Simultaneous Chemical and Sensory Analysis of Domestic Cat Urine and Feces with Headspace Solid-Phase Microextraction and GC-MS-Olfactometry

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Abstract: The association between humans and cats (Felis 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 preconcentrate 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 in the emission of VOCs for urine and feces. On aging, the emission increased to 34 detected chemicals for stale urine, whereas only 12 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 companionship between humans and cats (Felis catus) is more than 8000 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 builds importance in human life as a family member. While cat owners love their cats, they have a less positive relationship with their cats' litterboxes due to several factors, including the smell of urine and feces.

The potent odor of domestic cat urine causes a continually growing research interest (Table 1). Improved separation and identification techniques were used over the decades to report compounds with a catty smell. One of the responsible specific amino acids, 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 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-methylbutanol-glutathione (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 cat urine and feces, depending on the age and sex-related factors of cats.

Cat urine and feces contain several volatile and nonvolatile compounds that help to recognize sex and species [7]. These volatile compounds emitted through urine and feces also act as chemical signaling in mammals to define their territory, dominance, and reproduction [8,9]. Stray and domestic cats also use urine as chemical signaling and 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 cat age and sex irrespective of their food diet or habitat, such as 1-butanol in feces found significantly in lower concentrations in female cats, and indoles and phenols such as 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-like” and “savory-like”; however, on interaction with soil, the bacteria can emit a cat urine smell described as “intensely fishy” [12]. These experiments were set to find the cat species chemical signaling for habituation–dishabituation and may not guide the resolution of 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 has only been a decade since scientists started using solid-phase microextraction (SPME) for biological sample VOC extraction. It is considered a noninvasive sampling device that extracts biomarkers for the early diagnosis of advanced or chronic diseases or for reporting impurity in food samples to assess food quality [14]. That study also reported that SPME is 10–50 times more efficient than any static headspace sampling. Moreover, the use of SPME is able to extract volatile organic compounds (VOCs) from a biological sample both ex vivo and in vitro for analysis using gas chromatography–mass spectrometry (GC–MS). This approach of sample extraction can reduce sample preparation steps and can extract the chemicals without modifying their original form. The use of SPME fibers has only recently been reported for marking fluid extraction and identification for Panthera tigris subspecies [8]. To our knowledge, there is no research published on odorous chemicals emitted by urine and fecal samples of domestic cat species using SPME–GC–MS–olfactometry (SPME–GC–MS–O) chemical and sensory analysis, especially in the context of smell development over time. Simultaneous chemical and sensory analyses can facilitate linking conventional chemical speciation with specific odors. Chemical identification of odor-causing chemicals can be aided by odor databases [15,16]. This current study was designed to find out the temporal behavior of odorous compounds emitted from cat urine and feces in a noninvasive way for the betterment of the human environment.
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 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                                                              | To separate 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                   | To 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 nonvolatile chemicals synthesized and reported and 14 odor attributes reported | To identify the key odorants in cat urine                                   | Starkenmann, C. et al. 2014 [12] |
| Domestic Cat (Felis catus)   | Fresh feces                      | Gas sampling                                | GC–MS                           | Not conducted    | 24 volatile organic compounds                                                        | To determine sex and age 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                                                                 | To determine behavioral bioassays via olfactory habituation–dishabituation | 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                                                                           | To 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.
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. It may also answer the following question: Is it possible to predict 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.

2. Materials and Methods

2.1. Cat Urine and Feces Collection

The cat urine and feces samples were collected at Nestlé Purina pet facility. The cat urine and feces samples were collected from a group of healthy cats (N > 10). The urine and feces samples were immediately frozen after collection. The urine and feces samples in this study represent a typical healthy cat. Freely collected urine and feces samples were homogenized and immediately frozen at −20 °C upon collection. The urine and feces samples were shipped in a cooler box with dry ice protection via next day air transport to the Iowa State University lab for analysis.

2.2. Sample Storage, Preparation, and Extraction

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 h. Each sample was then weighed to approximately 1 g into an amber color 10 mL vial in duplicate using a disposable dropper or spatula. Temperature facilitates the organic molecules to move from the urine or feces to headspace and then SPME fiber coating. However, a high T can thermally decompose compounds such as trimethylamine (a potent odorant). Too high of a T could diminish the extraction efficiency of the SPME fiber coating. On the other hand, a low T might challenge the SPME extraction of the high-molecular-weight compounds. Thus, the temperature used in this experiment was close to body temperature. Considering these T factors, the aging of urine and feces was performed at room temperature, and the SPME extraction was performed at 37 °C.

We relied on previous methods developed in our lab (Soso and Koziel, 2016 [8]). For example, Soso and Koziel, 2016, [8] tested five types of SPME fiber coatings (75 µm Carboxen polydimethylsiloxane (PDMS), 85 µm Carboxen/PDMS, 65 µm PDMS/(divinylbenzene) DVB, 50/30 µm DVB/Carboxen/PDMS, and 100 µm PDMS), two time intervals (1 h and 24 h), two sample sizes, and two Ts. Among the fiber coatings, DVB/Carboxen/PDMS was the least effective in extracting the characteristic smell of marking fluid of the Siberian tiger. However, the DVB/PDMS/Carboxen fiber was chosen because our objective was to record all the odors and chemicals emitted by the fresh and stale cat urine or feces samples rather than any specific chemical.

