SUMMARY:
INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES

Daniel E. Meeroff (PI)[1]
Julia Roblyer and Mateja Vidovic

In 2015, the Bill Hinkley Center for Solid and Hazardous Waste Management funded FAU Lab.EES to find ways to improve and standardize odor identification, evaluate additional methods to establish reasonable, objective standards for odor severity, and explore other options for mitigation and detection including a novel technology that will attempt to use human odorant binding protein to quantify odors. Areas of application include policy development, land use strategic planning, odor regulation, complaint assessment, odor impact assessment, odor master planning, odor control efficiency assessment, and process design.

Nuisance odor levels produced by solid waste management operations such as landfill facilities, wastewater treatment plants and confined animal feeding operations are subject to regulatory standards because of their impacts on the quality of life of the public living within range. Failure to meet such standards may result in costly fines, litigation, inability to acquire permits, mitigation, and re-siting operations. Since measurement of environmental nuisance odors is currently limited to subjective techniques, monitoring odor levels to meet such standards is often problematic.

The objective of the proposed research is to develop a standardized, non-subjective measurement of nuisance odors using human odorant binding protein 2a (OBP2A) or similar analog. Since OBP2A binds a wide range of odorants, it may be used singularly as an odorant detection method for municipal solid waste facilities whose odors are caused by a vast array of chemicals in varying proportions.

The OBP2A will be synthesized and isolated using standard laboratory methods. Following isolation, OBP2A will be labeled with fluorescent markers to indicate when odorant molecules have been bound to the protein. After fluorescent marking, OBP2A will be exposed to known odorants within a vacuum chamber. Fluorescence will be measured using a fluorometer and analyzed for fluorescence – concentration responses during odorant binding. If the relationship follows Beer's Law, then concentrations of odorants can be accurately determined using fluorometric measurements.

As a starting point, the fluorescently tagged OBP2A will be exposed to model compounds that generate specific responses in human olfactory cells such as formic acid and dimethyl disulfide, detected at concentrations as low as 0.1 ppm, to determine a positive response and concentration dependence.

[1] Prof., Dept. of Civil, Environmental & Geomatics Engineering, Florida Atlantic University, 777 Glades Road, 36/206, Boca Raton, FL 33431-0091, Phone: (561) 297-3099, E-Mail: dmeeroff@fau.edu

PROGRESS REPORT
(August 2016)

Project Title: INVESTIGATION OF EFFECTIVE ODOR CONTROL STRATEGIES
Principal Investigators: Daniel E. Meeroff, Ph.D.
Affiliation: FAU
Phone number: (561) 297-2658
Project website: http://labees.civil.fau.edu/leachate.html
Students: Julia Roblyer, Mateja Vidovic

Methodology/Scientific Approach

- **TASK 1. Conduct literature review**. Mateja Vidovic is continuing to conduct and update an exhaustive literature review focused on identifying sources of odor in landfills, non-subjective odor monitoring techniques, and methods of odor control including best odor management practices. To date, databases have been created and are being constantly updated with: 1) lists of specific odor causing compounds in solid waste operations; 2) lists of parameters that can impact the efficiency of data collection; 3) lists of parameters which have the greatest influence on creating and spreading of nuisance odors; 4) lists of odor monitoring technologies that are used in solid waste operations; and 5) lists of case studies and best management practices for odor mitigation technologies.
- **TASK 2. Collect data on Florida-specific odor management strategies.** The strategy of this study is to target partner landfills located in an urban setting. Therefore, several solid waste management facilities in those locations have been contacted in order to collect data about odor complaints. So far, data has been provided by the Solid Waste Authority of Palm Beach County as well as the real-time access to their weather station has been approved. Meteorological parameters such as, temperature, wind speed, wind direction, precipitation accumulation and pressure can be monitored via a wireless connection. Also, the meteorological data from previous years can be retrieved as well. A meeting to secure data from Monarch Hill was held on July 19, 2016. Monarch Hill agreed on installing an FAU provided weather station and providing access to the secured odor complaint data. During this visit, the research team gathered valuable information on how odor complaints are managed, on what odor mitigation technologies are in use and what could be the other potential sources of odors. Another meeting with Craig Ash, Jeff Roccapriore and Jim Christiansen of Waste Management Inc. of Florida is planned to collect historical data sets and arrange installation of the weather station. The online ENVIROS system (Environmental Inquiry and Resource System) of Broward County will be reviewed as well, in order to collect more information on odor complaints to the county environmental protection unit. Mrs. Damaris Lugo has been contacted to assist with this endeavor, and she has agreed to assist the research team in collecting this data for further analysis.
- **TASK 3. Pattern identification and trend analysis.** Based on the odor complaints data received from the Solid Waste Authority of Palm Beach County and databases from Waste Management Inc. of Florida, as well as using appropriate qualifiers for meteorological measurements and landfill operations, the datasets can be analyzed to

determine the existence of patterns or trends that could lead to the development of effective management strategies.

