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Article

Simultaneous chemical and sensory analysis of cat urine and feces with headspace solid-phase microextraction and GC-MS-olfactometry

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Abstract: The association between human and cat (*F. catus*) is well known. This domestic animal is also known for its malodorous urine and feces. The complexity of the odorous urine and feces impacts human life by triggering the human sensory organ in a negative way. The objective of this research was to identify the volatile organic chemicals (VOCs) and associated odors in cat urine and feces using gas chromatography-mass spectrometry and simultaneous sensory analysis of fresh and aged samples. The solid-phase microextraction (SPME) technique was used to pre-concentrate the VOCs emitted from urine or feces samples. Twenty-one compounds were identified as emitted from fresh urine, whereas 64 compounds were emitted from fresh feces. A contrasting temporal impact was observed on the emission of VOCs for urine and feces. On aging, the emission increased to 36 detected chemicals for stale urine, whereas only 17 chemicals were detected in stale feces. Not all compounds were malodorous; some compounds had a pleasant hedonic smell to the human nose. Although trimethylamine, low molecular weight organic acids, and ketones were contributors to the odor to some extent, phenolic compounds and aromatic heterocyclic organic N compounds generated the most intense odors and substantially contributed to the overall malodor, as observed by this study. This work might be useful to formulate cat urine and feces odor remediation approaches to reduce odor impacts.

Keywords: feline, smell, odor, SPME, GC-MS-O, VOCs, felinine

1. Introduction

The companion between human and cat (*F. catus*) is more than 8,000 years old [1]. The market research statistics by the American Veterinary Medical Association counted 74 million domestic cats in the USA in a report presented in 2012 [2]. This popular companion of humans is building their importance in human life as a family member. While cat owners love their cats, they have a less positive relationship with their cats' litterbox due to several factors, including the smell of urine and feces.

The potent odor of domestic cat urine caused a continually growing research interest (Table1). Improved separation and identification techniques were used over the decades to report compounds with a catty smell. One of the responsible specific amino acid felinine excreted by the Felidae family does not have a specific odor, but the degradation products of felinine are odorous. Fractionation and separation of felinine and its derivatives were done using paper chromatographic techniques and spot tests in earlier studies [3]. Using GC-MS total ion chromatogram of the cat urine headspace analysis, Miyazaki et al. (2006) identified a total of 25 compounds in the male domestic cat urine [4]. They reported the urinary protein Cauxin to be involved in felinine production. Felinine, the sulfur-containing amino acid, is carried in the cat bloodstream as 3-methylbutanolglutathione (MBG) [5]. An increase in testosterone concentration can increase the free Felinine in the male and female cat [6] because testosterone increases the production of MBG and shifts the distribution of MBG metabolites towards the generation of free felinine. In addition to felinine, several organic chemicals can be emitted from the cat urine and feces, depending on the age and sex-related factors of cats.

Cat urine and feces contain several volatile and non-volatile compounds that help to recognize sex and species [7]. These volatile compounds emitted through the urine and feces also act as chemical signaling in mammals to define their territory, dominance, and reproduction [8,9]. The stray and domestic cats also use urine as chemical signaling and intend to bury their feces around the home range [10], and that odor of cat urine and feces can be annoying to humans. Research articles have been published that are focused more on the odorous components in cat urine and less on feces, although both waste products are putrid. The concentration of VOCs emitted by cat feces can significantly differ with the cat age and sex irrespective of the food diet or habitat, like 1-butanol in feces found significantly less in concentration in female cats and indole and phenol like odorous compounds can increase with the age of male cats. Moreover, the aging of the cat urine and feces emits odorous chemicals.

A recent study by Suzuki et al., 2019 reported a significant impact of time (fresh and 24 h) on VOC released from the same urine sample, and the reason was provided to be the degradation of VOCs by bacteria in urine, urinary enzymatic reaction, or oxidation [11]. Fresh cat urine does not emit a strong odor and can be described as 'ammonia-' and 'savory-like'; however, on interaction with soil, bacteria can emit cat urine smell describes as 'intensely fishy' [12]. These experiments were set to find the cat species chemical signaling for habituation-dishabituation and may not guide to resolve odor issues for human annoyance. Simultaneous chemical identification and sensory analysis of the VOC data from cat excrete by the human nose is still limited; moreover, a simple technique and temporal data set are always in need to build the odor profile emitted from cat urine and feces. The water intake by cats can vary with their food diet, and the volume of water intake can end up in a release of different amounts of urine or feces samples [13].

It is only a decade that scientist is using solid-phase microextraction (SPME) for biological sample VOC extraction. It is considered as a non-invasive sampling device that extracts biomarkers for early diagnosis of advanced or chronic diseases or reports impurity in food samples to assess food quality [14]. The study also reported that SPME is 10-50 times more efficient than any static headspace sampling. Moreover, the use of SPME able to extract volatile organic compounds (VOCs) from a biological sample in both *ex vivo* and *in vitro* and analyze using GC-MS. This approach of sample extraction can reduce sample preparation steps and extract the chemicals without modifying its original form. The use of SPME fiber has only recently been reported for marking fluid extraction and identification of *Panthera tigris* subspecies [8]. There is not any research data recorded as per our knowledge on odorous chemicals emitted by urine and fecal samples of domestic cat species using SPME-GC-MS-O chemical and sensory analysis. This current study designed is to find out the temporal behavior of odorous compounds emitted from cat urine and feces in a non-invasive way for the betterment of human territory.

The current study's objective is to use SPME fiber extraction to identify odorous VOCs emitted from fresh and aged urine and feces of domestic cat species. This SPME-GC-MS-O will simplify the chemical and sensory characterization of odorous compounds emitted from cat urine and feces. May also answer the question: Is it possible to predict the odor intensity using GC-MS chemical analysis? This study might also help formulate cleaners and other remediation techniques to reduce cat urine and feces odor problems in the long term.

