Supporting Information

Qualitative and Quantitative Analysis of Volatile Constituents from Latrines

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Summary

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Number of tables: 4

Chemicals

Two-centimeter custom SPME fibers, 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate, 85 μm carboxen/PDMS (CAR/PDMS), 65 μm PDMS/divinylbenzene (PDMS/DVB), and 55/30 μm DVB/CAR-PDMS, were purchased from Supelco (Bellefonte, PA, USA). Amber and clear headspace vials (20 mL) with screw caps were from MicroLiter Analytical Supplies, Inc. (Suwanee, GA, USA). Porapak Q sorbent (50/80 mesh) was from Supelco. An Alpha-2 pump from Ametek (Berwyn, PA, USA) was used for the dynamic headspace experiment. A stainless steel holder with a screen (2 cm diameter, 0.1 mm, 0.125 mm grid) from Supelco was used in the static headspace experiment. Oasis® HLB cartridges (60 μm, 6 g, 35 mL or 60 μm, 1 g, 20 mL) were purchased from Waters (Milford, MA, USA). The ceramic filter was a Katadyn Vario EU from Katadyne (Kemptthal, Switzerland) with the charcoal filter removed. The 20 L Ropak high-density polyethylene (HDPE) buckets were purchased from LINPAC Packaging (Birmingham, UK).

Trimethyl amine, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, allyl methyl sulfide, dimethyl trisulfide, butyric acid, isovaleric acid, 2-methyl butyric acid, 2-methoxy-4-methyl-1-phenol, phenol, *p*-cresol, 4-ethylguaiacol, *m*-cresol, 4-ethylphenol, eugenol, borneol, benzyl alcohol, caryophyllene, indole, and skatole were in-house flavor materials. d_6-Dimethyl disulfide, d_9-2-methyl butyric acid, d_9-3-methyl butyric acid, d_5-phenol, and d_7-indole were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). Ammonium hydroxide was purchased from EM Science (Gibbstown, NJ, USA), and d_6-dimethyl sulfide and methanol were from Sigma-Aldrich (St Louis, MO, USA).

Gas Chromatography (GC)-Mass Spectrometry (MS)

GC-MS analysis of solid phase microextraction (SPME) samples was performed on a GC-MS system with an Agilent 6890 gas chromatograph coupled with an Agilent model 5973N mass spectrometer. The system was installed with a polar Rtx®-Wax column (60 m × 0.32 mm ID × 1.00 μm film thickness, Restek, Bellefonte, PA, USA). Approximately 20% of the eluent of the column was diverted to the MS detector via an open split interface. Helium was used as carrier gas at a constant flow rate of 2.6 mL/min for the working column. The temperature of the GC injectors was 250 °C. Oven temperature program: 40 °C (1 min hold) to 100 °C at 5 °C/min, to 200 °C at 8 °C/min, and then to 240 °C (21.83 min hold) at 15 °C/min, giving a total run time of 50 min.

The mass spectrometer was operated in electron impact (EI) mode with an ionization voltage of 70 eV. The temperatures of the quadrupole and ion source were 150 °C and 230 °C, respectively. The MSD transfer line temperature was 280 °C. The mass scan range was from *m/z* 13 to *m/z* 450.

AMDIS deconvolution software version 2.65, NIST MS Search 2.0d (NIST, USA), commercial (NIST05 and Wiley 7) and in-house MS libraries, and in-house retention index libraries were used in the GC-MS data analysis. Table S1 lists all compounds detected.

GC-MS analysis of latrine volatile extracts obtained from Porapak Q or solid phase extraction (SPE) was performed on a GC 6890 (Agilent, Palo Alto, CA, USA) equipped with a fused silica SPWax-10 (30 m × 0.25 mm i.d., 0.25 μm film thickness) capillary column (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. The injection port was operated in split mode 1/10, the injection volume was 1 μL, and the carrier gas was helium at a constant flow rate of 0.7 mL/min. The

column was coupled to a 5973N Inert MS (Agilent). The mass spectra in EI mode were measured at 70 eV in a scan range from 30 to 300 *m/z*. The linear retention indices were calculated by linear extrapolation from the retention times of the analytes and the two closest alkanes eluting just before and just after the analyte.