Odor complaint data received from the Solid Waste Authority of Palm Beach County was used for preliminary analysis and to see if any patterns exist connected to meteorological conditions. Trend analysis was done in Excel, and the data from the weather station provided by SWA was used to get the information about the meteorological conditions in that specific time period (2005-2016). The initial dataset consisted of 423 points. An analysis was conducted to determine what year, month and day of the week as well as the time of the day in which the most odor complaints were logged. Also, the meteorological conditions on the most problematic days were compared to determine any patterns. Preliminary results showed that the year 2014 had the largest number of odor complaints (n=98). Average number of odor complaints per month in 2014 was 9, with the most number being logged in the month of September with 51 complaints; followed by December with 44 complaints and February with 43. When comparing the most problematic days, analysis showed that working days had more odor complaints than the weekends, potentially because most people were not home during the weekends. Most odor complaints were received in the afternoon hours, which can be related to people coming home from work in the afternoon and spending more time at home than in the morning hours.

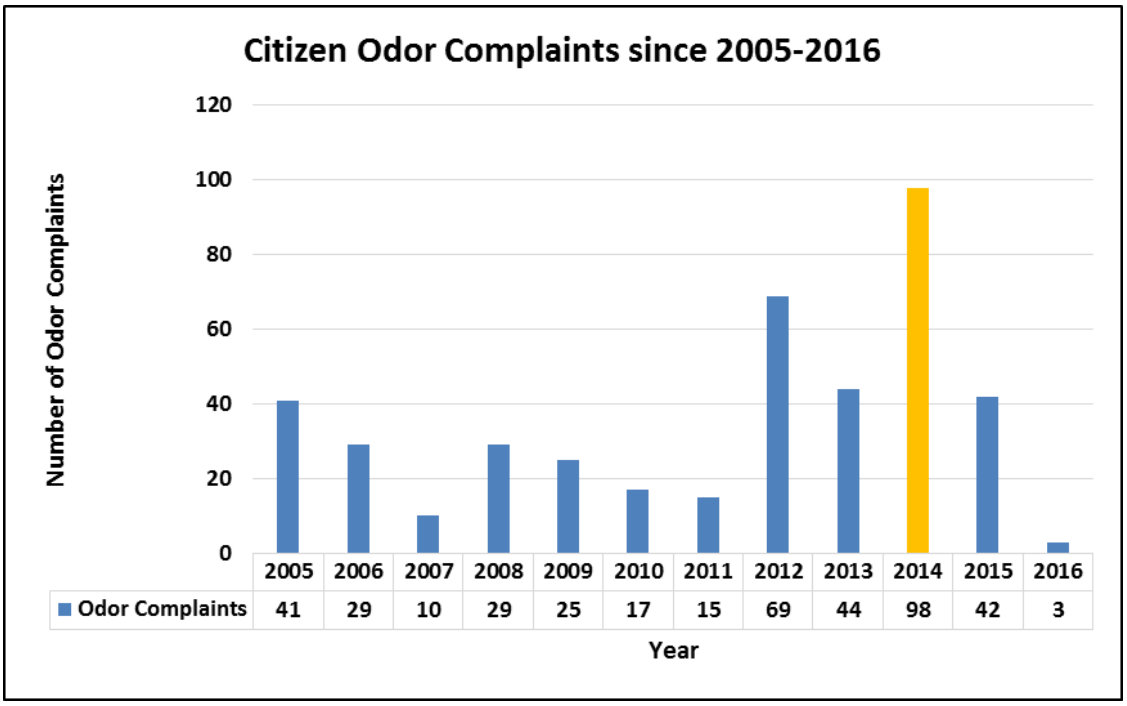

Figure 1. Year with the greatest number of odor complaints

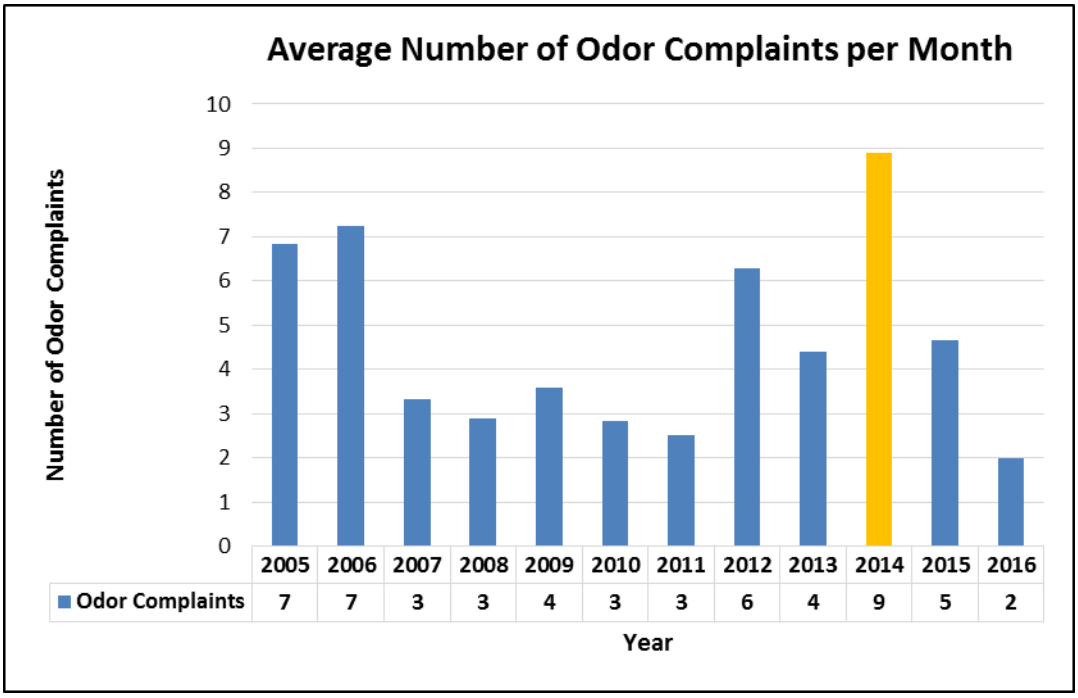

Figure 2. Average number of odor complaints per month in a year

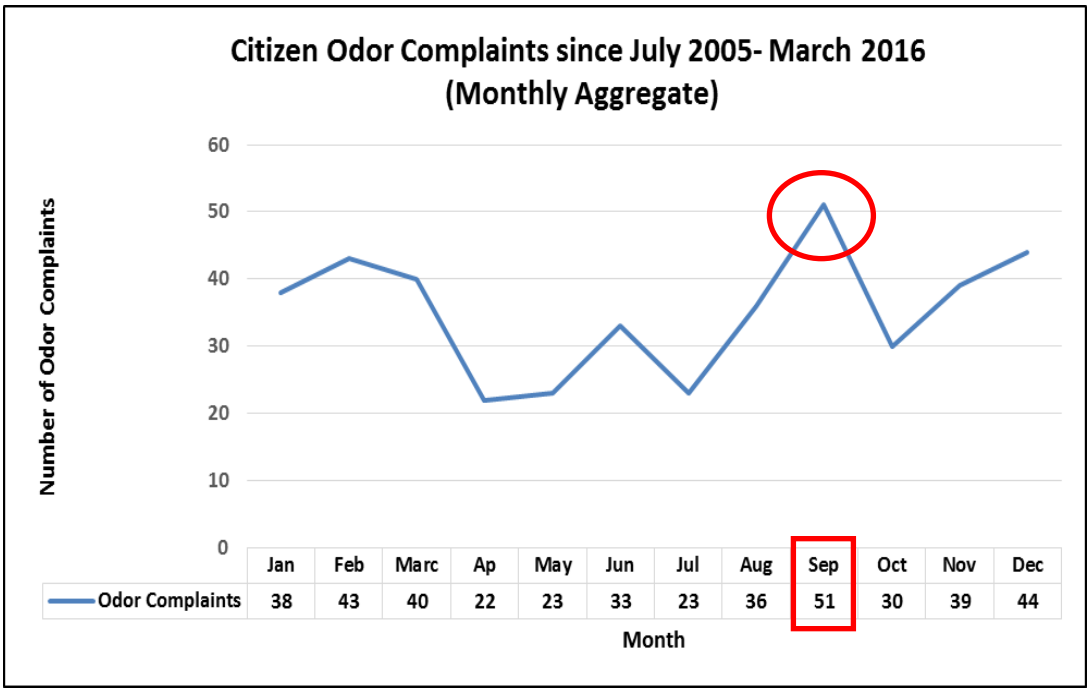

Figure 3. Month with the greatest number of odor complaints

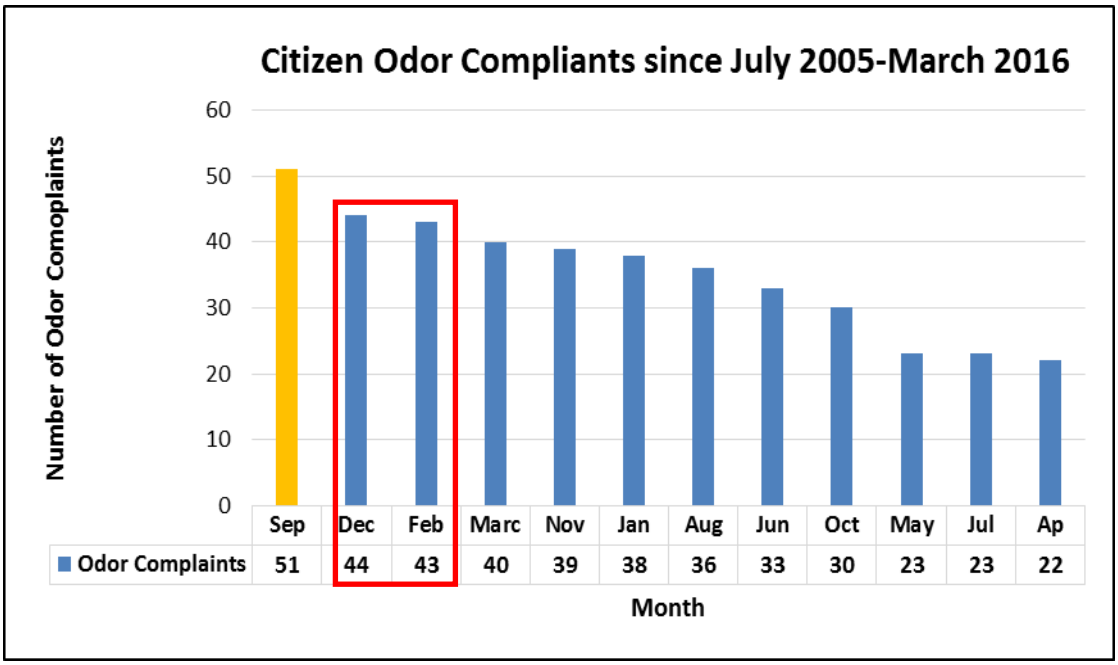

Figure 4. Month with the greatest number of odor complaints

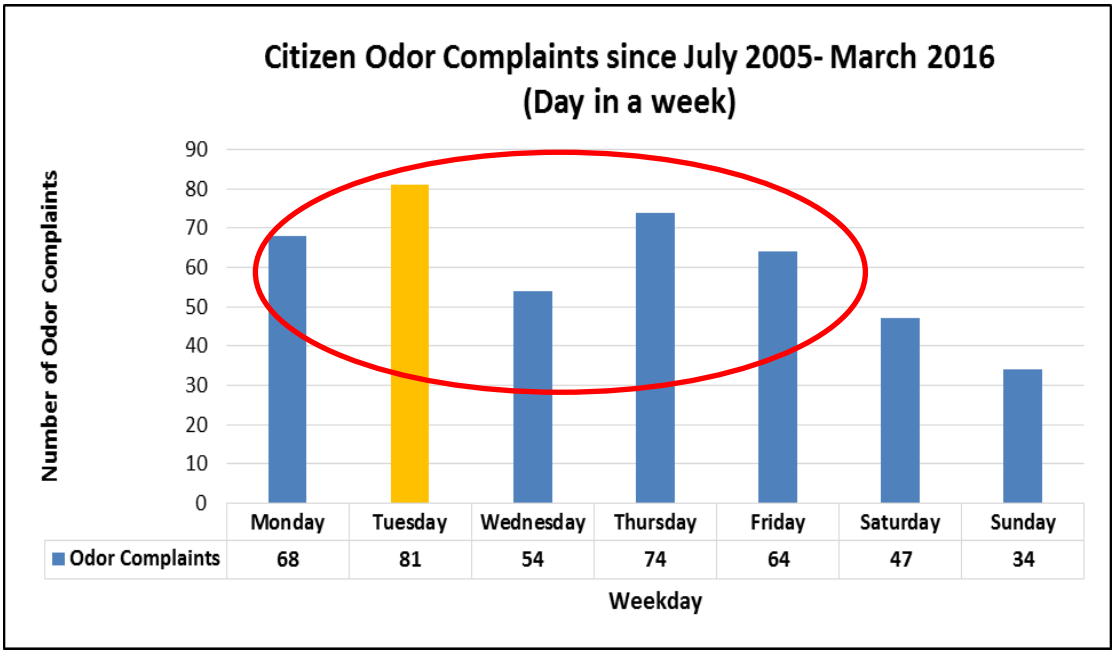