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Table 1: Literature related to domestic cat urine and feces sample preparation for chemical and sensory analysis to identify and compare the chemical constituents of the cat excretes

Species	Sample type	Sample preparation	Chemical* analysis	Sensory analysis	Identified compounds	Study purpose	Reference
Domestic Cat (Felis catus)	Urine	Solvent extraction and derivatization	Paper chromatography	Not conducted	Felinine and amino acids	Separation of felinine and its derivatives	Westall, R.G. 1953 [3]
Domestic Cat (Felis catus)	Urine	Solvent extraction and membrane concentration	HPLC, GC-MS-headspace	Not conducted	Carboxylesterases cauxin, felinine and felinine derivatives.	To identify hydrolyzed products of cauxin and degradative products of felinine.	Miyazaki, M. et al. 2006 [4]
Domestic Cat (Felis catus)	Urine and blood	Solvent extraction and derivatization	HPLC	Not conducted	Felinine, N-acetylfelinine, creatinine, testosterone, and estradiol	Quantify felinine and NAcFel and report effects of testosterone and estradiol on free felinine, NAcFel, and c-glutamylfelinylglycine	Hendricks, W.H. et al. 2008 [6]
Domestic cat (Felis catus)	Urine and soiled urine	Solvent extraction	UPLC-MS, GC-MS-O, and NMR	GC-MS-O	34 volatile and non-volatile chemicals synthesized and reported. 14 odor attributes reported.	Identify the key odorants in cat urine	Starkenmann, C. et al. 2014 [12]

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Domestic Cat (Felis catus)	Fresh feces	Gas sampling	GC-MS	Not conducted	24 volatile organic compounds	Sex and age determination using volatile compounds emitted from fecal samples	Uetake, K. et al. 2017[10]
Domestic Cat (Felis silvestris catus)	Anal sac secretions	Sorbed onto Tenax TA tube	TD-GC-MS	Olfactory of cats	10 free fatty acids	Olfactory habituation-dishabituation to determine behavioral bioassays	Miyazaki, T. et al. 2018 [17]
Domestic Cat (Felis silvestris catus)	Fresh and up to 24 h aged cat urine	GC X GC-MS VOC preconcentrator	GC-MS	Olfactory of cats	36 compounds	Discriminate temporal changes and individual differences in urine	Suzuki, S. et al. 2019 [11]

HPLC: High-performance liquid chromatography; GC-MS: Gas chromatography; UPLC-MS: Ultra-performance liquid chromatography-mass spectrometry, NMR: Nucleic magnetic resonance

2. Materials and Methods

2.1. Cat urine and feces collection

The cat urine and feces samples were collected at Nestlé Purina pet facility. Freely collected urine and feces samples were homogenized and immediately frozen at -20 °C upon collection. Urine and feces samples were shipped in a cooler box with dry ice protection via next day air to Iowa lab for analysis.

2.2. Sample storage

After receiving the samples from the sample collector, all urine and feces samples were stored at -20 ˚C until they were analyzed. A week before analysis, they were moved to a freezer at – 4 ˚C and thawed on the analysis day in the morning at lab temperature (24 ˚C) for 3-4 hours. Each sample was then weighed approximately 1g into an amber color 10 ml vial in duplicate using a disposable dropper or spatula. The aging of urine and feces was performed at lab temperature, and the extraction was performed at 37 ˚C.

2.3. Multidimensional gas chromatography-mass spectrometry olfactometry

All sample analysis of cat urine and feces were completed using the multidimensional gas chromatograph-mass spectrometer olfactometer (GC-MS-O). All compounds emitted from the sample vial headspace were extracted using SPME fiber of 2 cm 50/30 µm DVB/PDMS/Carboxen (57248-U, Supelco, Bellefonte, PA, USA). The samples were heated to 37 °C during extraction to enhance the emissions. A 50 min extraction time was used for all of the extractions except an additional 10 min extraction was done for a fresh urine sample for comparison purposes. A schematic of the method is given in the appendix (Figure A1).

All the cat urine vials were kept at lab temperature (24 °C) for two weeks, and the feces samples were aged at lab temperature for one week to do the extraction of the aged urine and feces samples. These vial caps were left closed to avoid samples drying out and opened for a couple of minutes in the air every other day to avoid complete anaerobic situations. On the analysis day, the vials were closed for the VOCs to equilibrate and accumulate for an hr under lab condition, and then the vial was put on a hot plate set at 37 °C for 10 minutes before inserting the SPME fiber to extract the headspace VOCs for 50 mins.

After the extraction, the SPME fiber loaded with odorants inserted into the 260 °C GC injector for thermal desorption of samples to the GC columns and separation and analysis using MS and olfactometer. The GC-MS-O analysis was performed on an Agilent 6890 GC with a restrictor guard column, non-polar capillary column (BP-5, 30.0 m x 530 µm inner diameter x 0.5 µm thickness, SGE, Austin, TX, USA) and polar capillary column (BP-20, 30.0 m x 530 µm inner diameter x 0.5 µm thickness, SGE, Austin, TX, USA) connected in series. The sample flow was split 3:1 via an open split interface to an olfactometry port and mass spectrometer, respectively. The GC oven temperature was programmed at the initial 40 °C for 3 min, followed by ramping up to 240 °C at a rate of 7 °C/min, where it was maintained for 8.43 min. The quadrupole MS was using electron ionization mode with ionization energy of 70 eV during operation, and the full scan range was 34 to 350 m/z.

The odor event was detected by the panelist, and the aromagram peak is the intensity of the aroma event. The trained panelist was recording the start and end of the odor event, a description of the odor event, and the odor intensity. The odor intensity was evaluated on a 0-100% scale, where 0% means no odor, and 100% means the strongest odor detected by the panelist. A humidified air was constantly delivered at a rate of 5.7 psi to the panelist's nose to reduce the dry out of the mucus membrane during the analysis. Aromagrams for odors were generated using Aromagram software (version 6.0, Microanalytics, Round Rock, TX, USA).

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Analysis of the compounds and data files were generated from Agilent Chemstation software, and the peaks were identified using PBM-Benchtop software and matched using Wiley 7 and NIST database library.

3. Results

3.1. Identification of volatile organic compounds in cat urine and feces using GC-MS-O:

The use of simultaneous sensory analyses (via GC-MS-Olfactometry) enabled the detection of malodors that GC-MS could not detect (Figure 1). For example, the 10 min equilibration and 50 minute SPME extraction of 1-week old stale feces headspace had only 16 detectable compounds (via GC-MS), while the use of human nose enabled detection of as many 35 distinct odors (via GC-MS-Olfactometry). However, for fresh feces, GC-MS detected as many as 64 compounds, but the human nose (via GC-MS-Olfactometry) detected 37 distinct compounds. Tables 1 and 2 contain the odor descriptions for the chemical compounds matched with the NIST and Wiley7 chemical library. A list of the odor description is provided in the supplementary material (Tables S1-S6). The difference in the number of events occurs because a human nose is sensitive compare to a chemical analyzer. Moreover, each chemical can possess a distinct aroma or aroma pattern, and more than one chemical could have a similar aroma.