2.3. Multidimensional Gas Chromatography–Mass Spectrometry Olfactometry

All sample analyses of cat urine and feces were completed using the multidimensional gas chromatograph–mass spectrometer olfactometer (GC–MS–O; Microanalytics, Round Rock, TX, USA). 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 for extraction of the aged urine and feces samples. These vial caps were left closed to avoid samples drying out and were opened for a couple of minutes of 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 hour under lab condition, and then, the vial was put on a hot plate set at 37 °C for 10 min before inserting the SPME fiber to extract the headspace VOCs for 50 min.

After extraction, the SPME fiber was 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 an 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 × 530 µm inner diameter × 0.5 µm thickness, SGE, Austin, TX, USA) and polar capillary column (BP-20, 30.0 m × 530 µm inner diameter × 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 olfactometer 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 used an 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 recorded 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).

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 min SPME extraction of 1-week-old stale feces headspace had only 12 detectable compounds (via GC–MS), while the use of human nose enabled the detection of as many as 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 descriptions is provided in the Supplementary Materials (Tables S1–S6). The difference in the number of events occurs because a human nose is more sensitive compared 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](image-url). Comparison of the number of compounds detected in the headspace of fresh/stale urine and feces using GC–MS analysis ((A) chromatogram) and GC–MS–olfactometry analysis ((B) aromagram): the solid-phase microextraction (SPME) fiber was exposed for 50 min at 37 °C.
Table 2. Compounds identified in the headspace of fresh and stale cat urine on short and long exposures to the SPME fiber.

