Olfactometer Parameters.[14] The airflow rate was 540 L/h, the N_2 flow rate was 60 L/h, and the total flow rate was 600

DOI: 10.1021/acs.est.5b00692
Environ. Sci. Technol. 2015, 49, 6134−6140

L/h (10 L/min). The molecules were delivered into the lower chamber of the olfactometer through a Teflon capillary connected to a 1 mL syringe (Codan, polypropylene syringe, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) filled with a mixture of propylene glycol and the target molecules. The flow rate was 0.101 mL/h delivered by a syringe pump from Infors AG (Basel, Switzerland) with the push piston set at 0.1 mm/min. The size of the solvent drop formed at the end of the capillary was stable. This means that an equal amount of solvent continuously evaporated and arrived by the capillary. The lower chamber was plunged into an oil bath maintained at 150 °C to force the evaporation. The lower chamber was flushed by the N_2 flow that was mixed with the airflow in the upper chamber. The temperature of the upper chamber was maintained at 29 °C with a water bath. The temperature of the air at the outlet of the olfactometer was 29.7 °C, measured with a Testo 650 temperature probe from VWR (Meyrin, Switzerland).

Trapping of Volatiles in Aqueous Trap. At the outlet of the olfactometer (2.5 L/min), or the pit latrine headspace, the air (350 L) was sucked through a glass line (diameter 0.5 cm) in a bottle (named: trap) (750 mL) filled with a 500 mL solution of *N*-ethyl maleimide (NEM, **1**) (5 mg/L), KH_2PO_4/K_2HPO_2 buffer at pH 8 (0.01 M) (Figure 3). For trimethyl amine, instead of the phosphate buffer, the bottle contained Amberlite IR H+ (5 g) in water (500 mL). The bottle was placed in a cooler filled with ice.

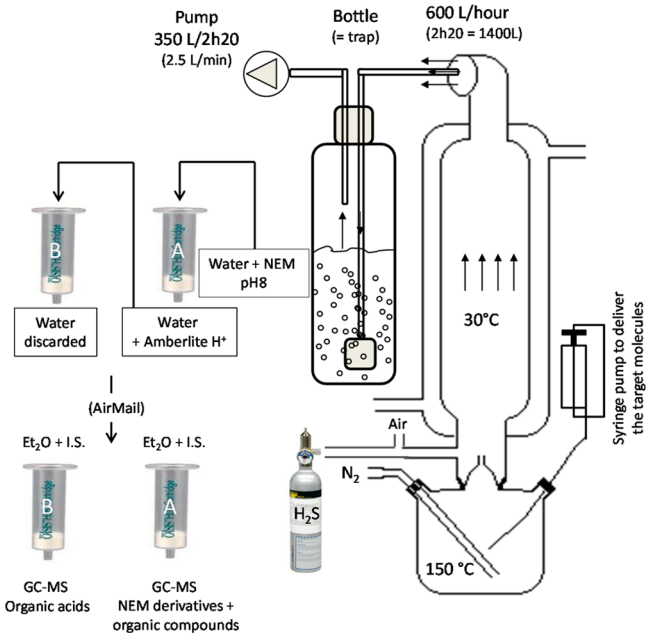

Figure 3. Laboratory setting used for the calibration with the olfactometer. Only the trap, the pump linked to the trap, and SPE were also used in the field.

Solid-Phase Extraction (SPE, Oasis HLB) of Volatile Constituents from the Trap. The SPE cartridges were conditioned with 10 mL of Et_2O, MeOH, and water each time. For the field trip, the cartridges were kept under EtOH. The water from the trap was then loaded on the SPE cartridge. The organic compounds were extracted from the cartridge by using 1 mL of Et_2O containing 1 μg of the IS and 8 mL of Et_2O (total 9 mL), and the organic phase was dried on anhydrous sodium sulfate, filtered, and concentrated carefully under argon flow to a volume of about 0.1 mL. The aqueous phase was then acidified at pH 3 with Amberlite IR-120 by hand shaking the bottle at room temperature until the pH became acidic. This solution was loaded on an SPE cartridge. The acidic compounds (butyric acid) were extracted from the cartridge by using 1 mL of Et_2O containing 1 μg of the IS and 8 mL of Et_2O, and the organic phase was dried on anhydrous sodium sulfate, filtered, and concentrated carefully to a volume of about 0.1 mL. These extracts were injected three times in the GC–MS, and the peak areas in SIM mode were registered as well as the IS peak area.