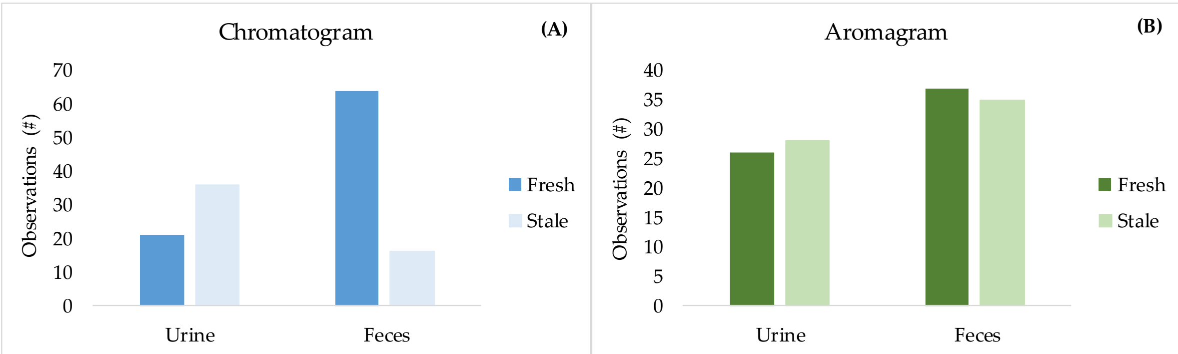

Figure 1. Comparison of the number of compounds detected in the headspace of fresh/stale urine and feces using: GC-MS analysis (Part A - Chromatogram) and GC-MS-Olfactometry analysis (Part B - Aromagram).

The refrigerated fresh urine produced 21 compounds and 26 odor events whereas, the 15 days old urine produces as many as 36 compounds, and the number of odor events was 28. Not all compounds could be classified as 'malodors.' Some of the compounds had a 'pleasant' hedonic smell, even in stale urine and feces samples. Phenolic compounds were among the most intense odors and a substantial contributor to malodors.

3.2. Temporal effect on volatile organic compounds in cat urine:

Exposure of SPME fiber to the urine sample improved the accumulation of several chemical compounds. A faint odor was recorded by the panelist, but the description was missing at this low concentration. The fresh urine had a mild odor, and upon short SMPE exposure did not reveal much information on odorous compounds. A short exposure of the fiber to the urine sample extracted most of the low molecular weight VOCs that long exposure time of SPME extracted. However, the high molecular weight VOCs were unable to reach the exposed fiber at short fiber exposer, and so the high molecular weight VOCs are absent in the list (Table 2). Moreover, the intensity of the many chemicals was low in concentration (low PAC) to distinguish the odor between different chemicals. The use of the GC-MS-olfactometer, however, senses the odors of phenolic compounds at short SPME exposure to the hedonic urine sample, although the odor intensity was low.

A dynamic aging temporal change was observed in the number of chemical compounds, odor events, and odor intensity observed in the headspace analysis of the urine and feces samples. Fresh cat urine has weak odor intensity and odors described as urine, indole, and animal-like. Stale urine has many intense odorous compounds (Table 2). A foul smell was recorded by the panelist at an RT of 2.7 min, possibly of the compound tri-methyl amine, although no compound was identified by the GC-MS. The identified compounds increased from 19 to 34 and worth mentioning that N containing compounds like pyrazine, pyrrole, pyrimidine, and some other ketones, aldehyde, and alcohols showed up with aging of the urine whereas dimethyl disulfide like malodors compound was only present in fresh urine. However, the intensity of the odor for the compounds present in the aged urine was higher than the fresh urine, as noticed by increasing the PAC. The presence of 2-Heptanone (fruity smell), limonene (mint-like smell) was observed in fresh urine samples for both long and short extraction time that was missing in the aged urine samples; however, jasmone (flowery smell) like good smell odor showed up in the aged urine sample absent in the fresh urine analysis (Table 2).

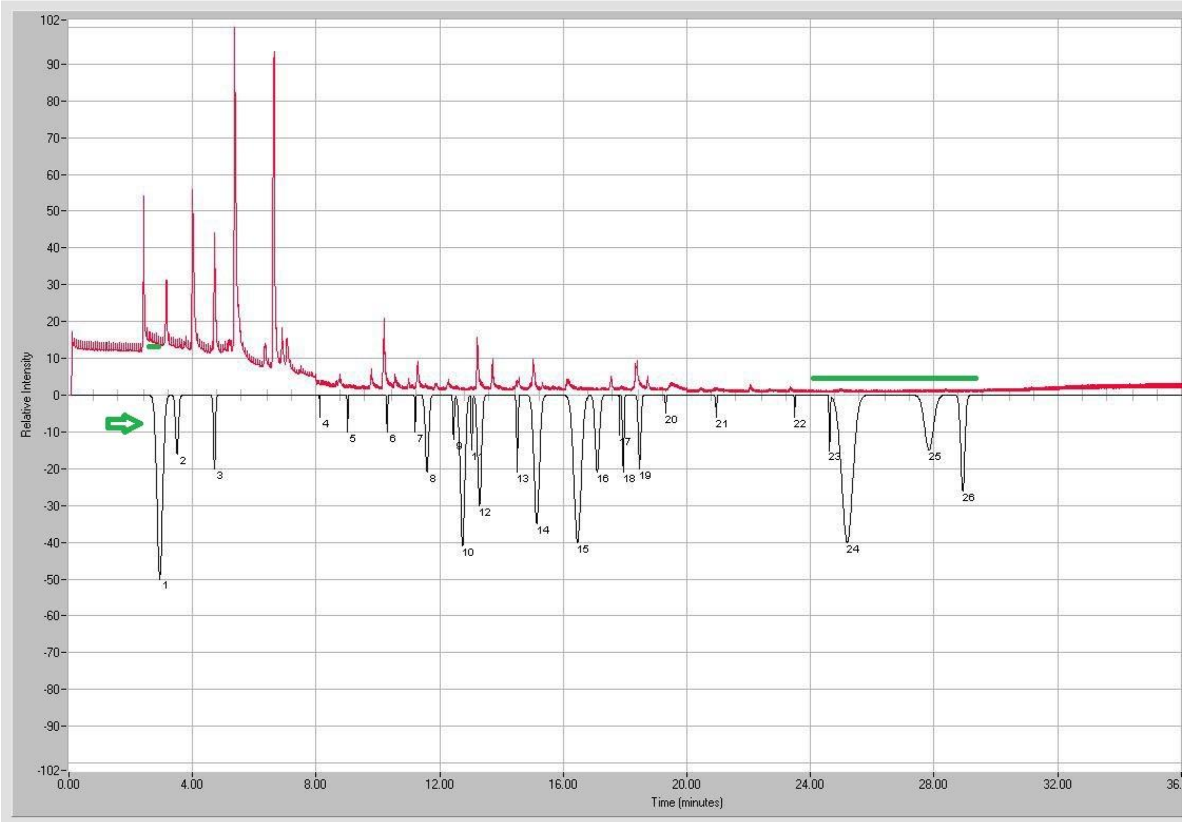

Figure 2. An overlay of aromagram and total ion chromatogram of the **fresh** cat urine exposed to SPME fiber for 50 min at 37 °C. An increase in black color signal height represents an increased intensity of odor, and the chromatogram is in the red color TIC signal. The green zone is showing that trace level concentration of malodors can produce an intense olfactory response detected by the panelist using GC-MS-O.