| Compounds                        | Retention Time (min) | Odor Description | Odor Description Panelist | % Match with Nist and WILEY7 | CAS # | Fresh Urine, 10 Min Exposure to SPME at 24 °C MS Detector Response, Peak Area Counts (PACs), and Arbitrary Units | Fresh Urine, 50 Min Exposure to SPME at 37 °C | Stale Urine, 50 Min Exposure to SPME at 37 °C | Ion (% Relative Intensity) |
|----------------------------------|----------------------|-------------------|---------------------------|-----------------------------|-------|---------------------------------------------------------------------------------------------------------------|---------------------------------------------|-----------------------------------------------|----------------------------------------------|
| Carbon disulfide                 | 3.09                 |                   |                           | 72                          | 75-15-0 | 236,687, 1,222,443                                                                                             | 52,104                                      | 76(100), 58(10), 78(8), 44(5)                | 43(100), 58(30), 42(8)                        |
| Acetone                          | 3.13                 | Fruity b, Camphor | Soil, fruity               | 74                          | 67-64-1 | 2,285,446                                                                                                      | 6,637                                       | 41(100), 43(70), 72(70), 40(40)              | 41(100), 42(30), 48(25)                        |
| Propanal, 2-methyl-              | 3.38                 | Pungent a, malt   |                           | 79                          | 78-84-2 | 66,637                                                                                                           | 43(100), 72(30), 57(8), 42(5)              | 41(100), 44(80), 43(78), 58(50), 39(45)       | 43(100), 86(20), 41(18)                       |
| 2-Butanone                       | 4.00                 | Fruity            |                           | 79                          | 78-93-3 | 4,102,112                                                                                                       | 2,778,630                                  | 41(100), 57(30), 48(25), 39(20)              | 43(100), 48(20), 41(12), 72(5)               |
| Butanal, 3-methyl-              | 4.62                 | Cocoa a, almond   |                           | 93                          | 590-86-3 | 378,438                                                                                                          | 58(100), 59(45)                            | 43(100), 86(20), 41(12), 72(5)               | 43(100), 58(40), 42(25), 57(25), 48(22)       |
| 2-Butanone, 3-methyl-            | 4.71                 | Sweet, chemical   |                           | 72                          | 563-80-4 | 516,109                                                                                                         | 1,126,561                                  | 43(100), 58(40), 42(25), 57(25), 48(22)       | 43(100), 86(20), 41(12), 72(5)               |
| Silanol, trimethyl-              | 5.08                 |                   |                           | 76                          | 106-60-6 | 381,302                                                                                                          |                                    |                                                |                                              |
| 2-Pentanone                      | 5.4                  | grassy            |                           | 72                          | 107-87-9 | 7,777,565                                                                                                       | 2,602,556                                  |                                                |                                              |
| 2-Pentanone, 4-methyl            | 6.34                 |                   |                           | 86                          | 108-10-1 | 518,121                                                                                                         | 59,465                                     |                                                |                                              |
| 2-Pentanone, 3 methyl            | 6.64                 |                   |                           | 83                          | 565-61-7 | 1,846,692                                                                                                       | 1,662,796                                  |                                                |                                              |
| Dimethyl disulfide               | 7.07                 | Onion a, putrid   |                           | 93                          | 624-92-0 | 684,042                                                                                                          |                                    |                                                |                                              |
| Pyrazine                         | 8.04                 |                   |                           | 83                          | 290-37-9 | 747,153                                                                                                          |                                    |                                                |                                              |
| 3-Pentanone, 2,2-dimethyl        | 8.7                  | Burnt             |                           | 58a                         | 564-04-5 | 121,125                                                                                                          |                                    |                                                |                                              |
| 3-Buten-1-ol, 3-methyl-          | 9.18                 |                   |                           | 63                          | 763-32-6 | 196,830                                                                                                          |                                    |                                                |                                              |
| 4-Heptanone                      | 9.80                 |                   |                           | 86                          | 123-19-3 | 730,516                                                                                                          |                                    |                                                |                                              |
| 2-Propanol, 1-propoxy           | 10.2                 |                   |                           | 50a                         | 1569-01-3 | 2,845,241                                                                                                       |                                    |                                                |                                              |
| Pyrazine, methyl-               | 10.36                | Popcorn a         | Sweet                     | 93                          | 109-08-0 | 329,275                                                                                                          |                                    |                                                |                                              |
| 2-Heptanone                      | 10.59                |                   |                           | 81                          | 110-43-0 | 420,707                                                                                                          |                                    |                                                |                                              |
| Prenol                           | 10.63                |                   |                           | 74                          | 556-82-1 | 226,665                                                                                                          |                                    |                                                |                                              |
| 3-Heptanone, 2-methyl-           | 11.03                | Roast beaf a, medicine, cocoa |                           | 74                          | 13019-20-0 | 225,824                                                                                                         | 111,557                                   |                                                |                                              |
| Pyrazine, 2,5-dimethyl           | 12.25                |                   |                           | 93                          | 123-32-0 | 2,865,550                                                                                                       |                                    |                                                |                                              |
| Cyclohexane, ethyl-             | 13.26                |                   |                           | 59a                         | 1678-91-7 | 1,793,164                                                                                                       | 952,512                                   |                                                |                                              |
| Limonene                         | 13.7                 | Camphor, lemon, orange, citrus |                           | 97                          | 138-86-3 | 1,182,493                                                                                                       |                                    |                                                |                                              |
| Compounds                          | Retention Time (min) | Odor Description                  | Odor Description Panelist | % Match with NIST and WILEY\(^7\) | CAS # | Fresh Urine, 10 Min Exposure to SPME at 24 °C MS Detector Response, Peak Area Counts (PACs), and Arbitrary Units | Fresh Urine, 50 Min Exposure to SPME at 37 °C | Stale Urine, 50 Min Exposure to SPME at 37 °C | Ion (% Relative Intensity) |
|-----------------------------------|----------------------|-----------------------------------|---------------------------|-----------------------------------|-------|------------------------------------------------------------------------------------------------|-----------------------------------------------|---------------------------------------------|---------------------------------------------|
| Pyrrole                           | 13.96                |                                   | 94                        | 109-97-7                          | 1,226,880 | 67(100), 39(48), 41(43), 40(31), 38(18)                                             | 40(100), 105(95), 77(90), 51(45), 50(30) | 67(100), 109(45), 43(32), 40(20), 39(20) | 57(100), 85(95), 43(85), 41(80)          |
| Benzaldehyde                      | 15.3                 | Sweet, fruity                     | 95                        | 100-52-7                          | 1,331,202 | 106(100), 105(95), 77(90), 51(45), 50(30)                                             | 67(100), 109(45), 43(32), 40(20), 39(20) | 57(100), 85(95), 43(85), 41(80)          |
| N-Acetyl pyrrole                  | 15.43                |                                   | 83                        | 609-41-6                          | 2,069,470 | 80(100), 43(50), 123(30), 57(25), 105(100), 77(80), 51(30), 120(28)                     | 133(100), 151(60), 153(22) | 125(100), 126(88), 97(60), 53(20), 45(20) | 69(100), 41(88), 134(30), 39(30), 89(20), 56(20) |
| 2-Methyl-4-decanone               | 17.60                |                                   | 70                        | 189,683                           | 104,228 | 88(100), 98(86-2)                                                                         | 104,228 | 104,228 | 93,069                                      |
| Pyrimidine                        | 17.86                | Must, flower, almond              | 50                        | 289-95-2                          | 497,501  | 120(100), 105(95), 77(90), 51(45), 50(30)                                             | 120(100), 105(95), 77(90), 51(45), 50(30) | 120(100), 105(95), 77(90), 51(45), 50(30) | 80(100), 43(50), 123(30), 57(25), 105(100), 77(80), 51(30), 120(28) |
| Acetophenone                      | 18.88                | Must, flower, almond              | 88                        | 98-86-2                           |                      | 88(100), 98-86-2                                                                         | 104,228 | 104,228 | 93,069                                      |
| Methoxy phenyl oxime              | 18.39                |                                   | 63                        | 422,536                           | 1,904,766 | 636,563                                                                                   | 133(100), 151(60), 153(22) | 125(100), 126(88), 97(60), 53(20), 45(20) | 69(100), 41(88), 134(30), 39(30), 89(20), 56(20) |
| 5-Methyl-2 thiophene-carboxaldehyde | 18.93               | Plastic, burnt                    | 74                        | 13679-70-4                        | 49,628   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| 3,3-dimethyl-4 thiapentan-1-ol    | 19.08                |                                   | 81                        |                                   | 93,069   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| 1,2-Ethandiol, 1-phenyl-           | 19.66                |                                   | 63                        | 93-56-1                           | 107,385  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Benzyl alcohol                    | 20.42                | Sweet, flower                     | 91                        | 100-51-6                          | 560,858  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Dimethyl sulfoxide                | 20.46                | Sulfur, burnt                     | 71                        | 67-71-0                            | 158,852  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Phenol                            | 22.12                | Phenol, Plastic, rubber           | 91                        | 108-95-2                          | 21,533   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| p-Cresol                          | 23.37                | Medicine, smoke                   | 93                        | 106-44-5                          | 228,817  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Jasmonate                         | 24.13                | Jasmine, flower                   | 96                        | 488-10-8                          | 358,180  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| 4-Hydroxy-2-nonenolic acid        | 24.68                | Minty                             | 63                        | 21963-26-8                        | 53,921   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Butylated hydroxytoluene          | 25.61                |                                   | 82                        | 128-37-0                          | 41,207   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| p-Acetylaniline                   | 25.9                 | Foul, urinous                     | 94                        | 99-92-3                           | 102,642  | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |
| Indole                            | 28.38                | Burnt, mothball                   | 93                        | 120-72-9                          | 60,593   | 104,228                                                                                   | 104,228 | 104,228 | 93,069                                      |