Figure 5. Odor complaints based on the day in a week

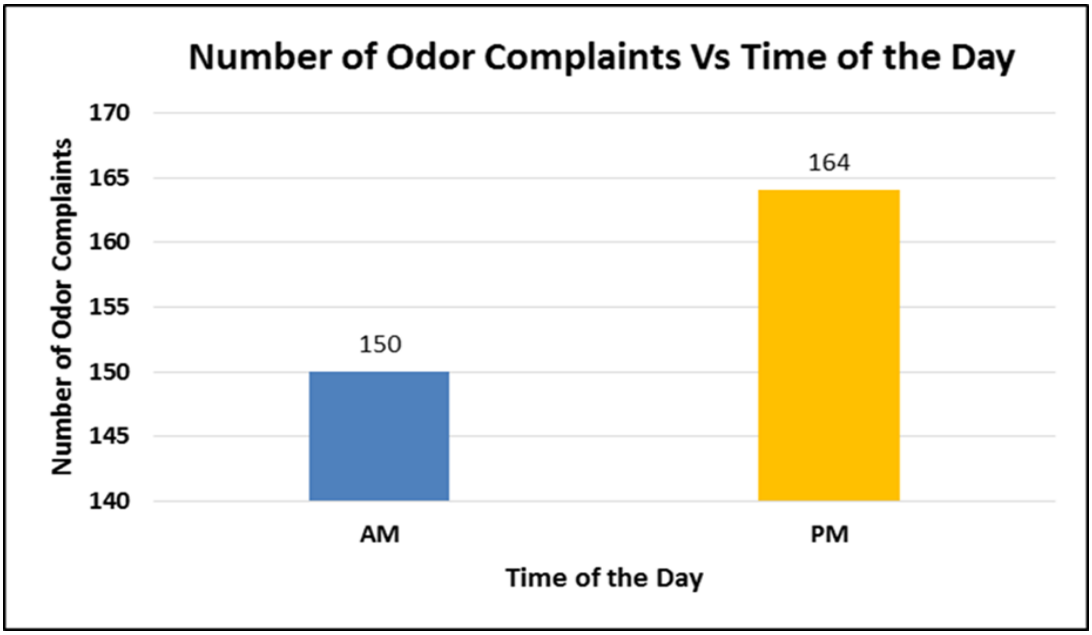

Figure 6. Odor complaints based on the time of the day

The next step was to see how many days in time period (2005-2016) had more than one odor compliant in the same day. Year 2005 had 6 odor complaints in the same day on 9th of December and 3 odor complaints on the 5th and 6th of December. Year 2006 on the 2nd of February had 4 odor complaints in the same day while 13th of February had 3 of them. Year 2009 had up to 4 odor complaints in almost consecutive days, on the 26th and 28th of May. Year 2012 had 4 odor complaints on the 7th of June. The most interesting situation was in the year 2014, in which September 16-19 had a total of 10 complaints. Also, 5 incidents in the same day were noted on the 25th of November. These would seem to indicate some operational change at the site.