Determination of SPE Recovery Factors at Various pHs

A tetrahydrofuran (THF) solution (0.1 mL) containing 0.4 mg or 0.1 mg of each compound was added to 50 mL of a buffer solution (pH 5, 6, or 7) in duplicate. The resulting solutions were loaded on two separate Oasis HLB cartridges (6 g). Each cartridge was eluted with 40 mL Et_2O containing 0.04 mg methyl octanoate as the internal standard (IS) and 8 mL (dead volume of the cartridge) Et_2O without IS. The eluent was concentrated to 1 mL and injected for GC-MS analysis. The same volume of the THF solution was directly added to 40 mL Et_2O containing 0.04 mg IS. This solution was also concentrated to 1 mL and injected for GC-MS analysis. The peak area ratios of each compound to IS were obtained for the two solutions and used to calculate the recovery factors of the compounds (Table S2).

Single Stool Analysis

A sample (70 g) of fresh stool, collected in a bucket, was transferred in a 500-mL glass beaker, which was loosely covered with aluminum foil to allow air to circulate. The volatile constituents in the stool were extracted by SPE at Day 0, Day 2, and Day 7 using the following procedure and analyzed by GC-MS. An aliquot of the stool (5 g) was taken from the beaker and mixed with water (50 mL) in a Falcon tube. The top layer of the turbid heterogeneous solution was filtered on sand and cotton wool. An aliquot of the filtrate (20 mL) was loaded on an Oasis

HLB cartridge (1 g). The cartridge was then washed twice with water (10 mL) and eluted with 10 mL Et_2O containing 1 μg/mL IS and 2 mL Et_2O without the IS. The eluent was dried over anhydrous Na_2SO_4, filtered, and concentrated under argon to about 0.1 mL.

To another aliquot of the filtrate (5 mL), concentrated HCl was added to adjust pH to 4 to extract the carboxylic acids. Since gel formation took place with the addition of HCl, SPE was not possible. Instead, water (10 mL) and Et_2O (10 mL, IS 1 mg/L) were added to perform solvent extraction of the acids. The mixture was thoroughly shaken and then centrifuged at 8400g to obtain an organic layer, which was separated, dried, and concentrated for GC-MS analysis.

The preceding process was repeated twice over a period of 3 weeks.

Isotope Dilution Assay of Latrine Malodor Compounds by SPME

Establishment of calibration curves

Non-labeled dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, butyric acid, 2-methyl butyric acid, 3-methyl butyric acid, phenol, *p*-cresol, indole, and skatole solutions at concentrations of ~10,000 mg/L were prepared in ethanol in separate 10-mL volumetric flasks. A mixture of these standards was prepared by diluting the stock solutions in a 10-mL volumetric flask using ethanol as the solvent. The final working standard solution mixture contained 50.0 mg/L dimethyl sulfide, 50.1 mg/L dimethyl disulfide, 50.1 mg/L dimethyl trisulfide, 505.4 mg/L butyric acid, 504.4 mg/L 2-methyl butyric acid, 501.0 mg/L 3-methyl butyric acid, 504.6 mg/L phenol, 505.5 mg/L *p*-cresol, 505.3 mg/L indole, and 505.5 mg/L skatole in ethanol.

Labeled d_6-dimethyl sulfide, d_6-dimethyl disulfide, d_9-2-methyl butyric acid, d_9-3-methyl butyric acid, d_5-phenol, and d_7-indole solutions at concentrations of ~10,000 mg/L were prepared in ethanol in separate 10-mL volumetric flasks. A mixture of these standards was prepared by diluting the stock solutions in a 10-mL volumetric flask using ethanol as the solvent. The final working standard solution mixture contained 50.1 mg/L d_6-dimethyl sulfide, 50.1 mg/L d_6-dimethyl disulfide, 507.3 mg/L d_7-butyric acid, 499.9 mg/L d_9-2-methyl butyric acid, 503.1mg/L d_9-3-methyl butyric acid, 505.4 mg/L d_5-phenol, and 501.3 mg/L d_7-indole.