The trace amount of odorous phenolic compounds had high odor intensity in both fresh and stale urine. The odor intensity of some compounds present in trace amount was substantially higher in the green region in Figure 2; this odor intensity was also recorded in the stale cat urine as revealed by the aromagrams from GC-MS-olfactometer. It is quite evident that time and possibly temperature are factors in releasing odor to the atmosphere from cat urine or feces samples as both time and temperature are drivers to diffuse these VOCs and help to move from a source to a sensory organ.

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Table 2: Compounds identified in the headspace of fresh and stale cat urine on short and long exposure to the SPME fiber

Compounds	Retention time (min)	Odor description*	Odor description panelist	% Match with NIST & WILEY7	CAS #	Fresh urine, 10 min exposure to SPME at 24 °C	Fresh urine, 50 min exposure to SPME at 37 °C	Stale urine, 50 min exposure to SPME at 37 °C	Ion (% relative intensity)
						MS Detector response, peak area counts (PACs), arbitrary units			
Carbon disulfide	3.09			72	75-15-0			52,104	76(100), 58(10), 78(8), 44(5)
Acetone	3.13	Fruity[b], Camphor	Soil, fruity	63/74	67-64-1	236,687	1,222,443	2,285,446	43(100), 58 (30), 42(8)
Propanal, 2-methyl-	3.38	Pungent[a], malt		79	78-84-2			66,637	41(100). 43(70), 72(70), 40(40)
2-Butanone	4.00		Fruity	68/79	78-93-3	632,475	4,102,112	2,778,630	43(100), 72(30), 57(8), 42(5)
Butanal, 3 methyl-	4.62	Cocoa[a], almond		93	590-86-3			378,438	41(100), 44(80), 43(78), 58(50), 39(45)
2-Butanone, 3methyl-	4.71		Sweet, chemical	72	563-80-4	516,109	2,132,140	1,126,561	43(100), 86(20), 41(18)
Silanol, trimethyl-	5.08			76	1066-40-6			381,502	75(100), 45(30), 47(12), 76(5)
2-Pentanone	5.4		grassy	72	107-87-9	1,448,327	7,777,565	2,602,556	43(100), 86(20), 41(12), 71(10)
2-Pentanone, 4-methyl	6.34			86	108-10-1		518,121	59,465	43(100), 58(40), 42(25), 57(25),85(22)
2-Pentanone, 3 methyl	6.64			83	565-61-7	1,846,692	7,433,319	1,662,796	43(100), 57(40), 41(35), 72(30)
Dimethyl, disulfide	7.07	Onion[a], putrid		93	624-92-0		684,042		94(100), 45(50), 79(50), 46(25)
Pyrazine	8.04			83	290-37-9			747,153	80(100), 53(45), 58(40), 52(15), 51(10)
3-Pentanone 2,2-dimethyl	8.7		Burnt	58	564-04-5			121,125	57(100), 41(22), 114(10), 86(5)
3-Buten-1-ol, 3-methyl-	9.18			63	763-32-6			196,830	41(100), 56(90), 68(80), 86(25)
4-Heptanone	9.80			86	123-19-3	172,967	730,516		43(100), 71(90), 41(30), 114(20)
2-Propanol, 1-propoxy	10.2			50	1569-01-3			2,845,241	45(100), 43(95), 41(50), 73(48), 42(40)

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Pyrazine, methyl-	10.36	Popcorn[a]	Sweet	93	109-08-0			329,275	94(100), 67(50), 40(20), 39(18), 53(12)
2-Heptanone	10.59			81	110-43-0	94,433	420,707		43(100), 58(75), 71(20), 114(7)
Prenol	10.63			74	556-82-1			226,665	71(100), 41(60), 39(55), 53(50), 67(30), 68(30)
3-Heptanone, 2-methyl-	11.03			74	13019-20-0	69,259	225,824	111,557	57(100), 43(75), 85(75), 41(60), 71(50)
Pyrazine, 2,5-dimethyl	12.25	Roast beaf[a], medicine, cocoa		93	123-32-0			2,865,550	108(100), 42(70), 39(35), 40(20), 81(15)
Cyclohexane, ethyl-	13.26			59	1678-91-7	181,554	1,793,164	952,512	83(100), 55(70),112(45), 41(40), 56(40)
Limonene	13.7	Camphor, lemon, orange, citrus		97	138-86-3	142,286	1,182,493		68(100), 93(85), 67(80), 79(45)
Pyrrole	13.96			94	109-97-7			1,226,880	67(100), 39(48), 41(43), 40(31), 38(18)
Benzaldehyde	15.3		Sweet, fruity	95	100-52-7			1,331,202	106(100), 105(95), 77(90), 51(45), 50(30)
N-Acetyl pyrrole	15.43			83	609-41-6			2,069,470	67(100), 109(45), 43(32), 40(20), 39(20)
?	17.60					189,683	104,228		57(100), 85(95), 43(85),95(85), 41(80)
Pyrimidine	17.86			50	289-95-2			497,501	80(100), 43(50), 123(30), 57(25)
Acetophenone	17.88	Must, flower, almond	smoke	88	98-86-2		104,228		105(100), 77(80), 51(30), 120(28)
Methoxy phenyl oxime	18.39			63		422,536	1,904,766	636,563	133(100), 151(60), 135(22)
5-Methyl-2 thiophenecarboxaldehyde	18.93		Plastic, burnt	74	13679-70-4			49,628	125(100), 126(88), 97(60), 53(20), 45(20)
3,3-dimethyl-4 thiapentan-1ol	19.08			81				93,069	69(100), 41(88), 134(30), 39(30), 89(20), 56(20)
1,2-Ethanediol, 1-phenyl-	19.66			63	93-56-1			107,385	79(100), 107(95), 77(80), 51(40)
Benzyl alcohol	20.42	Sweet, flower		91	100-51-6			560,858	79(100), 108(40), 77(32), 94(30)

Dimethyl sulfone	20.46	Sulfur, burnt	Smoke, butter	71	67-71-0	158,852		79(100), 94(45), 45(20), 108(15)
Phenol	22.12	Phenol, Plastic, rubber		91	108-95-2		21,533	94(100), 66(30), 39(25), 65(20)
p-Cresol	23.37	Medicine, smoke	Smokey	93	106-44-5	228,817	651,731	107(100), 108(80), 77(20), 39(20)
Jasmone	24.13	Jasmine, flower		96	488-10-8		358,180	79(100), 164(80), 91(70), 110(60), 41(50), 149(50)
4-Hydroxy-2nonenoic acid	24.68	Minty		63	21963-26-8		53,921	84(100), 55(80), 43(50), 125(40), 41(40)
Butylated hydroxytoluene	25.61			82	128-37-0	41,207		205(100), 57(48), 220(20), 41(20)
p-Acetylaniline	25.9		Foul, urinous	94	99-92-3		102,642	120(100), 135(60), 92(48), 65(35), 43(10)
Indole	28.38	Burnt, mothball	Smokey, animal	93	120-72-9	60,593	155,847	117(100), 90(40), 89(39), 45(20)

* [15] & [16]

3.3. Temporal effect on volatile organic compounds in cat feces:

Several volatile fatty acids (VFAs) and phenolic compounds contributed to overall cat feces odors. However, the phenolics appreciably contributed to the overall feces odor. The aged feces (1week old) showed a significant drop in the number of emitted compounds in the headspace (Table 3), and so the aging process was not further carried out. The VFAs dominated in the cat feces were isobutyric, propanoic, butanoic, hexanoic, and acetic. Among the phenolics, phenol, p-cresol, and guaiacol, and the aromatic heterocyclic 1H-indole and 3-methyl indole were the contributors to the overall odor. The stale cat feces had trimethylamine, a rotten fish-like odor that chemical was not identified by GC-MS in fresh feces sample. However, a fish-like foul odor was recorded by the odor panelist (Figures 3 and 4). The observation supports the fact that a trace level concentration can be sensed by living sensory organs.