To analyze trimethylamine, we used a second bottle (trap) containing water and the resin Amberlite IR 120 H^+ (5 g). After being passed through air in the same conditions as described above, the resin was filtered out. Deuterated trimethylamine ($(CD_3)_3N$ DCl) (250 μL, 25 ng) was added as IS to the resin in an SPME vial in water (5 g) and NaOH (600 mg) to reach pH >10. The headspace was analyzed by SPME. The vial was automatically stirred for 15 min at 22 °C for the SPME extraction.

Calibration. The gas phase concentration and the ratio of the peak area in SIM mode of each volatile, as well as the peak area of the IS, which was added in a fixed concentration, were log transformed, and linear models were applied to the transformed data. The resulting calibration curves for each compound are shown in Figure SI 1 of the Supporting Information. Following the linear models, we used the inverse prediction function (chemCal package, R) to predict the gas phase concentration of volatiles in each sample from the triplicate injections. The solutions for gas-phase delivery in the olfactometer are described in Table SI 1 of the Supporting Information. The trapping and extraction on SPE was performed in the same way in each latrine and with the olfactometer in the laboratory for calibration. The peak areas were recorded in SIM mode. For the neutral fraction: *p*-cresol, m/z 107 (Time window 14.5–16.2 min); IS, m/z 74 (16.2–20.8 min); indole, m/z 117 (20.9–22.8 min); NEM-S-CH_3 **2** and skatole, m/z 127 + m/z 130 (22.9–25.0 min); NEM–S-C_2H_5 **3**, m/z 127 (25.1–28.0 min); NEM_2-S, m/z 127 (28.1–42.0 min); for the acidic fraction, butyric acid, m/z 60 (5–10 min). For trimethyl amine, the peak ratios were obtained in SIM mode by using m/z 58 for trimethyl amine and m/z 66 for the $(CD_3)_3N$ DCl used as a deuterated standard.

Equipment Required for Analysis in the Field. The coolers, stainless-steel food-grade bottles, and water were bought locally. We carried with us bent glass tubes with fritted glass at one end wrapped in plastic and stacked into a graduated cylinder (500 mL) to protect this most fragile part of the equipment. We took the four pumps (Gilian Air Plus, Sensidyne, Clearwater, USA) (size: 250 cm^3 each), the tubing and inert plastic connectors for the glass tube, a 1 L polypropylene filter flask and hand pumps (Lincoln Ind. Corp., St Louis, USA), and preconditioned SPE. Reagent, buffers, and resins were preconditioned in small vials at the required amount for one bottle. The SPE cartridges were loaded in the hotel room and then shipped without temperature control to our laboratories (2–4 days) and immediately treated.

RESULTS

Quantitative Analysis of Latrine Volatile Organic Compounds in the Field. The toilets located in Ahamadabad, India were blocks of four to eight units connected to a

DOI: 10.1021/acs.est.5b00692
Environ. Sci. Technol. 2015, 49, 6134–6140

Figure 4. Latrines analyzed in India. Ahmadabad Khada Vadaj and Ahmadabad Behrampura, Mohannathu. In Mohannathu, two identical samplings were performed.