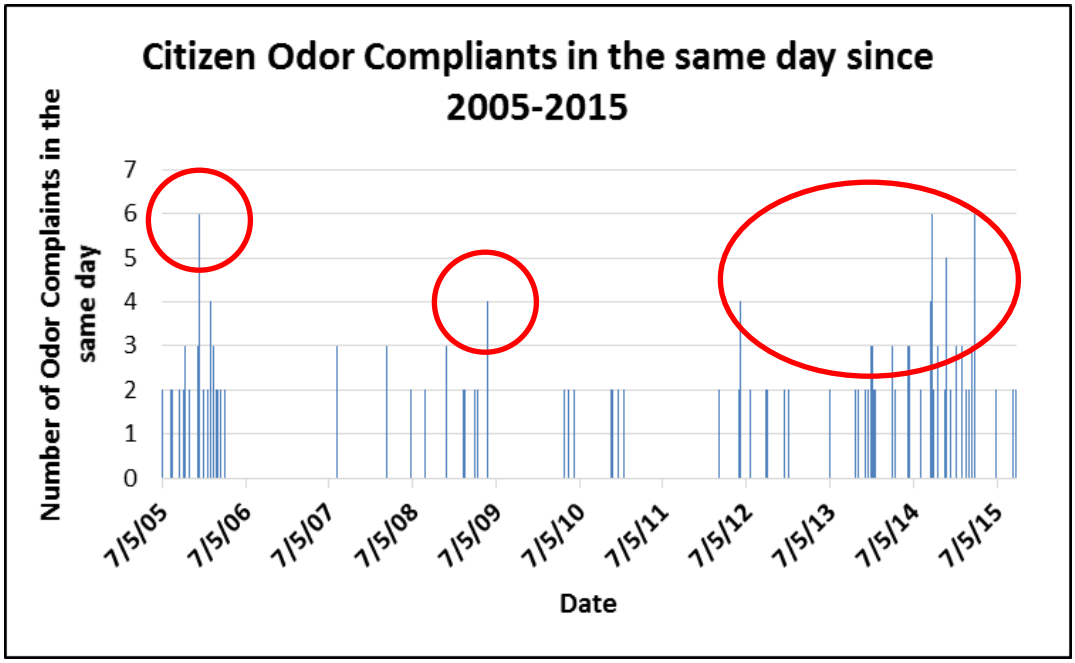

Figure 7. Years with greatest number of odor complaints in the same day

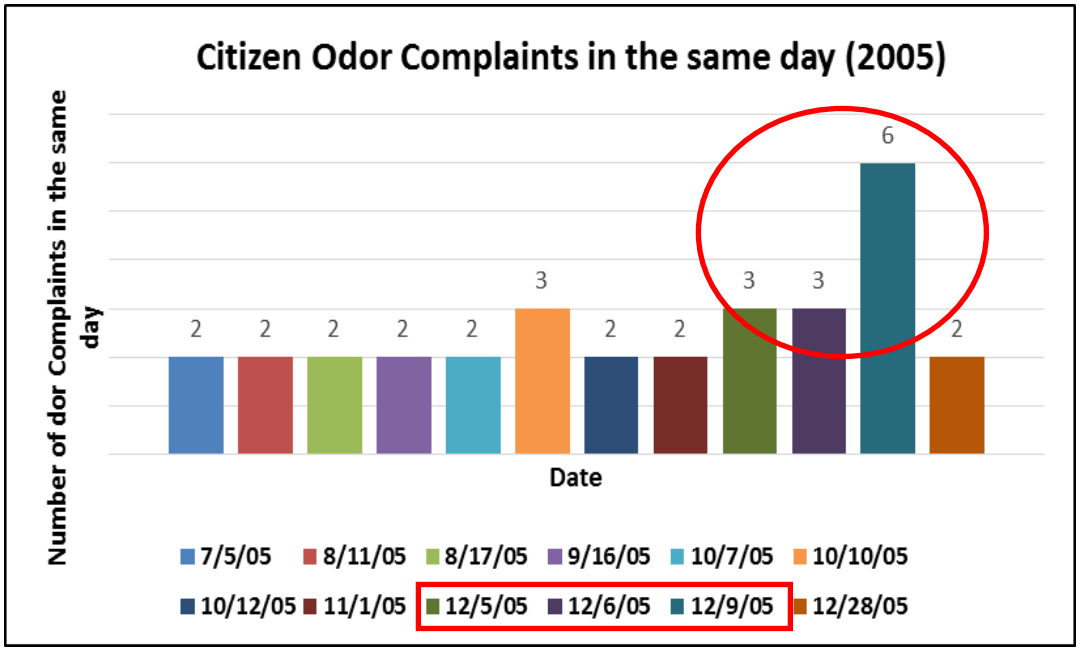

Figure 8. Odor complaints in the same day for 2005

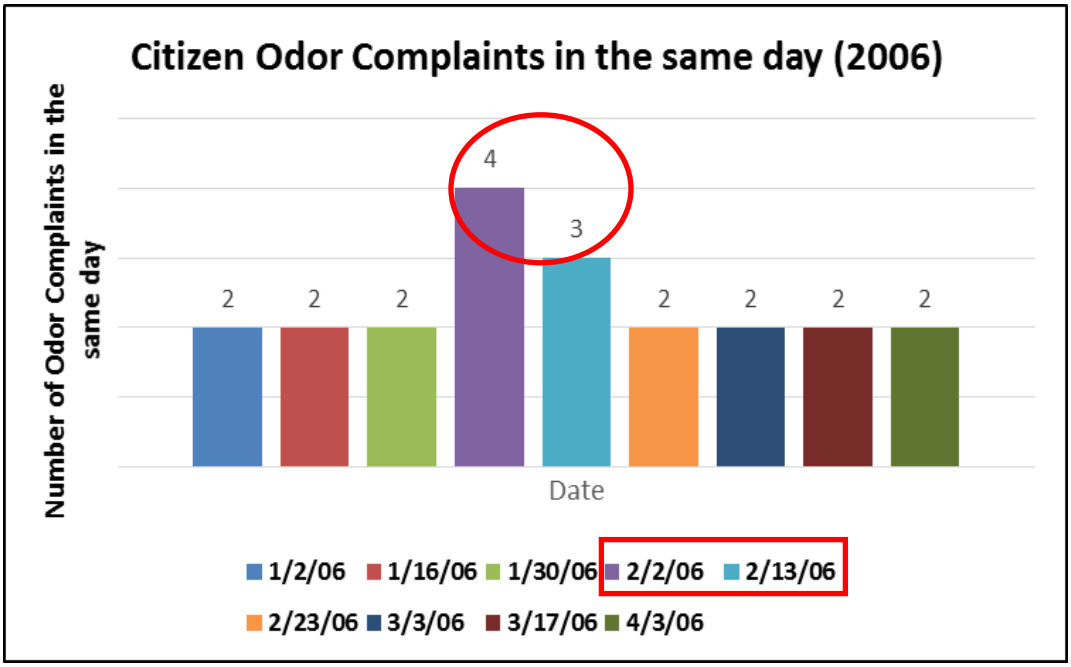

Figure 9. Odor complaints in the same day for 2006

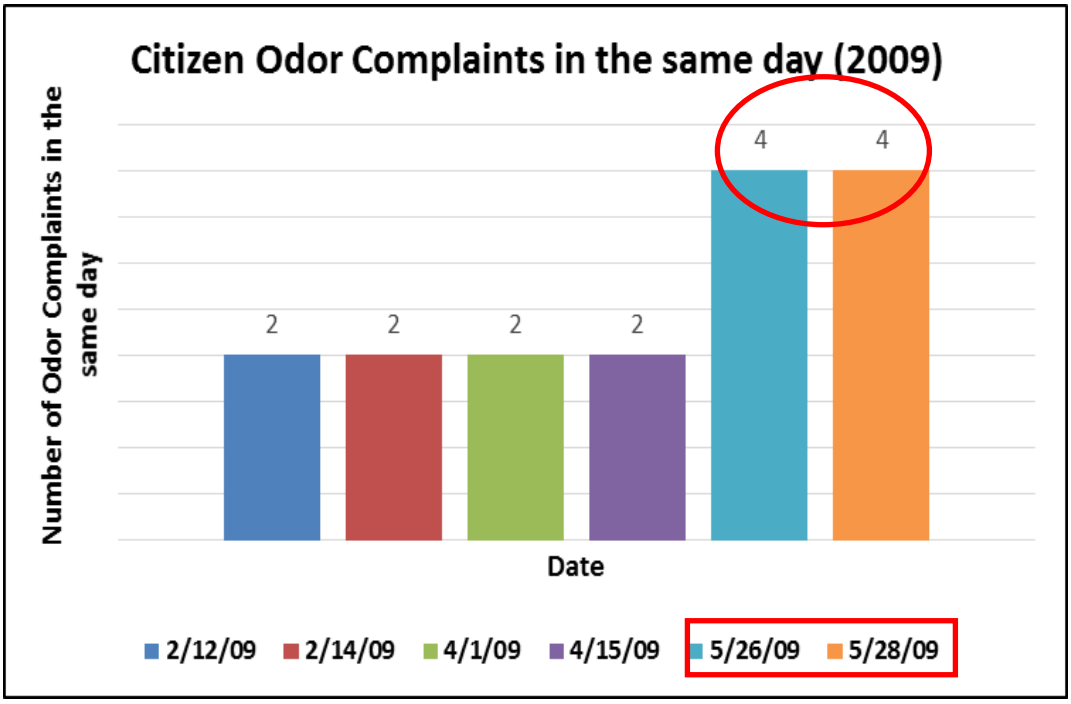

Figure 10. Odor complaints in the same day for 2009

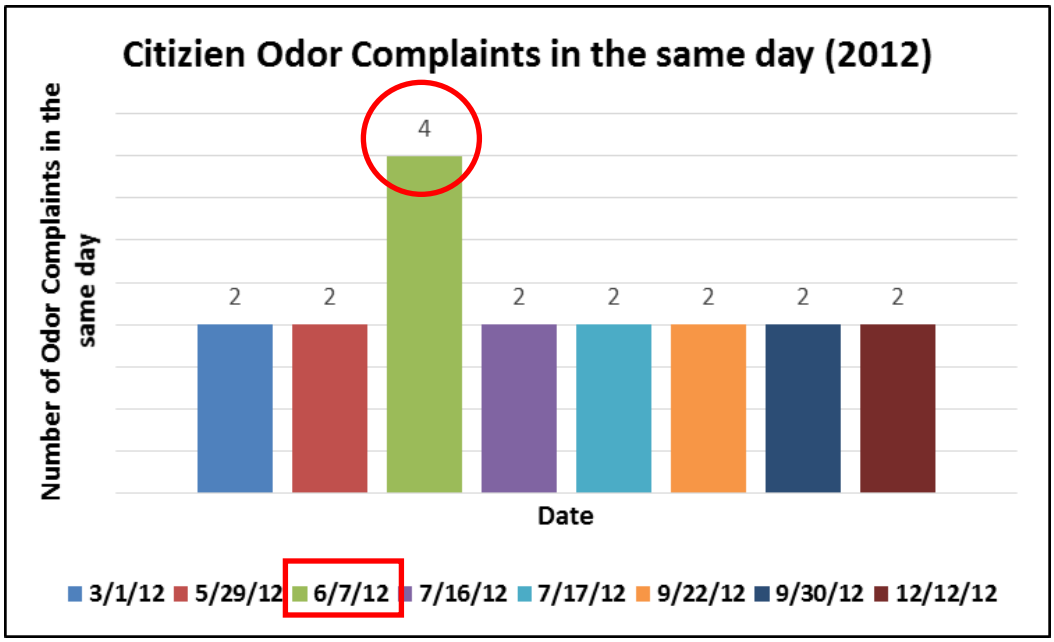

Figure 11. Odor complaints in the same day for 2012

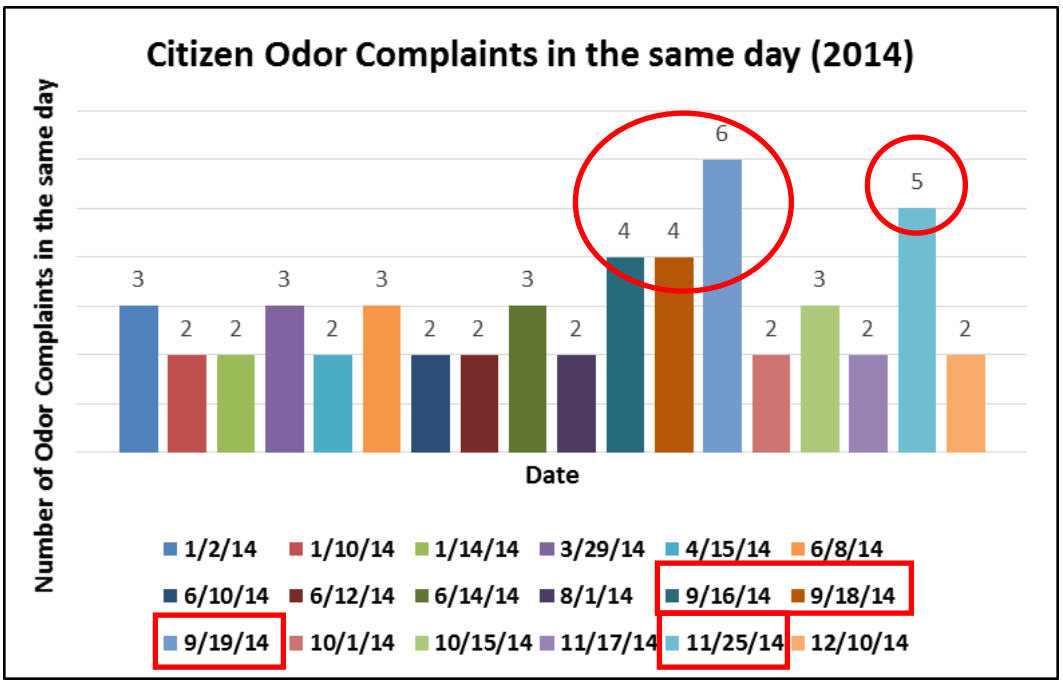

Figure 12. Odor complaints in the same day for 2014