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Table 3: Compounds identified in the headspace of fresh and stale cat feces.

Compounds	Retention time (min)	Odor description published work*	Description by panelist	% Match library	CAS #	Fresh Feces*	Stale Feces** short equilibrium	Stale Feces^^ long equilibrium	Ion (% relative intensity)
						MS Detector response, peak area counts (PACs), arbitrary units			
Trimethyl amine	2.76	Fish[a]	Foul, fishy	83	75-50-3		1,395,179	1,237,586	58(100), 59(40), 42(32), 57(5)
Acetone	3.14	Fruity[b], Camphor		63	67-64-1	2,036,156			43(100), 58(30), 42(5)
Acetic acid, methyl ester	3.28		Chemical, sweet	80	79-20-9	1,774,983			43(100), 74(30), 59(10)
2-Butanone	4.01			70	78-93-3	4,100,865			43(100), 72(20), 57(5),42(5)
Methyl propionate	4.26		butter	88	554-12-1	4,300,327			57(100), 88(40), 59(30), 45(5)
Butanal, 3-methyl-	4.62	Fruity[a], nutty		68	590-86-3			172,874	43(100), 39(62), 44(60), 58(35), 71(20), 86(20)
Butanal, 2-methyl-	4.70			59	96-17-3			64,220	41(100), 57(75), 58(60)
Butanoic acid, methyl ester	4.90			74	623-42-7	474,339			43(100), 71(55),87(40), 41(40), 59(30)
Propanoic acid, ethyl ester	5.49			85	105-37-3	2,831,917			57(100), 75(18), 74(15), 102(15), 45(10)
n-Propyl acetate	5.66			76	109-60-4	1,738,458			43(100), 61(40), 73(20), 42(10), 41(8)
Butanoic acid, methyl ester	5.88			95	623-42-7	8,965,644			74(100), 43(90), 71(70), 41(40), 87(30)
Propanoic acid, 2-methyl-, ethyl ester	6.3	Citrus[b], fruity, buttery	Herbaceous	81	97-62-1	348,148			43(100), 71(40), 41(30), 116(20)
2-Pentanone, 3-methyl-	6.6			63	565-61-7	121,781			43(100), 57(40), 41(35), 72(30)
3-Octene, (E)-	6.7		Mint	75	14919-01-8	127,564			41(100), 55(98), 70(40), 112(40)

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Butanoic acid, 2-methyl-, methyl ester	6.96			86	868-57-5	439,900	88(100), 57(80), 41(50), 85(25)
Methyl isovalerate	7.04			88	556-24-1	1,341,790	74(100), 43(40), 59(35), 85(30), 41(25)
1-Butanol	7.13			72	71-36-3	612,423	56(100), 41(70), 43(40), 42(30), 55(20)
Butanoic acid, ethyl ester	7.62			95	105-54-4	6,640,131	71(100), 43(80), 88(55), 41(30), 60(20)
Propanoic acid, propyl ester	7.86			90	106-36-5	9,397,738	57(100), 75(50), 43(20), 87(10)
Acetic acid, butyl ester	8.16			72	123-86-4	416,938	43(100), 56(40), 73(20), 41(19), 61(15)
Methyl valerate	8.48		Foul	93	624-24-8	4,637,137	74(100), 85(38), 57(35), 43(30), 41(30)
Butanoic acid, 2-methyl-, ethyl ester	8.77		Floral	93	7452-79-1	498,859	57100), 102(70), 41(40), 85(35), 74(20)
Butanoic acid, 3-methyl-, ethyl ester	8.96			85	108-64-5	242,635	43(100), 88(68), 41(60), 71(50), 85(45)
1-Pentanol	9.46			76	71-41-0	1,917,299	42(100), 55(85), 41(70), 70(60)
Acetoin	9.76			63	513-86-0	584,798	43(100), 45(60), 70(15), 55(10), 88(8)
2-Propanol, 1-propoxy-	10.19			83	1569-01-3	125,926	45(100), 43(90), 73(42), 41(30), 59(28)
Butanoic acid , propyl ester	10.27		Chemical, sweet	95	105-66-8	7,807,628	71(100), 43(70), 89(60), 41(30), 42(20)
Pentanoic acid, ethyl ester	10.40			95	539-82-2	3,496,629	88(100), 85(95), 57(70), 60(40), 101(30)
2-Heptanone	10.52	Soap[a,b], Fruity, sweet, cheese		79	110-43-0	55,224	43(100), 58(75), 71(10), 114(7)
Propanoic acid, butyl ester	10.6			76	590-01-2	1,373,814	57(100), 56(35), 75(30), 41(20)

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Heptanal	10.8	Citrus[a], fat, rancid		83	111-71-7	68,145			70(100), 44(97), 41(82), 43(75), 55(60)
Acetic acid, pentyl ester	10.9			79	628-63-7	246,282	88,580		43(100), 70(40), 61(25), 55(22), 42(20)
Butanoic acid, 2-methyl-, propyl ester	11.42			70	37064-20-3	836,785			57(100), 103(80), 85(85), 41(60), 42(45)
Butanoic acid, 3-methyl-, propyl ester	11.6	Bitter[b], sweet, apple fruity		90	557-00-6	3,074,917			85(100), 103(68), 41(60), 43(59), 57(58)
1-Hexanol	11.9	Resin[a], flower, green	Floral, banana	72	111-27-3	812,614			56(100), 43(62), 55(50), 41(48), 42(40)
Pentanoic acid, 4-methyl-, ethyl ester	12.11			56	25415-67-2	61,216			88(100), 101(70), 43(60), 99(55), 55(35)
Propanoic acid, pentyl ester	12.21			79	624-54-4	528,515			57(100), 70(80), 43(45), 55(40), 41(25)
Pyrazine, 2,6-dimethyl-	12.39	Cocoa[a], meat		88	108-50-9	490,854	49,044		108(100), 42(55), 39(35), 40(30),
2-Heptanone 5-methyl-	12.52		Herbaceous, grassy, earthy	81	18217-12-4	127,769			43(100), 58(40), 71(38), 70(25), 41(20)
Acetic acid	12.6	sour[a]	sour, nutty	96	64-19-7	33,638,253	159,486,602	74,310,817	43(100), 45(88), 60(60)
Pentanoic acid, propyl ester	12.95			68	141-06-0	4,421,968			85(100), 103(75), 57(70), 41(60)
Propanoic acid, pentyl ester	13.25		Grassy, soil	63	624-54-4	741,201			57(100), 70(40), 75(40), 43(40), 55(25)
5-Hepten-2-one-6-methyl-	13.52		Old cheese	95	110-93-0	228,324			43(100), 41(60), 108(40), 69(40), 39(28)
3-Octanol	14.08			72	589-98-0	171,440			59(100), 83(60), 55(60), 43(55), 44(48)
Butanoic acid, 3-methyl-, butyl ester	14.16			63	109-19-3	157,326			85(100), 57(85), 41(80), 103(72), 56(70)