\(^{8}\) Below 60% library match considered as semi-confirmative. Odor verified with \(^{a}\) Flavornet [15] and \(^{b}\) Good Scent Company [16].
The refrigerated fresh urine produced 21 compounds and 26 odor events, whereas the 15-day-old urine produced as many as 34 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. Phenols and indoles were among the most intense odors and were a substantial contributor to malodors.

3.2. Temporal Effect on Volatile Organic Compounds in Cat Urine

Exposure of the 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 a long exposure time of SPME extracted. However, we were not able to detect the high-molecular-weight VOCs with short SPME extractions, and therefore, the high-molecular-weight VOCs are absent in the list (Table 2). Moreover, the intensity of the many chemicals was low in concentration (low Peak Area Count (PAC)) to distinguish the odor between different chemicals. The use of the GC–MS–olfactometer, however, sensed the odors of phenolic compounds at a 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 a 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 retention time (RT) for 2.7 min, possibly of the compound trimethyl amine, although no compound was identified by the GC-MS. The identified compounds increased from 19 to 34, and it is worth mentioning that N-containing compounds like pyrazine; pyrrole; pyrimidine; and some other ketones, aldehyde, and alcohols emerged with the aging of the urine, whereas dimethyl disulfide-like malodor compounds were 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) and limonene (“mint-like” smell) was observed in fresh urine samples for both long and short extraction times that was missing in the aged urine samples; however, jasmone (“flowery” smell), a pleasant aroma, emerged in the aged urine sample and was absent in the fresh urine (Table 2).

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 line region in Figure 2; this odor intensity was also recorded in stale cat urine, as revealed by the aromagrams from GC–MS–olfactometry. 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.

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 (1 week old) showed a significant drop in the number of emitted compounds in the headspace (Table 3), and therefore, the aging process was not further carried out. The VFAs that 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 overall odor. The stale cat feces had trimethylamine, a rotten fish-like odor that 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 of concentration can be sensed by living sensory organs.
Figure 2. An overlay of the aromagram and total ion chromatogram of the fresh cat urine exposed to SPME fiber for 50 min at 37 °C: an increase in the black signal height represents an increased intensity of odor, and the chromatogram is in red for total ion chromatogram (TIC) signal. The green zone shows that trace levels of the concentration of malodors can produce an intense olfactory response detected by the panelist using GC–MS–O.

Figure 3. An overlay of the aromagram and total ion chromatogram of the fresh cat feces exposed to the SPME fiber for 50 min at 37 °C: an increase in the black signal height represents an increased intensity of odor, and the chromatogram is in red for the total ion chromatogram (TIC) signal. The green zone shows that trace levels of the concentration of malodors can produce an intense olfactory response detected by the panelist using GC–MS–O.
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) |
|----------------------------------|----------------------|---------------------------------|--------------------------|-----------------|----------------|---------------|-------------------------------|-------------------------------|-----------------------------|
| Trimethylamine                   | 2.76                 | Fish *                          | 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               |                          |                 | 67-64-1        | 2,036,156     |                               |                               | 43(100), 58(30), 42(5) |
| Acetic acid, methyl ester        | 3.28                 | Chemical, sweet                 |                          |                 | 79-20-9        | 1,774,983     |                               |                               | 43(100), 74(30), 59(10) |
| 2-Butanone                       | 4.01                 |                                 |                          |                 | 78-93-3        | 4,100,865     |                               |                               | 43(100), 72(20), 57(5), 42(5) |
| Methyl propionate                | 4.26                 |                                 |                          |                 | 554-12-1       | 4,300,327     |                               |                               | 57(100), 88(40), 59(30), 45(5) |
| Butanal, 3-methyl-               | 4.62                 | Fruity *, nutty                 |                          |                 | 590-86-3       | 172,874       |                               |                               | 43(100), 39(62), 44(60), 58(35), 71(20), 86(20) |
| Butanal, 2-methyl-               | 4.70                 |                                 |                          |                 | 96-17-3        | 64,220        |                               |                               | 41(100), 57(75), 58(60) |
| Butanoic acid, methyl ester      | 4.90                 |                                 |                          |                 | 623-42-7       | 474,339       |                               |                               | 43(100), 71(55), 87(40), 41(40), 59(30) |
| Propanoic acid, ethyl ester      | 5.49                 |                                 |                          |                 | 105-37-3       | 2,831,917     |                               |                               | 57(100), 75(18), 74(15), 102(15), 45(10) |
| n-Propyl acetate                 | 5.66                 |                                 |                          |                 | 109-60-4       | 1,738,458     |                               |                               | 43(100), 61(40), 73(20), 42(10), 41(8) |
| Butanoic acid, methyl ester      | 5.88                 |                                 |                          |                 | 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 |                          |                 | 97-62-1        | 348,148       |                               |                               | 43(100), 71(40), 41(30), 116(20) |
| 2-Pentanone, 3-methyl-            | 6.6                  |                                 |                          |                 | 565-61-7       | 121,781       |                               |                               | 43(100), 57(40), 41(35), 72(30) |
| 3-Octene, (E)-                   | 6.7                  | Mint                            |                          |                 | 14919-01-8     | 127,564       |                               |                               | 41(100), 55(98), 70(40), 112(40) |
| Butanoic acid, 2-methyl-, methyl ester | 6.96                 |                                 |                          |                 | 868-57-5       | 439,900       |                               |                               | 88(100), 57(80), 41(50), 85(25) |
| Methyl isovalerate               | 7.04                 |                                 |                          |                 | 556-24-1       | 1,341,790     |                               |                               | 74(100), 43(40), 59(35), 85(30), 41(25) |
| 1-Butanol                        | 7.13                 |                                 |                          |                 | 71-36-3        | 612,423       |                               |                               | 56(100), 41(70), 43(40), 42(30), 55(20) |
| Butanoic acid, ethyl ester       | 7.62                 |                                 |                          |                 | 105-54-4       | 6,640,131     |                               |                               | 71(100), 43(80), 88(55), 41(30), 60(20) |
| Propanoic acid, propyl ester     | 7.86                 |                                 |                          |                 | 106-36-5       | 9,397,738     |                               |                               | 57(100), 75(50), 43(20), 87(10) |
Table 3. Cont.