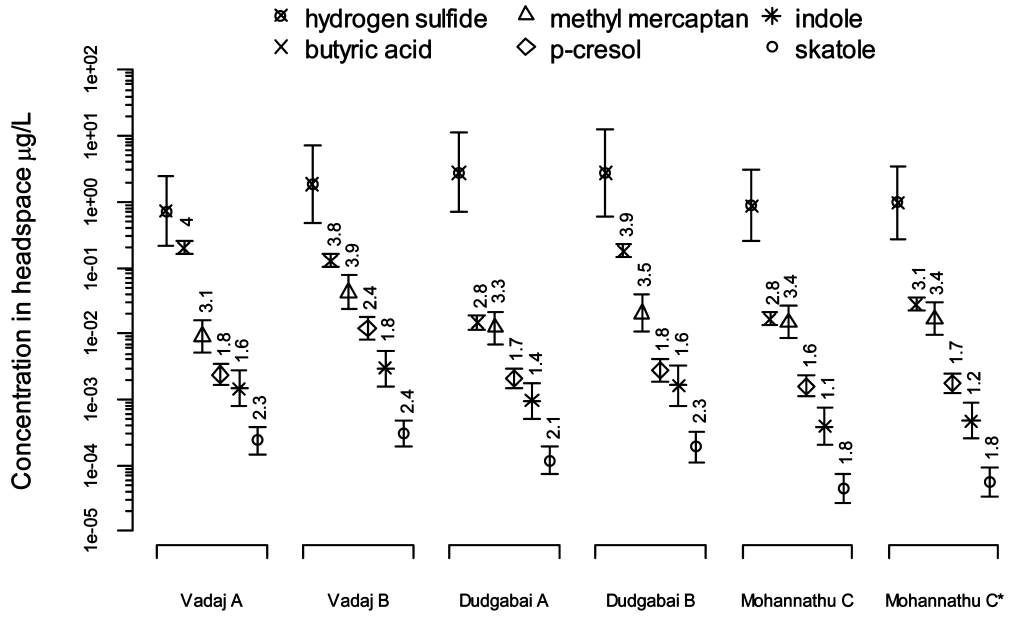

Figure 5. Mean ±95% CI of concentrations for H_2S, butyric acid, methyl mercaptan, *p*-cresol, indole, and skatole in the toilet headspace in Ahmadabad Khada Vadaj (toilets A and B from the same block), Ahmadabad Behrampura Dudgabai (toilets A and B from the same block), and Ahmadabad Behrampura Mohannathu (C and C*, two devices on which testing was repeated). Numbers above the 95% CI bars are the intensity that would be perceived if the compounds were measured alone, according to Figure 1).

sewage drain system. Toilets Khada Vadaj A and B were in poor condition and totally blocked (Figure 4). Comparing the quantity of volatiles in both toilets, we found similar concentrations of hydrogen sulfide, butyric acid, indole, and skatole at 7.2×10^{-1} µg/L (detransformed mean), 1.3×10^{-1} µg/L, 1.5×10^{-3} µg/L, and 2.4×10^{-4} µg/L, respectively (Figure 5). However, we found that the amounts of methyl mercaptan and *p*-cresol were about five times higher in toilet Vadaj B than in Vadaj A (Figure 5). In both toilets, the predicted intensities were in a range of 3.9 for butyric acid, 1.6 for indole, and 2.3 for skatole. The predicted values were interpolated from the relationship between intensity and concentration shown in Figure 1 (results in Figure 5). The intensity for *p*-cresol was 1.8 in toilet Vadaj A and 2.4 in toilet Vadaj B, and the intensity for methyl mercaptan was 3.1 in toilet Vadaj A and 3.9 in toilet Vadaj B. This correlated perfectly with the odor description of Vadaj A as vomit and rancid and that of Vadaj B as more manure, barnyard, and sewage due to the high amount of butyric acid in Vadaj A and a higher amount of *p*-cresol and methyl mercaptan in Vadaj B. The odor description in the field was performed by two scientists, and the odor descriptors were discussed with perfumers by using the olfactometers prior to the field trip.

The toilets in Behrampura Dudgabai A and B were also in poor condition but apparently not fully blocked. We could not see single stools but instead observed a dark liquid (liquid sludge), probably resulting from partially clogged pipes. These toilets smelled more sulfury, sewage, manure, and cabbage compared with the previous toilets. Behrampura Dudgabai B was described as even more sulfury and sewage compared with Dudgabai A, and this was confirmed by analytical quantifications. Dudgabai A and B contained 1.3×10^{-2} µg/L air and 3.2×10^{-2} µg/L air of methyl mercaptan, respectively, as well as 7.3×10^{-2} µg/L air and 1.1×10^{-1}µg/L air of H_2S (Figure 5), respectively.