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Propanoic acid	14.31	Pungent[a], rancid	Unpleasant, butter	93	79-09-4	54,686,812	9,605,716	66,087,807	74(100), 45(72), 73(60), 57(40)
Propanoic acid, 2-methyl-	14.93		Medicinal	85	79-31-2	11,986,638	13,857,212	16,723,011	43(100), 41(55), 73(42), 39(25), 88(10)
Benzaldehyde	15.30	almond[b]	Butter	93	100-52-7	2,215,240			106(100), 105(95), 77(95), 51(45), 50(25)
Butanoic acid	16.04			95	107-92-6	113,602,470	123,858,474	85,788,896	60(100), 73(40), 41(22), 40(20)
Butanoic acid, 3-methyl-	16.84		Sweet, fruity	83	503-74-2	76,585,646	61,915,734	38,769,616	60(100), 41(60), 74(42), 87(30)
2-Methyl-4-decanone	17.60			70 (NIST only)	6628-25-7	116,081			57(100), 85(95), 41(85), 95(85), 43(80), 113(30)
Acetophenone	17.88	Must[a,b], flower, almond	Smokey	88	98-86-2	359,940			105(100), 77(80), 120(30), 51(28)
Pentanoic acid	18.02			76	109-52-4	108,458,001	52,809,697	24,373,275	60(100), 73(45), 41(20), 45(18)
Pentanoic acid, 4-methyl-	19.13		Butter, basmati rice, butter	85	646-07-1	3,286,558			57(100), 60(80), 41(75), 73(75), 55(62)
Hexanoic acid	19.81			83	142-62-1	3,822,922	325,897		60(100), 73(55), 41(32), 87(12)
o-Guaiacol	20.42	Smoke[a], medicine	Woody, wild	95	90-05-1	4,755,984	283,513		109(100), 124(90), 81(70), 53(20)
Benzeneethanol	21.24			91	60-12-8	384,960			91(100), 92(50), 122(25), 65(20)
Benzene propanoic acid, methyl ester	21.93			93	103-25-3	210,723			104(100), 91(60), 164(30), 105(30)
Phenol	22.10	phenol[a]	Medicinal	96	108-95-2	10,114,684	2,023,698	315,371	94(100), 66(35), 65(25), 39(25)
2-Dodecanone	22.5			85	6175-49-1	77,879			58(100), 43(90), 71(35), 59(30), 41(22)
Benzenepropanoic acid, ethyl ester	23.01			85	2021-28-5	148,940			104(100), 91(45), 105(30), 107(28), 178(20)
p-Cresol	23.30	Smoke[a], medicine	Medicinal	93	106-44-5	39,957,137	3,947,249	1,376,506	107(100), 108(80), 77(20), 39(20)

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Phenol, 4-ethyl-	24.80	Must[a]	Foul, unpleasant	94	123-07-9	1,021,035	103,774		107(100), 122(30), 77(20),106(8)
Butylated hydroxytoluene	25.10	-		96	128-37-0	182,612	52,706		205(100), 220(25), 57(15), 204(15)
Indole	28.38	Burnt[a]	Medicinal, unpleasant	96	120-72-9	69,332,928	195,879	810,599	117(100), 90(40), 89(39), 45(20)
Diethyl Phthalate	29.08	-		94	84-66-2	81,563			149(100), 177(23), 117(20), 150(10), 176(7)
Indole, 3-methyl-	29.2	Fecal[a]	Urinous, animal	71	83-34-1	78,683			130(100), 131(50), 149(20), 117(15), 77(10)

* 50 min exposure to SPME at 37 ˚C; **15 min equilibrium & 50 min exposure to SPME at 37 ˚C; ^^24 hrs equilibrium and 50 min exposure to SPME at 37 ˚C; odor verified with [a]Flavornet and [b]Good Scent Company * [15] & [16]

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The fresh feces on 50 minutes exposure to SPME fiber caused several ketones, aldehydes, esters, acids phenols to accumulate to SPME fiber. Among these identified chemicals, most of the high molecular compounds have a smoke, medicinal, animal, and foul smell, whereas the low molecular weight chemicals are more of a chemical, sweet, fruity, and grassy or earthy smell. From this, it is more obvious that the high molecular weight phenolic and N aromatic heterocyclic compounds are the contributors to the overall smell of the fresh feces.

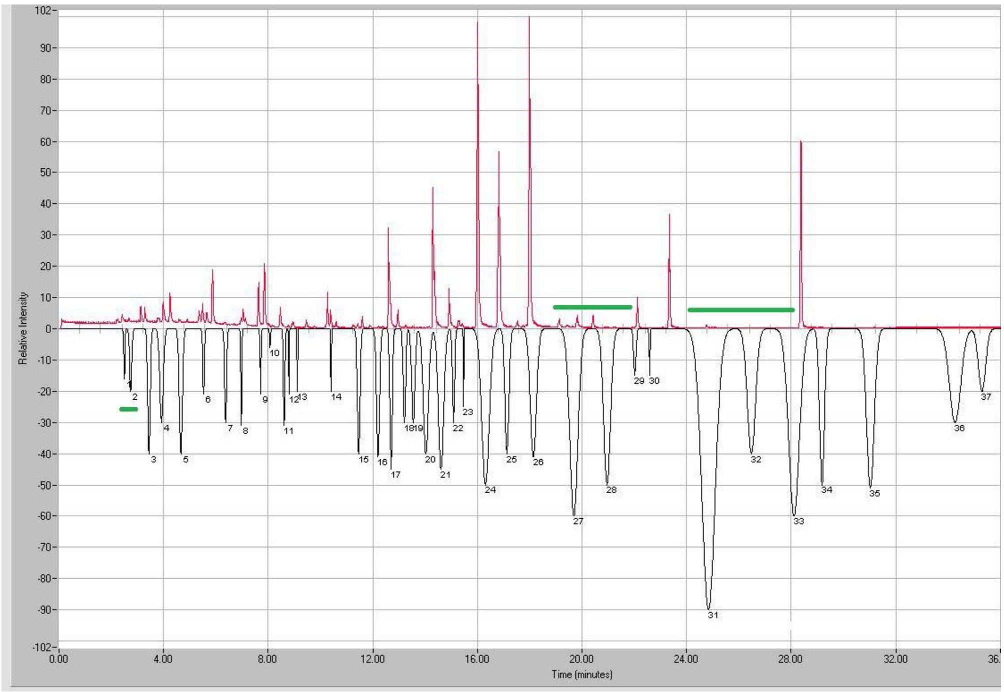

Figure 3. An overlay of aromagram and total ion chromatogram of the **fresh** cat feces exposed to SPME fiber for 50 min at 37 °C. An increase in black color signal height represents an increased intensity of odor, and the chromatogram is in the red color TIC signal. The green zone is showing that trace level concentration of malodors can produce an intense olfactory response detected by the panelist using GC-MS-O.