| 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) |
|--------------------------------|----------------------|---------------------------------|--------------------------|----------------|-------|---------------|---------------------------------|-------------------------------|-----------------------------|
| Acetic acid, butyl ester       | 8.16                 |                                 |                          | 72             | 123-86-4 | 416,938       |                                 |                               |                             |
| Methyl valerate                | 8.48                 | Foul                            |                          | 93             | 624-24-8 | 4,637,137     |                                 |                               |                             |
| Butanoic acid, 2- methyl-, ethyl ester | 8.77               | Floral                          |                          | 93             | 7452-79-1 | 498,859       |                                 |                               |                             |
| Butanoic acid, 3- methyl-, ethyl ester | 8.96            |                                 |                          | 85             | 108-64-5 | 242,635       |                                 |                               |                             |
| 1-Pentanol                     | 9.46                 |                                 |                          | 76             | 71-41-0  | 1,917,299     |                                 |                               |                             |
| Acetoin                        | 9.76                 |                                 |                          | 63             | 513-86-0 | 584,798       |                                 |                               |                             |
| 2-Propanol, 1-propoxy-         | 10.19                |                                 |                          | 83             | 1569-01-3 | 125,926       |                                 |                               |                             |
| Butanoic acid, propyl ester    | 10.27                | Chemical, sweet                 |                          | 95             | 105-66-8 | 7,807,628     |                                 |                               |                             |
| Pentanoic acid, ethyl ester    | 10.40                |                                 |                          | 95             | 539-82-2 | 3,496,629     |                                 |                               |                             |
| 2-Heptanone                    | 10.52                | Soap *, Fruity, sweet, cheese   |                          | 79             | 110-43-0 | 55,224        |                                 |                               |                             |
| Propanoic acid, butyl ester    | 10.6                 |                                 |                          | 76             | 590-01-2 | 1,373,814     |                                 |                               |                             |
| Heptanal                       | 10.8                 | Citrus *, fat, rancid           |                          | 83             | 111-71-7 | 68,145        |                                 |                               |                             |
| Acetic acid, pentyl ester      | 10.9                 |                                 |                          | 79             | 628-63-7 | 246,282       | 88,580                          |                               |                             |
| Butanoic acid, 2-methyl-, propyl ester | 11.42        |                                 |                          | 70             | 37064-20-3 | 836,785 | 3,074,917     | 85(100), 103(68), 41(60), 43(59), 57(58) | 56(100), 43(62), 55(50), 41(48), 42(40) |
| Butanoic acid, 3-methyl-, propyl ester | 11.6            | Bitter *, sweet, apple fruity, Resin *, flower, green | 90             | 557-00-6 | 812,614        | 61,216                          | 25415-67-2 | 528,515 |
| 1-Hexanol                      | 11.9                 | Floral, banana                  |                          | 72             | 111-27-3 | 812,614        |                                 |                               |                             |
| Pentanoic acid, 4-methyl-, ethyl ester | 12.11        |                                 |                          | 56             | 25415-67-2 | 61,216 | 56(100), 103(68), 41(60), 43(59), 57(58) | 56(100), 43(62), 55(50), 41(48), 42(40) |
| Propanoic acid, pentyl ester   | 12.21                |                                 |                          | 79             | 624-54-4 | 490,854       | 49,044                          | 108(100), 42(55), 39(35), 40(30), |                             |
| Pyrazine, 2,6-dimethyl-         | 12.39                | Cocoa *, meat                   |                          | 88             | 108-50-9 | 490,854       |                                 |                               |                             |
Table 3. Cont.