Thirteen calibration solutions were prepared by transferring different volumes of these working solutions of the labeled (L) and non-labeled (NL) standard mixtures into 20-mL headspace vials containing 4 mL of DI water, the pH of which was adjusted to pH 6 with 1N HCl. The volumes of the L and NL standard mixtures transferred to the 13 samples were as follows: 5 μL of L + 195 μL of NL, 10 μL of L + 190 μL of NL, 20 μL of L + 180 μL of NL, 40 μL of L + 160 μL of NL, 60 μL of L + 140 μL of NL, 80 μL of L + 120 μL of NL, 100 μL of L + 100 μL of NL, 120 μL of L + 80 μL of NL, 140 μL of L + 60 μL of NL, 160 μL of L + 40 μL of NL, 180 μL of L + 20 μL of NL, 190 μL of L + 10 μL of NL, and 195 μL of L + 5 μL of NL.

Each of the freshly prepared calibration solutions was analyzed by SPME GC-MS using the same method as for sample analysis to determine the peak area ratios of the analytes versus the corresponding labeled standards. Calibration curves were established by plotting concentration ratios versus area ratios.

Labeled standard mixture for spiking latrine samples

d_9-3-Methylbutyric acid (263.0 mg), d_9-2-methylbutyric acid (261.5 mg), d_7-butyric acid (1258.8 mg), and d_5-phenol (51.2 mg) were weighed into a 10-mL volumetric flask, 2 mL of ethanol was

added to the flask, and the contents in the flask were carefully mixed. Subsequently, 100 μL of 10.78 mg/mL d_6-dimethyl sulfide, 1 mL of 113.2 ug/mL d_6-dimethyl disulfide, and 1 mL of 10.78 mg/mL d_7-indole were pipetted into the same flask. Additional ethanol was added to the flask to reach the mark. About 0.8 mL of the resulting well-mixed labeled standard mixture was transferred into 10 ampoules and the solutions were vacuum sealed for storage and transport. The solutions were kept in a freezer until the dates of field trips. About 50 g of latrine samples collected prior to dilution with water and SPME sampling was spiked with 200 μL of this solution. Ethyl esters of d_7-butyric acid, d_9-3-methylbutyric acid, and d_9-2-methylbutyric acid were seen in the SPME profiles of latrine volatiles, especially in those of the three Pune samples. However, it was estimated that neligible percentages of the deuterated acids, i.e., less than 2% of d_7-butyric acid and less than 0.3% of d_9-methylbutyric acid, were transformed into the corresponding ethyl esters.

Calculation of analyte concentrations

Integration of each selected ion peak was carried out by Chemstation software. After obtaining all the selected ions peak area values for each analyte and its labeled standard, we calculated peak area ratios for each analyte by dividing the peak area of the analyte ion by the peak area of the standard ion. The ratios of analyte concentration over labeled standard concentration in the samples were calculated by using the calibration equations and the area ratios. Finally, the analyte concentrations were determined from the concentration ratios and the concentrations of the labeled standards in the samples.

Development of Isotope Dilution Assay Method

The first step in the development of an isotope dilution assay (IDA) method is to acquire appropriate isotope-labeled IS for each analyte. A minimum of two labeled atoms and non-exchangeable deuterium are the criteria for labeled ISs. The labeled standards used in this work are listed in Table S3. All of them had more than two labeled atoms, but one deuterium in d_7-indole is exchangeable. However, it is still an appropriate labeled IS as long as the ion generated from exchanged d_7-indole is taken into consideration in the calculation, because the resulting ion can still be differentiated from the corresponding ion generated from non-labeled indole due to the high number of labeled atoms. Isotope-labeled standards for dimethyl trisulfide, *p*-cresol, and skatole were not available. As a compromise, they were quantified by a pseudo IDA method, where d_6-dimethyl disulfide, d_5-phenol, and d_7-indole were used as their ISs, respectively.