The compounds with high molar masses that appeared after the retention time of 20 minutes or higher had intense malodors. It is interesting to mention that for stale feces, a short equilibration time of 10 min resulted in missing many low molecular weight compounds, and several high molecular weight compounds had high PAC like p-cresol, phenol, and guaiacol than long equilibration time of 24 h. In stale feces, the primary contributor like 3-methyl indole was missing, and phenolics had lower PAC (4-ethyl phenol, p-cresol, and phenol) than phenolics in fresh feces headspace. The odor intensity of the many chemicals, including phenolic compounds, were similar in the stale cat feces, as revealed by the GC-MS-olfactometer. The aromagrams of feces samples show that the presence of odorants even in the below detection limit concentration for GC-MS can cause a considerable odor impact (green arrow in Fig 3 & 4) problem. Aromagram also revealed that compounds that not in the GC-MS data can contribute to overall odor, thus, trigger the human nose to react to the odorants.

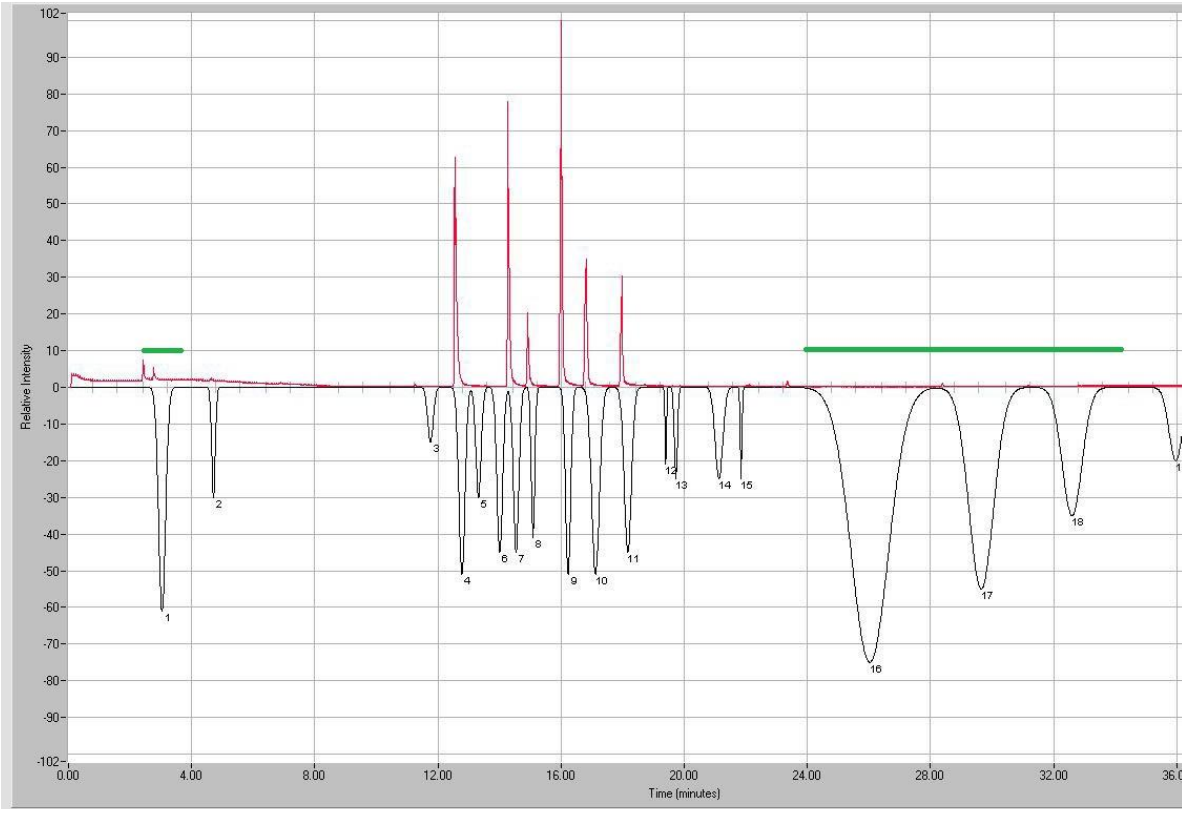

Figure 4: An overlay of aromagram and total ion chromatogram of the **stale** cat feces, equilibrated for 24 h and exposed to SPME fiber for 50 min at 37 °C. An increase in black color signal height represents an increased intensity of odor, and the chromatogram is in the red color TIC signal. The green zone is showing that trace level concentration of malodors can produce an intense olfactory response detected by the panelist using GC-MS-O.

4. Discussion

The focus of this study is to report the malodorous VOCs in domestic cat urine and feces and the influence of aging of urine and feces on the emitted VOCs. Domestic cats spray urine to mark their territory, and the odor is unpleasant to many people. The concentration of volatile compounds can differ by age and sex of the species [10]. This study reported several VOCs emitted from fresh cat urine and feces that were not reported in previous studies. The precursor of many sulfur-containing compounds, felinine, was not identified in this research [6]. As reported by Miyazaki et al., (2018), the presence of fishy odor in the headspace of domestic cat anal sac secretions suggests that cat urine can produce distinct fishy odorous trimethylamine is also present in our findings of the distinctive fishy odor in the fresh and stale feces but not in fresh or aged urine headspace [17]. Feral male cat sprays urine more often than females [18] and uses unburied feces as scent-marking [19], and the released volatiles in both cases could be offensive to humans; the odor gets even worse with time although the VOC concentrations get into trace level in feces sample with time (Fig 4). This current study recorded the odor description of the emitted VOCs that were never reported before for a cat urine and feces study. In addition to phenolics as a major odor contributor, indoles are prominent in stale urine, and organic acids and indoles are prominent in fresh urine odor.