| 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) |
|----------------------------------|----------------------|---------------------------------|--------------------------|-----------------|-------|---------------|---------------------------------|-------------------------------|---------------------------|
| 2-Heptanone 5-methyl-            | 12.52                |                                 | Herbaceous, grassy, earthy sour, nutty | 81              | 18217-12-4 | 127,769       | 33,638,253                     | 159,486,602                  | 43(100), 58(40), 71(38), 70(25), 41(20) |
| Acetic acid                      | 12.6                 | sour a                          |                          | 96              | 64-19-7  | 63,623,253    | 159,486,602                     | 43(100), 45(88), 60(60)       | 85(100), 103(75), 57(70), 41(60) |
| Pentanoic acid, propyl ester     | 12.95                |                                 |                          | 68              | 141-06-0 | 4,421,968    | 4,421,968                       | 4,421,968                    | 57(100), 70(40), 75(40), 43(40), 55(25) |
| Propanoic acid, pentyl ester     | 13.25                | Grassy, soil                    |                          | 63              | 624-54-4 | 741,201       | 741,201                         | 741,201                      | 43(100), 41(60), 108(40), 69(40), 39(28) |
| 5-Hepten-2-one-6-methyl-         | 13.52                | Old cheese                      |                          | 95              | 110-93-0 | 228,324       | 228,324                         | 228,324                      | 74(100), 41(60), 57(70), 44(48) |
| 3-Octanol                        | 14.08                |                                 |                          | 72              | 589-98-0 | 170,440       | 171,440                         | 171,440                      | 74(100), 41(60), 57(70)       |
| Butanoic acid, 3-methyl-, butyl ester | 14.16          |                                 |                          | 63              | 109-19-3 | 157,326       | 157,326                         | 157,326                      | 74(100), 45(72), 73(60), 57(40) |
| Propanoic acid                   | 14.31                | Pungent a, rancid               | Unpleasant, butter       | 93              | 79-09-4  | 54,686,812    | 9,505,716                       | 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                     | 13,857,212                   | 74(100), 45(72), 73(60), 57(40) |
| Benzaldehyde                     | 15.30                | almond b                        | Butter                   | 93              | 100-52-7 | 2,215,240     | 2,215,240                       | 2,215,240                    | 74(100), 45(72), 73(60), 57(40) |
| Butanoic acid                    | 16.04                |                                 |                          | 95              | 107-92-6 | 113,602,470  | 123,858,474                     | 123,858,474                  | 74(100), 45(72), 73(60), 57(40) |
| Butanoic acid, 3-methyl-         | 16.84                | Sweet, fruity                   |                          | 83              | 503-74-2 | 76,585,646    | 61,915,734                     | 38,769,616                   | 74(100), 45(72), 73(60), 57(40) |
| 2-Methyl-4-decanone              | 17.60                |                                 |                          | 70 (NIST only)  | 6628-25-7 | 116,081       | 116,081                         | 116,081                      | 74(100), 45(72), 73(60), 57(40) |
| Acetophenone                     | 17.88                | Must a, b, flower, almond       | Smokey                   | 88              | 98-86-2  | 359,940       | 359,940                         | 359,940                      | 74(100), 45(72), 73(60), 57(40) |
| Pentanoic acid                   | 18.02                |                                 |                          | 76              | 109-52-4 | 108,458,001   | 52,809,697                      | 52,809,697                   | 74(100), 45(72), 73(60), 57(40) |
| Pentanoic acid, 4- methyl-       | 19.13                | Butter, basmati rice, butter    |                          | 85              | 646-07-1 | 3,286,558     | 3,286,558                       | 3,286,558                    | 74(100), 45(72), 73(60), 57(40) |
| Hexanoic acid                    | 19.81                |                                 |                          | 83              | 142-62-1 | 3,822,922     | 3,822,922                       | 3,822,922                    | 74(100), 45(72), 73(60), 57(40) |
| o-Guaiacol                       | 20.42                | Smoke a, medicine               | Woody, wild              | 95              | 90-05-1  | 4,755,984     | 283,513                         | 283,513                      | 74(100), 45(72), 73(60), 57(40) |
Table 3. Cont.

| 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) |
|----------------------------------------|----------------------|---------------------------------|--------------------------|-----------------|-------|--------------|--------------------------------|-------------------------------|-----------------------------|
| Benzeneethanol                         | 21.24                |                                 |                          | 91              | 60-12-8 | 384,960      |                                |                               |                             |
| Benzene propanoic acid, methyl ester   | 21.93                |                                 |                          | 93              | 103-25-3 | 210,723      |                                |                               |                             |
| Phenol                                 | 22.10                | phenol a                        | Medicinal                | 96              | 108-95-2 | 10,114,684   | 2,023,698                        | 315,371                      |                             |
| 2-Dodecanone                           | 22.5                 |                                 |                          | 85              | 6175-49-1 | 77,879       |                                |                               |                             |
| Benzenepropanoic acid, ethyl ester     | 23.01                |                                 |                          | 85              | 2021-28-5 | 148,940      |                                |                               |                             |
| p-Cresol                               | 23.30                | Smoke a, medicine               | Medicinal                | 93              | 106-44-5 | 39,957,137   | 3,947,249                        | 1,376,506                    |                             |
| Phenol, 4-ethyl-                       | 24.80                | Must a                          | Foul, unpleasant         | 94              | 123-07-9 | 1,021,035    | 103,774                          |                               |                             |
| Butylated hydroxytoluene               | 25.10                |                                 |                          | 96              | 128-37-0 | 182,612      | 52,706                          |                               |                             |
| Indole                                 | 28.38                | Burnt a                         | Medicinal, unpleasant    | 96              | 120-72-9 | 69,332,928   | 195,879                          | 810,599                      |                             |
| Diethyl Phthalate                      | 29.08                | -                               |                          | 94              | 84-66-2  | 81,563       |                                |                               |                             |
| Indole, 3-methyl-                      | 29.2                 | Fecal a                         | Urinous, animal          | 71              | 83-34-1  | 78,683       |                                |                               |                             |