The next step was to select proper quantifying ions by comparing the mass spectra of the analytes with those of the corresponding labeled ISs. Ions of high intensity are preferred for better sensitivity, and ions of high specificity, such as molecular ions, ions with high *m/z*, and even-numbered ions, are preferred to minimize ion interferences. The quantifying ions selected for the analytes and labeled ISs are listed in Table S3. Clusters of ions, i.e., *m/z* 116, 117 and *m/z* 121, 122, 123, were selected for indole and d_7-indole, respectively, due to an exchangeable deuterium in d_7-indole. Since three of the compounds were to be quantified by a pseudo IDA method, calibration solutions were prepared in water, and then the same SPME method used for sample analysis was used to collect both non-labeled and labeled standard compounds for GC-MS analysis. Calibration curves over a wide concentration of ratio ranges (from 0.02 to 20, 4 orders of magnitude) were established because we had no idea of the levels of the analytes in field latrines. Linearity of the curves was not always good over this wide range. Therefore, during sample quantification, calibration curves over appropriate ranges were used to calculate

analyte concentrations to improve accuracy, depending on the area ratios obtained in each sample.

During sample analyses, the amounts of d_6-dimethyl disulfide used to spike a few of the latrine samples were extremely low compared with the amounts of dimethyl disulfide present in the sample. Consequently, no signals of d_6-dimethyl disulfide were observed and dimethyl disulfide in those samples had to be quantified by pseudo IDA, using d_6-dimethyl sulfide as the IS. In a few other cases, *m/z* 79 from d_9-2-methyl butyric acid suffered seriously from interference by a coeluting compound, leading to significant errors in the IDA of 2-methyl butyric acid. Conversely, pseudo IDA of 2-methyl butyric acid, using d_9-3-methyl butyric acid as the IS, was much more accurate because both quantifying ions were well resolved and the physical chemical properties of the analyte and the IS were very similar. All of the calibration curves, both IDA and pseudo IDA, and their parameters used in this work are listed in Table S3. As expected, the slopes of the IDA calibration curves often fall between 0.9 and 1.1 because their physical chemical properties and ionization behaviors are very similar. On the other hand, the slopes of the pseudo IDA calibration curves can be far from 1, which results from the differences in physical chemical properties and ionization behaviors between analyte and IS.

The reproducibility of the method was checked using a model latrine sample. Since pHs of latrines were found to be either slightly acidic or slightly basic, the quantification experiment was performed at both pH 6 and pH 8 with duplicates. The results obtained are listed and compared in Table S4. At pH 8, the reproducibility of the method was very good, except for very low level dimethyl disulfide and dimethyl trisulfide, where the relative standard deviation (RSD) was 17% and 42%, respectively. The imperfect RSD of indole and skatole determined at pH 6 was attributed to slightly different procedures applied to the duplicates. The pH of one sample

was adjusted before the addition of ISs, while that of the other was adjusted after the addition of IS. The concentrations determined at two different pHs agreed with each other quite well, indicating that the method was not pH dependent. The lower concentrations of carboxylic acid obtained at pH 8 was due to more severe interference of ion *m/z* 63 and especially *m/z* 79 under more basic conditions, where the signals of carboxylic acids, both analytes and ISs, are much weaker. Since the matrices of latrine sludge are complex and inhomogeneous, and the dynamics of microbiological and chemical activities may continue or be altered by sampling, it is critical to be highly consistent in sample preparation to ensure reproducibility. In addition, because volatile constituents of latrine sludge are complex and vary from one another, careful investigation of possible interference is necessary for accuracy. This method is advantageous in that interferences can be recognized by inspection of mass spectra and ion chromatograms. In addition, the method is flexible in that another IS or quantifying ion can be chosen when needed.