DOI: 10.1021/acs.est.5b00692
Environ. Sci. Technol. 2015, 49, 6134−6140

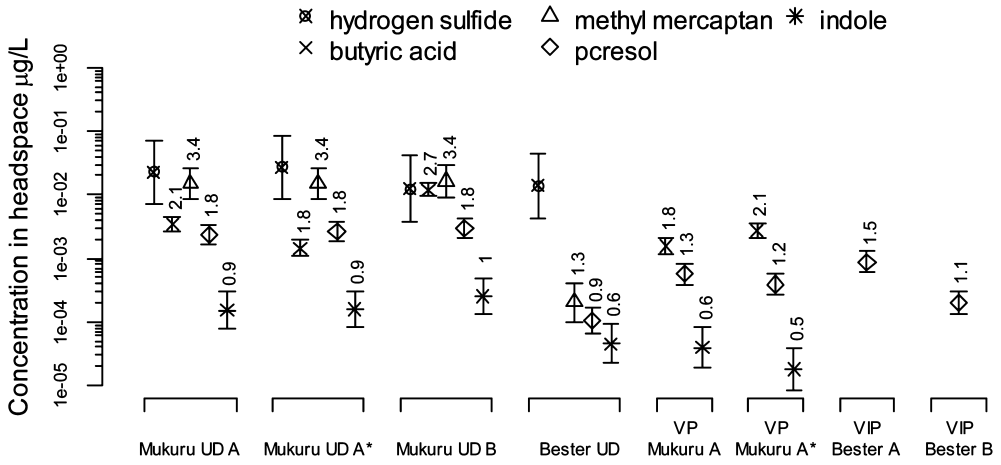

Figure 6. Mean ±95% CI of concentrations for H_2S, butyric acid, methyl mercaptan, *p*-cresol, and indole in UD pits in Nairobi, Kenya (Mukuru A and repetition A*, Mukuru B) and in Durban, South Africa (Bester area). Data obtained from ventilated pit (VP) latrine and ventilated improved pit (VIP) latrine in Mukuru (Mukuru A and repetition A*) and Bester are also shown. Only concentrations above the LOQ are shown. H_2S was detected in VP Mukuru A and A*. Skatole not detected. Numbers above the 95% CI bars are the intensity that would be perceived if the compounds were measured alone, according to Figure 1.

The cleanest toilets were in Behrampura Mohannathu, and the smell was mainly of sewage (Figure 4). The quantitative analytical results of the headspace confirmed that methyl mercaptan and H_2S were the major contributors to the malodor. The same toilet was analyzed twice, and we found H_2S at 8.8×10^{-1} µg/L and 9.8×10^{-1} µg/L; butyric acid at 1.7×10^{-2} µg/L air and 2.8×10^{-2} µg/L air; methyl mercaptan at 1.5×10^{-2} µg/L air and 1.7×10^{-2} µg/L air; *p*-cresol at 1.6×10^{-3} µg/L and 1.8×10^{-3} µg/L; indole at 3.9×10^{-4} µg/L air and 4.7×10^{-4} µg/L air; and skatole at 4.5×10^{-5} µg/L and 5.6×10^{-5} µg/L, which demonstrated how reproducible the method is (Figure 5) and that the headspace showed a certain level of homogeneity. These results are also in line with the odor profile description for these toilets that indicated they smelled more of sewage due to CH_3SH and H_2S and contained less butyric acid, *p*-cresol, indole, and skatole.

The organic extract (pH 8) eluted from the SPE cartridge was also analyzed by GC−MS in scan mode for qualitative information, and we found the same compounds as described previously.[1] GC−MS coupled to an olfaction port showed the importance of di(tri)-methyl disulfide, guaiacol, and other aromatic compounds such as alkyl phenols, also described previously.[1] However, NEM derivatives were also present; therefore, these organic extracts were used only for quantification, and no intensive GC-O was performed. The organic extract of the acidified water showed the occurrence of branched short chain fatty acids, phenyl acetic acid, and phenyl propionic acid.[1]

The pit latrines visited in Nairobi were well maintained and connected to sewage pipes. There was a block of six latrines and a urinal. The urinal had a strong ammonia and trimethyl amine smell, but trimethyl amine was not analyzed. The latrine smell was weak and described as slightly barnyard. An important air flux flowed across these toilets. The typical odor of sewage was absent, and this was confirmed with the analytical results, as no methyl mercaptan was detected, and H_2S was detected but at a concentration below the limit of quantification (LOQ) (<8.7×10^{-4} µg/L). In this toilet, the contribution of indole was minor, as it was measured at 4×10^{-5} µg/L near its ODT (see VP Mukuru A in Figure 6). In the VIP Bester A and B latrines, only *p*-cresol was in a sufficient amount for detection and quantification, corresponding to the weak animal and fecal odor that we described (Figure 6).