The use of SPME sampling techniques has allowed easy pre-concentrating of sample preparation without the use of a solvent or derivatization of the VOCs responsible for the nuisance to human sensory organs. The volatile compound types and the relative PACs emitted by urine and feces significantly changed over time, even under laboratory conditions. The total number of compounds increased for the aged urine sample and decreased for the aged feces samples. Many esters and acidic VOCs were dropped in number for the aged feces, either degraded to other compounds by microbial community present in the sample, oxidized, or lost to the atmosphere during the aging process [11].

A number of phenol, alcohol, aldehyde, amines, s-compounds, ketones, and acids reported in this study were emitted from the urine of lion, tiger, and domestic cat species as reported in previous studies (Table 4) [4,8,20]. Many VOCs emitted from the cat feces samples, as reported in this study, were also reported to be emitted from swine manure samples [21]. In addition, a few VOCs extracted by SPME and reported in this study are found to be common in the SPME extraction from cold-hardy grapes samples [22]. Characteristic odor compounds 2,5 dimethyl pyrazine from the lion urine indicates the evolutionary similarities between animals. These observations also indicate the fact that SPME in a combination of GC-MS-O for the headspace extraction is better than only GC-MS. Overall, SPME reduces the sampling time for volatile or semivolatile compound determination than a traditional sampling technique [23], and this technology can be developed more to the future assessment of quantitative analysis of these odorous constituents characteristic to the overall smell of urine or feces sample.

Table 4: Some literature recorded VOCs emitted from urine, feces, and fruits of different biological species common finding in the current study

Compounds	CAS #	Lion urine (Soso &Koziel 2017) [20]	Tiger urine (Soso &Koziel 2016) [8]	Cat urine (Miyazaki 2006) [4]	Swine manure (Yin-cheyung et al., 2006) [21]	Grapes (Rice et al., 2019) [22]	Current study
Phenol	108-95-2	X	X		X		X
p-Cresol	106-44-5	X	X	X	X		X
Phenol,4-ethyl-	123-07-9				X		X
3-Octanol	589-98-0				X		X
1-Butanol	71-36-3		X		X	X	X
1-Hexanol	111-27-3		X		X	X	X
Benzyl alcohol	100-51-6					X	X
3-Buten-1-ol, 3-methyl-	763-32-6			X			X
Benzeneethanol	60-12-8	X	X		X		X
Dimethyl disulfide	624-92-0	X			X		X
Dimethyl sulfone	67-71-0			X			X
Benzaldehyde	100-52-7	X	X	X	X	X	X
Butanal, 3-methyl-	590-86-3	X	X				X
Isobutyraldehyde	78-84-2					X	X
Trimethyl amine	75-50-3	X	X				X
2-Dodecanone	6175-49-1				X		X
2-Heptanone	110-43-0		X		X		X
2-Pentanone	107-87-9	X					X
3-methyl 2-pentanone	565-61-7				X		X
2-Butanone	78-93-3	X	X	X	X	X	X
Acetone	67-64-1	X	X	X	X	X	X
5-Hepten-2-one, 6-methyl-	110-93-0				X		X
Acetophenone	98-86-2				X	X	X
Jasmone	488-10-8			X	X		X
Indole	120-72-9	X	X		X		X
3-methylindole	95-20-5				X		X

Pyrazine, 2,6 dimethyl -	108-50-9	X	X				X
Acetic acid	64-19-7			X	X	X	X
Butanoic acid	107-92-6			X	X		X
Pentanoic acid, 2-methyl-	79-31-2				X		X
Butanoic acid, 3-methyl-	503-74-2		X		X		X
Pentanoic acid	109-52-4				X	X	X
Propanoic acid	79-09-4				X		X
Hexanoic acid	142-62-1					X	X
Butanoic acid, 3-methyl	503-74-2			X			X
Carbon disulfide	75-15-0					X	X
Butanoic acid, ethyl ester	105-54-4					X	X

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1,

Table S1: Aroma description as recorded by the panelist for fresh cat urine 10 min extraction,

Table S2: Aroma description as recorded by the panelist for fresh cat urine 50 min extraction,

Table S3: Aroma description as recorded by the panelist for stale cat urine 50 min extraction,

Table S4: Aroma description as recorded by the panelist for fresh feces 50 min extraction,

Table S5: Aroma description as recorded by the panelist for stale feces 15 min equilibrium, 50 min extraction,

Table S6: Aroma description as recorded by the panelist for stale cat feces, 24 h equilibrium and 50 min extraction

Author Contributions: conceptualization, J.K and J.L.; methodology, J.K., J.L. and C.B.; formal analysis, C.B.; investigation, C.B.; resources, J.K.; data curation, C.B.; writing—original draft preparation, C.B.; writing—review and editing, J.K., and J.L.; visualization, C.B..; supervision, J.K.; project administration, J.K.; funding acquisition, J.K."

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Conflicts of Interest: The authors declare no conflict of interest.

Data Availability Statement: The original contributions presented in the study are included in the article and the Supplementary Materials; further inquiries can be directed to the corresponding author.

Appendix A

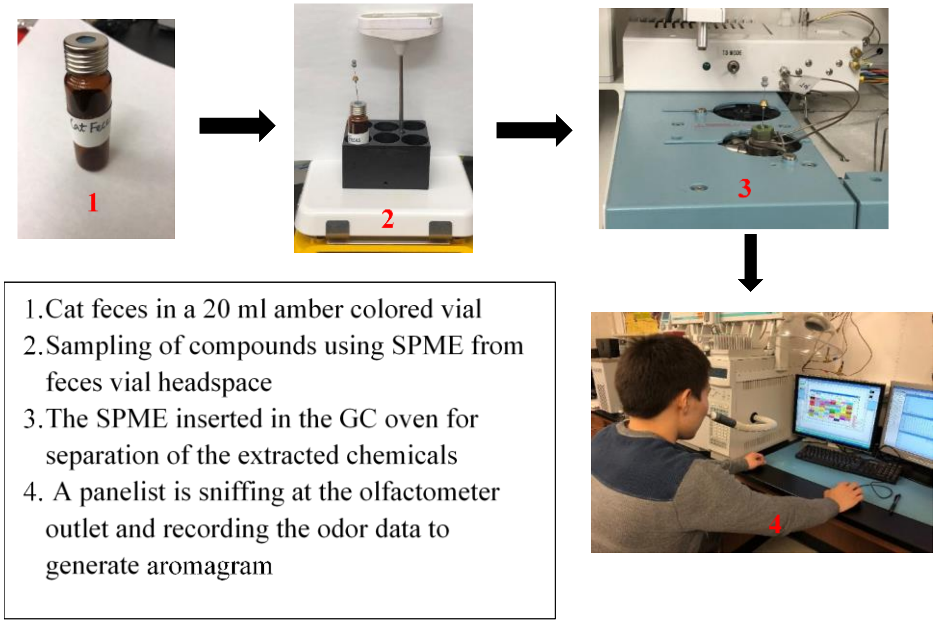

Figure A1: Schematic representation of the cat urine, feces and soiled litter sample preparation and analysis

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