Spiking each latrine sample with appropriate amounts of ISs was critical to successful quantification of the 10 selected analytes in field latrines because repeating experiments can be costly or even impossible. This challenge was partially overcome by using the levels in the model latrine to guide the amounts of ISs for spiking. The approximate levels of ISs for spiking and the concentrations of ISs in the labeled standards solution are also listed in Table S4. The concentrations of the analytes in the model system ranged from 0.01 to 1000 μg/g, the differences covering 5 orders of magnitude. Therefore, to manage these expected enormous differences in analyte concentrations, the appropriate amounts of the labeled ISs for spiking were arbitrarily determined to be from 0.05 to 500 μg/g for different analytes.

Table S1. Occurrence and abundance of 198 volatile constituents detected in 16 latrines.

Compound Name	ID criteria	Name of latrines																Frequency of occurrence
		Durban VIP	Durban UD	Nairobi VP1	Nairobi VP2	Nairobi Fresh life 1	Nairobi Fresh life 2	Kampala VP1	Kampala VP2	India A	India B	India C	Model latrine A	Model latrine B	Model latrine C	Model latrine D	Model latrine E	
Nitrogen-Containing Compounds																		
trimethylamine	MS,RI																	5
ammonia	MS,RI																	5
1-methylpiperidine	MS																	1
butanenitrile	MS																	3
1-methylpyrrole	MS,RI																	2
2-butenenitrile	MS																	1
pyridine	MS,RI																	5
2-methylpyridine	MS, RI																	1
4-pentenenitrile	MS																	2
3-methylpyridine	MS																	1
1H-pyrrole	MS,RI																	3
2-methylbutyronitrile	MS																	1
indole	MS,RI																	14
skatole	MS,RI																	11
Sulfur-Containing Compounds																		
methyl mercaptan	MS,RI																	7
carbon disulfide	MS																	13
dimethyl sulfide	MS,RI																	10
1-propanethiol	MS,RI																	1
sulfur dioxide	MS																	8
allyl mercaptan	MS																	1
propyl methyl sulfide	MS																	2
allyl methyl sulfide	MS,RI																	4
(Z)-1-propenyl methyl sulfide	MS																	3
(E)-1-propenyl methyl sulfide	MS																	2
methyl thioacetate	MS																	3
dimethyl disulfide	MS,RI																	13
S-methyl propanethioate	MS																	2
methyl thiobutyrate	MS,RI																	3
S-methyl 3-methylbutanethioate	MS,RI																	2
S-methyl 2-methylbutanethioate	MS																	1
propyl methyl disulfide	MS,RI																	1
allyl methyl disulfide	MS,RI																	3
(E)-1-propenyl methyl disulfide	MS																	1
S-methyl pentanethioate	MS																	1
2,4-dithiapentane	MS																	4
ethyl methanesulfinate	MS																	1
1-(methylthio)-2-propanone	MS																	1
allyl isothiocyanate	MS,RI																	3
1,1-bis(methylthio)propane	MS																	3
dimethyl trisulfide	MS,RI																	10
3-methyl-2,5-dithiahexane	MS																	4