The odors of urine-diverting (UD) toilets in Nairobi and Durban were stronger than those in the pit latrine of Nairobi described above. The presence of methyl mercaptan was in the range of 2.0×10^{-4}−1.6×10^{-2} µg/L air for all UD toilets, which confirmed its strong contribution to the odor. We also found H_2S at 1.2×10^{-2}−2.7×10^{-2} µg/L air, with butyric acid, *p*-cresol, and indole in the headspace giving a typical latrine odor profile (Figure 6). The smell of urine was also distinctive. For this reason, trimethyl amine was analyzed in the last UD toilet visited in South Africa. To specifically analyze trimethyl amine, we prepared a second trap containing an acidic ion-exchange resin.

The smell of urine was important in the UD toilets in Durban (Bester area) on the DEWATS (Decentralized-Wastewater-Treatment System) experimental site. This was probably due to the dried urine on the urinal or the two-way pit to separate urine from feces. We found 3.6×10^{-4} µg/L air of trimethyl amine, a concentration near the ODT (5×10^{-4} µg/L air).

Determination of ODT of Target Compounds. The ODT of butyric acid, *p*-cresol, indole, skatole, and methyl mercaptan was measured in our laboratory in two steps. First, the dose−intensity curve was established for each compound with 35 panelists to find a range of concentrations near the ODT. Second, the ODT for each compound was established with 35 panelists from an alternative forced-choice triangle test by using the concentrations found in the first step.[14] We established that for butyric acid, the ODT is 9×10^{-4} µg/L air; for *p*-cresol, 2×10^{-5} µg/L air; for indole, 6×10^{-5} µg/L air; for skatole, 5×10^{-6} µg/L air; and for methyl mercaptan, 4×10^{-5}µg/L. The ODT for trimethyl amine is 5×10^{-4} µg/L air (Figure 1).

DISCUSSION

Analysis. Hydrogen sulfide and methyl mercaptan are highly volatile and not stable; therefore, their derivatization under a more stable form was our first challenge. Many methods have been published, but our constraints included the kinetics of the reaction, stability on solid support (for shipping

DOI: 10.1021/acs.est.5b00692
Environ. Sci. Technol. 2015, 49, 6134−6140

by air back to our laboratories), and solubility in water. For example, *N*-(1-pyrene) maleimide was too hydrophobic, and acrylodan was not stable enough. NEM is probably not the best derivative reagent for H_2S, as explained previously, but it is the best for coping with our constraints.[15]

A promising alternative could be to use a honeycomb-patterned microchannel scrubber to improve extraction efficiency.[17] In their report, Toda et al. described using a microreactor for derivatization of methyl mercaptan with 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole and a portable fluorimeter. The LOQ was $4-6 \times 10^{-4}$ μg/L for methyl mercaptan.[17] Usually there are two ways to analyze volatile thiols. The first step is a concentration step, and the second is detection in liquid phase by LC−MS or gas phase by GC−MS, or with specific sulfur or fluorometric detectors. For example, selenium derivatives were used to quantify the methyl mercaptan in coffee by LC−MS with an LOQ of 1×10^{-3} μg/kg coffee, but we do not know the concentration factors.[18] An alternative would be to use *p*-hydroxy mercury benzoic acid to trap the methyl mercaptan. The conjugate could be trapped on a cationic ion-exchange resin,[19] but we preferred to avoid selenium and mercury in our study.

Many papers describe the analyses of thiol by GC−MS. The headspace concentration by SPME is the most common method. Derivatization on fibers is possible, and Mateo-Vivaracho et al. analyzed methyl mercaptan in wine at 5×10^{-4} μg/L.[20] However, this method is not applicable to quantification in an open toilet space. Dynamic headspace analysis with solid polymers, such as Tenax or Porapak adsorbent, could have been used as an alternative, but they are sensitive to humidity or temperature differences. In Ahmadabad, the temperature was 45 °C. Several reviews summarize the analytical methodologies for malodor assessment.[21−23] After an intensive literature search, we found no existing methods to precisely quantify methyl mercaptan, butyric acid, *p*-cresol, indole, or skatole in air.