* Fifty min exposure to SPME at 37 °C; ** 15 min equilibrium and 50 min exposure to SPME at 37 °C; °° 24 h equilibrium and 50 min exposure to SPME at 37 °C; odor verified with a Flavornet [15] and b Good Scent Company [16]. # Below 60% library match considered as semi-confirmative.
Figure 4. An overlay of the aromagram and total ion chromatogram of the stale cat feces, equilibrated for 24 h and exposed to the SPME fiber for 50 min at 37 °C: an increase in the black signal height represents an increased intensity of odor, and the chromatogram is in red for the total ion chromatogram (TIC) signal. The green zone shows that trace levels of the concentration of malodors can produce an intense olfactory response detected by the panelist using GC–MS–O.

The fresh feces on 50 min exposure to SPME fiber caused several ketones, aldehydes, esters, acids, and phenols to accumulate in the SPME fiber. Among these identified chemicals, most of the high-molecular-weight compounds have “smoke”, “medicinal”, “animal”, and “foul” smells, whereas the low-molecular-weight chemicals had more “chemical”, “sweet”, “fruity”, and “grassy” or “earthy” smells. 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.

The compounds with high molar masses that appeared after a retention time of 20 min 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 compared to long equilibration times of 24 h. In stale feces, the primary contributors such as 3-methyl indole were missing, and phenolics had lower PAC (4-ethyl phenol, p-cresol, and phenol) than phenolics in fresh feces headspaces. 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 ofodorants even in the below-the-detection-limit concentration for GC-MS can cause a considerable odor impact (the green line in Figures 3 and 4) problem. The aromagram also revealed that compounds not in the GC-MS data can still contribute to the overall odor, thus triggering the human nose to react to the odorants.
4. Discussion

The focus of this study is reporting the malodorous VOCs in domestic cat urine and feces and the influence of aging 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 previously in research [6]. As reported by Miyazaki et al. (2018), the presence of a fishy odor in the headspace of domestic cat anal sac secretions suggesting 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]. In contrast, Banik et al., 2020, reported the odor of trimethylamine released from a carpet contaminated with cat urine upon two weeks of aging; however, the “fishy” smell was absent in the emissions from fresh urine-treated carpets [18]. Feral male cats spray urine more often than females [19] and use unburied feces as scent-marking [20], and the released volatiles in both cases could be offensive to humans; the odor becomes even worse with time, although the VOC concentrations become trace levels in feces sample with time (Figure 4). This current study recorded the odor description of emitted VOCs that were never reported before for aged cat urine and feces studies. 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. Our work supports that 2,5-dimethyl pyrazine and other pyrazines are the product of cat urine aging [11]. Additionally, this work also reports that longer (e.g., 50 min) exposure of the SPME to the aged urine headspace allowed the pyrazines, pyrrole, and pyrimidine to be absorbed onto the fiber and to be detectable in GC–MS.

The use of SPME sampling has allowed easy preconcentrating of sample 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 dropped in number for the aged feces, either degraded to other compounds by the microbial community present in the sample, oxidized, or lost to the atmosphere during the aging process [11].

A number of phenol, alcohol, aldehyde, N-compounds (amines, pyrazines, and indoles), S-compounds (dimethyl disulfide and dimethyl sulfone), 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) [4,8,21]. Highly odorous gases emitted from urine or feces pose likely low inhalation risks. The human nose is very sensitive to many impactful odorants emitted from feces and urine in general. The nuisance odor experienced by homeowners or visitors can be attributed to stale urine. The aged urine is difficult to clean and completely remove from carpets and subflooring materials. Some homeowners experience these stale urine-like smells from floor areas that linger for years even after the cats are removed and no longer contributing urine. Once the carpet is contaminated with cat urine, many of the VOCs emitted are different compared to the VOCs emitted initially by urine itself; however, several VOCs emitted from the contaminated carpet are common to those reported here [18].
Table 4. Some literature recorded the VOCs emitted from urine, feces, and fruits of different biological species commonly found that are also in the current study.