Compound Name	ID criteria	Name of latrines																Frequency of occurrence
		Durban VIP	Durban UD	Nairobi VP1	Nairobi VP2	Nairobi Fresh life 1	Nairobi Fresh life 2	Kampala VP1	Kampala VP2	India A	India B	India C	Model latrine A	Model latrine B	Model latrine C	Model latrine D	Model latrine E	
2-(methylthio)ethanol	MS																	1
propyl methyl trisulfide	MS																	2
dimethyl sulfoxide	MS																	5
allyl methyl trisulfide	MS																	1
Carboxylic Acids																		
acetic acid	MS,RI																	13
formic acid	MS,RI																	1
propionic acid	MS,RI																	11
isobutyric acid	MS,RI																	8
butyric acid	MS,RI																	10
isovaleric acid	MS,RI																	8
2-methylbutyric acid	MS,RI																	8
pentanoic acid	MS,RI																	7
2(E)-butenoic acid	MS																	1
4-methylvaleric acid	MS																	3
hexanoic acid	MS,RI																	7
2-ethylhexanoic acid	MS,RI																	1
heptanoic acid	MS,RI																	4
octanoic acid	MS,RI																	3
cyclohexanecarboxylic acid	MS																	4
benzoic acid	MS																	2
dodecanoic acid	MS,RI																	1
Phenols																		
guaiacol	MS,RI																	4
phenol	MS,RI																	12
p-cresol	MS,RI																	14
m-cresol	MS,RI																	3
thymol	MS,RI																	1
4-ethylphenol	MS,RI																	1
carvacrol	MS,RI																	2
Alcohols																		
methanol	MS,RI																	3
2-propanol	MS,RI																	4
2-butanol	MS,RI																	9
1-propanol	MS,RI																	8
isobutanol	MS,RI																	7
2-pentanol	MS,RI																	2
1-butanol	MS,RI																	11
isoamyl alcohol	MS,RI																	7
2-methyl-1-butanol	MS,RI																	5
1-pentanol	MS,RI																	11
3-methyl-3-buten-1-ol	MS,RI																	2

Compound Name	ID criteria	Name of latrines																Frequency of occurrence
		Durban VIP	Durban UD	Nairobi VP1	Nairobi VP2	Nairobi Fresh life 1	Nairobi Fresh life 2	Kampala VP1	Kampala VP2	India A	India B	India C	Model latrine A	Model latrine B	Model latrine C	Model latrine D	Model latrine E	
4-heptanol	MS																	2
4-methyl-1-pentanol	MS,RI																	2
1-hexanol	MS,RI																	10
3(Z)-hexen-1-ol	MS,RI																	1
4-methyl-3-penten-1-ol	MS,RI																	1
cyclohexanol	MS,RI																	2
1-octen-3-ol	MS,RI																	2
1-heptanol	MS,RI																	5
linalool	MS,RI																	4
2-ethyl-1-hexanol	MS,RI																	8
1-octanol	MS,RI																	2
beta-fenchol	MS,RI																	1
isomenthol	MS,RI																	3
menthol	MS,RI																	9
3(Z)-nonen-1-ol	MS,RI																	1
2-pinen-4-ol	MS																	1
alpha-terpineol	MS,RI																	1
borneol	MS,RI																	2
dihydrocarveol	MS,RI																	1
citronellol	MS,RI																	1
1,4-butanediol	MS,RI																	4
phenethanol	MS,RI																	1
epicubenol	MS																	1
T-cadinol	MS																	1
1-hexadecanol	MS,RI																	2
Aldehydes																		
acetaldehyde	MS,RI																	6
propanal	MS,RI																	3
butanal	MS,RI																	3
2-methylbutanal	MS,RI																	2
3-methylbutanal	MS,RI																	1
pentanal	MS,RI																	2
hexanal	MS,RI																	2
nonanal	MS,RI																	3
2(E)-nonenal	MS,RI																	1
benzaldehyde	MS																	8
2(E)-decenal	MS,RI																	1
Ketones																		
acetone	MS,RI																	12
2-butanone	MS,RI																	12
3-methyl-2-butanone	MS,RI																	1
2,3-butanedione	MS,RI																	6