It was also not possible to find high-quality studies describing the controlled release of precise concentrations of volatiles into the headspace. Usually H_2S and CH_3SH are liberated from their salts from calibrated solutions, which is unsatisfactory because of the equilibrium between the liquid phase and the gas phase. Suppliers of pressurized gas offer pressurized bottles of H_2S precisely diluted in N_2. A published and validated method for the controlled release of volatiles in the headspace was developed based on olfactometers and used for a study of perfumery raw materials to help develop perfume creations.[14,24] Therefore, we decided to develop our quantitative analysis of trace compounds in the air by pumping a fraction of the air coming from these olfactometers. To our knowledge, this is the first time that 350 L of air was analyzed by using a water scrubber to precisely quantify sulfur compounds, nitrogen-containing compounds, phenols, and organic acids at the same time. In our method, we concentrated 350 L of headspace to about 100 μL of diethyl ether for GC−MS injections.

Link between Odor Profile and Quantitative Data. The smell of toilet Vadaj A was described as fecal, butyric, manure, and vomit, and that of toilet B was clearly more animalic. These observations correlated with the analytical results. The significant difference between these two toilets was the ratio between methyl mercaptan and *p*-cresol versus butyric acid, which was five times higher for Vadaj B than Vadaj A. The presence of fresh fecal material explained the high content of butyric acid (2.0×10^{-1} μg/L air for toilet A, corresponding to an intensity of 4 (Figure 1)). The intensity values express the intensity of a similar concentration of the compound alone, but it is not possible to directly link these values to the odor tone in the mixture. The inlet of our sampling device was about half a meter from the ground, which is about the height of the nose of a squat pit user; therefore, we measured what a pit user smells while using these latrines. A well-designed ventilation port significantly decreases the concentration of odorant molecules in the toilet headspace, which explains the low concentration observed in the African pit latrine (Figure 6).

Odor Detection Thresholds. The Gemert odor threshold database[9] compiles all published ODTs; the differences can be up to six orders of magnitude for methyl mercaptan. This wide discrepancy can be explained by the variety of methods used to determine these ODTs. Here, in addition to the dose−intensity curves, we present ODTs that were established with the same method, allowing pertinent comparisons between ODTs for compounds found in the toilet malodors. For safety reasons, we did not determine the ODT and the dose−intensity curve for H_2S. From our data, skatole is the most potent odorant in toilet malodor compared with the other compounds among the molecules that we selected. Comparative ODTs and dose−intensity curves have been measured by using the same methodology and reported for the key odorants of latrine malodors.

In conclusion, methyl mercaptan and hydrogen sulfide are important for toilet malodor, as they push the odor in the sewage direction. In a well-ventilated pit latrine, they were not detected, and less volatile compounds such as butyric acid, *p*-cresol, and indole were more important. A difference in *p*-cresol of $2.4 \times 10^{-3}-1.2 \times 10^{-2}$ μg/L air (five times) turned the odor to barnyard and animalic. These analytical results helped in latrine malodor reconstitutions, and the appreciation of these odors was validated in India, Africa, and Europe via a careful sensory survey.[25] The precise understanding of the odor profile and the contribution of each compound to malodor is critical to design targeted systems for odor control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Descriptive texts and tables of experimental procedures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00692.

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Funding

This research was equally cofunded by Firmenich and the Bill and Melinda Gates Foundation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to express our appreciation to the following people: Dr. Myles F. Elledge, Senior Director of RTI International (USA), for helping us organize the survey in Ahmadabad; Ritu A. Sinha, Head-Strategic Alliances, RTI India; the team from SEWA (Self Employed Women Association) in Ahmadabad for organizing the odor sampling; in Durban, South Africa, Prof. Chris Buckley of the University of KwaZulu-

DOI: 10.1021/acs.est.5b00692
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Natal for coordinating the odor sampling, along with Dr. Lungi Zuma from e Thekwini Municipality. We would also like to thank Dr. Laurent Wünsche, Director of the Analytical Innovation group, for the critical remarks; M. Pierre-André Rebetez and Natacha Freiburghaus for dose-response relationship and odor threshold measurements; and M. Robert Brauchli for NMR measurements and descriptions.

■ ABBREVIATIONS

odor detection threshold	(ODT)
solid-phase microextraction	(SPME)
solid-phase extraction	(SPE)
internal standard	(IS)
single-ion monitoring	(SIM)
gas chromatography	(GC)
olfaction	(O)
mass spectrometry	(MS)
nuclear magnetic resonance	(NMR)
urine diverting	(UD)
limit of quantification	(LOQ)
N-ethyl maleimide	(NEM)
confidence interval	(CI)

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DOI: 10.1021/acs.est.5b00692
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