| Compounds                  | Lion Urine (Soso and Koziel, 2017) [21] | Tiger Urine (Soso and Koziel, 2016) [18] | Cat Urine (Miyazaki, 2006) [4] | Swine Manure (Lo et al., 2006) [22] | Grapes (Rice et al., 2019) [23] | Cat Urine-Carpet (Banik et al., 2020) [18] | Current Study |
|----------------------------|---------------------------------------|----------------------------------------|-------------------------------|-----------------------------------|---------------------------------|---------------------------------------------|---------------|
| Phenol                     | 108-95-2                              | X                                      | X                             | X                                 | X                               |                              |               |
| p-Cresol                   | 106-44-5                              | X                                      | X                             | X                                 | X                               |                              |               |
| Phcnol, 4-ethyl-3-Octanol  | 123-07-9                              | X                                      | X                             | 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                             | X                                 | X                               |                              |               |
| 3-Buten-1-ol, 3-methyl-Benzene ethanol | 763-32-6 | X                                      | X                             | X                                 | X                               |                              |               |
| Dimethyl disulfide         | 624-92-0                              | X                                      | X                             | X                                 | X                               |                              |               |
| Dimethyl sulfone           | 67-71-0                               | X                                      | X                             | X                                 | X                               |                              |               |
| Benzaldehyde               | 100-52-7                              | X                                      | X                             | X                                 | X                               |                              |               |
| Butanal, 3-methyl-Isobutyraldehyde | 78-84-2 | X                                      | X                             | X                                 | X                               |                              |               |
| Trimethyl amine            | 75-50-3                               | X                                      | X                             | X                                 | X                               |                              |               |
| 2-Dodecanone               | 6175-49-1                             | X                                      | X                             | X                                 | X                               |                              |               |
| 2-Heptanone                | 110-43-0                              | X                                      | X                             | X                                 | X                               |                              |               |
| 2-Pentanone                | 107-87-9                              | X                                      | X                             | X                                 | X                               |                              |               |
| 3-methyl 2-pentanone       | 565-61-7                              | X                                      | X                             | X                                 | X                               |                              |               |
| Butanone                   | 78-93-3                               | X                                      | X                             | X                                 | X                               |                              |               |
| Acetone                    | 67-64-1                               | X                                      | X                             | X                                 | X                               |                              |               |
| 5-Hepten-2-one, 6-methyl-Acetophenone | 98-86-2  | X                                      | X                             | X                                 | X                               |                              |               |
| Jasmonne                   | 488-10-8                              | X                                      | X                             | X                                 | X                               |                              |               |
| Indole                     | 120-72-9                              | X                                      | X                             | X                                 | X                               |                              |               |
| 3-methylindole            | 95-20-5                               | X                                      | X                             | X                                 | X                               |                              |               |
| Pyrazine, 2,6 dimethyl-Acetic acid | 108-50-9 | X                                      | X                             | X                                 | X                               |                              |               |
| Acetic acid                | 64-19-7                               | X                                      | X                             | X                                 | X                               |                              |               |
| Butanoic acid              | 107-92-6                              | X                                      | X                             | X                                 | X                               |                              |               |
| Pentanoic acid, 2-methyl-Butanoic acid, 3-methyl- | 79-31-2 | X                                      | X                             | X                                 | X                               |                              |               |
| Pentanoic acid             | 109-72-4                              | X                                      | X                             | X                                 | X                               |                              |               |
| Propanoic acid             | 78-09-4                               | X                                      | X                             | X                                 | X                               |                              |               |
| Hexanoic acid              | 142-62-1                              | X                                      | X                             | X                                 | X                               |                              |               |
| Butanoic acid, 3- methyl   | 503-74-2                              | X                                      | X                             | X                                 | X                               |                              |               |
| Carbon disulfide           | 75-15-0                               | X                                      | X                             | X                                 | X                               |                              |               |
| Butanoic acid, ethyl ester | 105-54-4                              | X                                      | X                             | X                                 | X                               |                              |               |

CAS# = Chemical Abstracts Service; X represents the VOCs common to this study and previously published work.
Many VOCs emitted from the cat feces samples, as reported in this study, were also reported to be emitted from swine manure samples [22]. 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 grape samples [23]. The characteristic odor compound 2,5 dimethyl pyrazine from lion and cat urine indicates the evolutionary similarities between animals. Chemicals such as phenol and p-cresol in human urine are important biomarkers, and they are challenging to measure due to their presence at a low detection limit [24]. However, the use of GC–MS–O can verify their presence by their distinct “ruinous” or “barnyard” odor even at trace levels (Figures 3 and 4). These observations also indicate the fact that SPME in a combination of GC–MS–O for the headspace extraction is better suited for assessing odor than only GC–MS. Overall, SPME reduces the sampling time for volatile or semi-volatile compound determination compared to a traditional sampling technique [25], and this technology can be developed more for future assessment of quantitative analysis of these odorous constituents characteristic to the overall smell of urine or feces sample.

Several VOCs were common in urine and feces samples, such as acetone, 2-butanone, 3-methyl butanal, 3 methyl 2-pentanone, phenol, p-cresol, and indole. Chemicals such as phenol and indole are commonly produced by cat species irrespective of their sex and age [8,10]. N-heterocycles like pyrimidine and pyrroles were only recorded in the urine after aging but not in the fresh nor stale feces. Similarly, o-guaiacol, 4-ethyl phenol, and 2-methyl indole were only present in the feces samples but not in the urine. This reveals that some chemicals possibly carry urine and feces samples’ characteristic VOCs and substantially contribute to their specific odor. This research reported chemicals emitted from domestic cat fresh urine and feces to the vial headspace and upon aging. However, there are limitations to this work as the research did not test different SPME fiber coatings and extraction regimes (time \(T\)). There is still plenty of opportunities to explore specific questions, e.g., the effectiveness of cleaning products for cat urine and the mitigation of malodors from cat litter boxes.

**Supplementary Materials:** The following are available online at [https://www.mdpi.com/2297-8739/8/2/15/s1](https://www.mdpi.com/2297-8739/8/2/15/s1), Table S1: Aroma description as recorded by the panelist for fresh cat urine at 10 min extraction, Table S2: Aroma description as recorded by the panelist for fresh cat urine at 50 min extraction, Table S3: Aroma description as recorded by the panelist for stale cat urine at 50 min extraction, Table S4: Aroma description as recorded by the panelist for fresh feces at 50 min extraction, Table S5: Aroma description as recorded by the panelist for stale feces at 15 min equilibrium and 50 min extraction, Table S6: Aroma description as recorded by the panelist for stale cat feces at 24 h equilibrium and 50 min extraction.

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Appendix A

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

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