Compound Name	ID criteria	Name of latrines																Frequency of occurrence
		Durban VIP	Durban UD	Nairobi VP1	Nairobi VP2	Nairobi Fresh life 1	Nairobi Fresh life 2	Kampala VP1	Kampala VP2	India A	India B	India C	Model latrine A	Model latrine B	Model latrine C	Model latrine D	Model latrine E	
2-pentanone	MS,RI																	7
3-methyl-3-buten-2-one	MS,RI																	3
3-hexanone	MS																	2
2-methyl-3-pentanone	MS, RI																	2
4-methyl-2-pentanone	MS, RI																	1
2,3-pentanedione	MS, RI																	2
2-methyl-1-penten-3-one	MS																	2
2-hexanone	MS,RI																	3
4-heptanone	MS,RI																	6
2-heptanone	MS,RI																	5
cyclopentanone	MS																	1
3-octanone	MS,RI																	2
2-octanone	MS,RI																	2
3-hydroxy-2-butanone	MS,RI																	2
2-methylcyclopentanone	MS																	1
cyclohexanone	MS,RI																	1
6-methyl-5-hepten-2-one	MS,RI																	4
2-nonanone	MS,RI																	2
fenchone	MS																	2
camphor	MS																	1
trans-dihydrocarvone	MS																	1
piperitone	MS																	2
Esters																		
ethyl formate	MS,RI																	3
methyl acetate	MS,RI																	3
ethyl acetate	MS,RI																	8
methyl propionate	MS,RI																	4
ethyl propionate	MS,RI																	6
propyl acetate	MS,RI																	4
methyl butyrate	MS,RI																	3
ethyl butyrate	MS,RI																	6
propyl propionate	MS,RI																	4
n-butyl acetate	MS,RI																	3
methyl pentanoate	MS,RI																	1
propyl butyrate	MS,RI																	4
ethyl valerate	MS,RI																	1
butyl propionate	MS,RI																	1
pentyl acetate	MS,RI																	1
methyl caproate	MS,RI																	2
propyl valerate	MS																	1
butyl butyrate	MS,RI																	3
ethyl caproate	MS,RI																	1

Compound Name	ID criteria	Name of latrines																Frequency of occurrence
		Durban VIP	Durban UD	Nairobi VP1	Nairobi VP2	Nairobi Fresh life 1	Nairobi Fresh life 2	Kampala VP1	Kampala VP2	India A	India B	India C	Model latrine A	Model latrine B	Model latrine C	Model latrine D	Model latrine E	
ethyl tiglate	MS,RI																	1
methyl heptanoate	MS,RI																	1
n-amyl butyrate	MS,RI																	1
propyl caproate	MS,RI																	2
ethyl heptanoate	MS,RI																	1
butyl caproate	MS,RI																	1
ethyl caprylate	MS,RI																	1
4-terpinenyl acetate	MS,RI																	1
alpha-terpinyl acetate	MS,RI																	1
Hydrocarbons																		
pentane	MS																	5
3-methylpentane	MS																	1
octane	MS,RI																	5
3,7-dimethyl-2-octene	MS																	1
alpha-pinene	MS,RI																	6
toluene	MS,RI																	6
beta-pinene	MS,RI																	6
sabinene	MS,RI																	1
ethylbenzene	MS																	1
dimethylbenzene	MS																	4
delta-3-carene	MS,RI																	2
1-p-menthene	MS																	1
limonene	MS,RI																	9
beta-phellandrene	MS,RI																	1
p-cymene	MS,RI																	8
alpha-longifolene	MS																	2
beta-caryophyllene	MS																	5
delta-cadinene	MS																	1
naphthalene	MS																	2
gamma-cadinene	MS																	1
Miscellaneous																		
2,3-dihydrofuran	MS																	3
tetrahydrofuran	MS																	3
1,8-cineole	MS,RI																	1
gamma-butyrolactone	MS																	3
trans-anethole	MS																	1
Unknown	na																	1
Unknown	MS																	1
Unknown	na																	1

Peak area $<10^{+5}$, not listed; peak area$<10^{+6}$, faint blue; peak area $<10^{+7}$, light blue; peak area<5 10^{+7}, blue; peak area >5 10^{+7}, dark blue. MS = mass spectrometry, RI = retention index.

Table S2. Recovery factors of the compounds from SPE at different pHs.

Compound	**pH 5**		**pH 6**		**pH 7**	
	Rec (%)	**SDev**	**Rec (%)**	**SDev**	**Rec. (%)**	**SDev**
Butyric acid	69	0.3	3	0.6	2	0.1
3-Methylbutyric acid	100	7	69	16	1	0.03
Dimethyl trisulfide	99	8	77	1	88	0.3
Hexanoic acid	93	6	97	7	32	1
p-Cresol	103	5	97	0.5	103	11
Indole	89	2	90	16	96	2
Skatole	96	5	97	9	100	0.2

Rec = Recovery; SDev = Standard Deviation

Table S3. Labeled IS, quantifying ions, and calibration curves used to quantify all analytes.

Analyte and quantifying ion	IS and quantifying ion	Equation	R^2	Area ratio range
Isotope dilution assay calibration curves				
Dimethyl sulfide, *m/z* 62	d_6-dimethyl sulfide, *m/z* 68	y = 1.0796x	0.990	0.004-1
Dimethyl sulfide, *m/z* 62	d_6-dimethyl sulfide, *m/z* 68	y = 0.8541x	0.990	1-10
Dimethyl disulfide, *m/z* 94	d_6-dimethyl disulfide, *m/z* 100	y = 0.9125x	0.996	0.80-10
Butyric acid, *m/z* 60	d_7-butyric acid, *m/z* 63	y = 0.9963x	0.998	0.09-19
3-Methylbutyric acid, *m/z* 60	d_9-3-Methylbutyric acid, *m/z* 63	y = 0.8935x	0.997	0.07-1
3-Methylbutyric acid, *m/z* 60	d_9-3-Methylbutyric acid, *m/z* 63	y = 0.8565x	0.990	1-10
2-Methylbutyric acid, *m/z* 74	d_9-2-Methylbutyric acid, *m/z* 79	y = 1.1385x	0.998	0.06-4
Phenol, *m/z* 94	d_5-phenol, *m/z* 99	y = 0.9341x	0.992	0.04-1.5
Indole, *m/z* 116, 117	d_7-indole, *m/z* 121, 122, 123	y = 0.9718x	0.998	0.06-4
Indole, *m/z* 116, 117	d_7-indole, *m/z* 121, 122, 123	y = 0.9283x	0.980	1-20
Pseudo isotope dilution assay calibration curves				
Dimethyl disulfide, *m/z* 94	d_6-dimethyl sulfide, *m/z* 68	y = 0.0628	0.997	7-150
Dimethyl trisulfide, *m/z* 126	d_6-dimethyl disulfide, *m/z* 100	y = 0.3584x	0.997	0.05-2.71
Dimethyl trisulfide, *m/z* 126	d_6-dimethyl sulfide, *m/z* 68	y = 0.0288x	0.998	10-300
2-Methylbutyric acid, *m/z* 74	d_9-3-Methylbutyric acid, *m/z* 63	y = 1.0353	0.999	0.03-1
p-Cresol, *m/z* 107	d_5-phenol, *m/z* 99	y = 0.7279x	0.991	0.04-1.5
p-Cresol, *m/z* 107	d_5-phenol, *m/z* 99	y = 0.6384x	0.990	1-15
Skatole, *m/z* 130	d_7-indole, *m/z* 121, 122, 123	y = 0.4656x	0.993	0.135-5

Table S4. Levels of the 10 analytes in the model latrine determined at two pHs, the approximate levels of ISs for spiking, and the concentrations of ISs in the labeled standards solution.

Analyte	Determined at pH 8		Determined at pH 6		Approximate IS conc. for spiking (μg/g)	Conc. in the IS solution (μg/μL)
	Average conc. (μg/g)	RSD (%)	Average conc. (μg/g)	RSD (%)		
Dimethyl sulfide	0.59	5	0.5	5	0.5	0.11
Dimethyl disulfide	0.05	17	0.05	3	0.05	0.011
Dimethyl trisulfide	0.002	42	0.01	18	n.a.	n.a.
Butyric acid	874.7	5	924	9	500	125.9
3-Methylbutyric acid	136.7	2	150.5	1	100	26.3
2-Methylbutyric acid	108.9	6	148.7	5	100	26.2
Phenol	14.4	6	13.5	7	20	5.1
p-Cresol	70.1	4	62.8	1	n.a.	n.a.
Indole	5.0	4	4.6	22	5	1.1
Skatole	7.4	2	6.2	11	n.a.	n.a.

RSD = Relative Standard Deviation; n.a. = not applicable