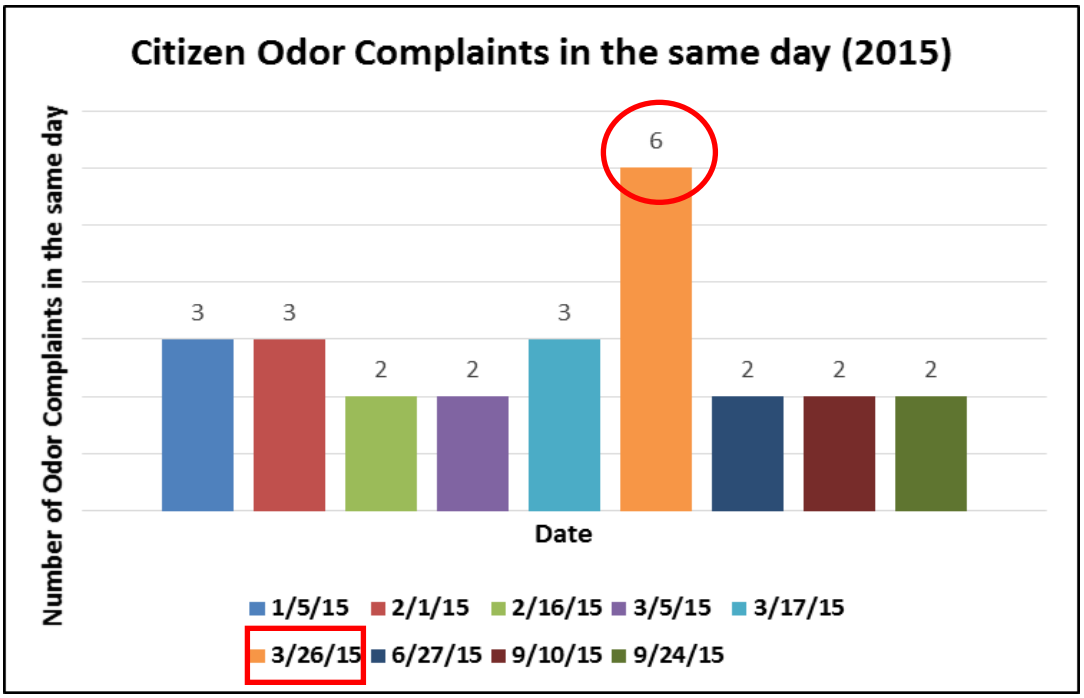

Figure 13. Odor complaints in the same day for 2015

Going through the literature review, four or more verified odor complaints in the same day triggers corrective action, so for further analysis, only days with the largest number of complaints (6) in the same day were taken into consideration. Since in 2014, only a small number of continuous days in a row had a large number of complaints in the same day and might be related. Dates taken for analysis were: 12/09/05 (6 complaints); 9/16/14 (4 complaints); 9/18/14 (4 complaints); 9/19/14 (6 complaints) and 3/26/15 (6 complaints). When mapping the locations of the odor complaints received for each date, it was concluded that the largest number of complaints is coming from the same neighborhood for all cases. Next step was to examine the meteorological conditions in the time of the call as well as for the previous day, and information was gathered from the nearest weather station. Year 2005 was excluded from the analysis since the weather station did not provide information on meteorological conditions prior to 2008. Meteorological conditions showed that there is a possibility for correlation between the number of odor complaints and weather conditions since for all cases they were very similar: wind speed was weak, wind direction was south, temperature was high, pressure dropped, and there were no precipitation, also the weather conditions were stable. Only in the case of September 19, 2014, according to weather station data, average wind direction was ENE, but the exact time of the odor complaint reported showing that the wind direction was also south.

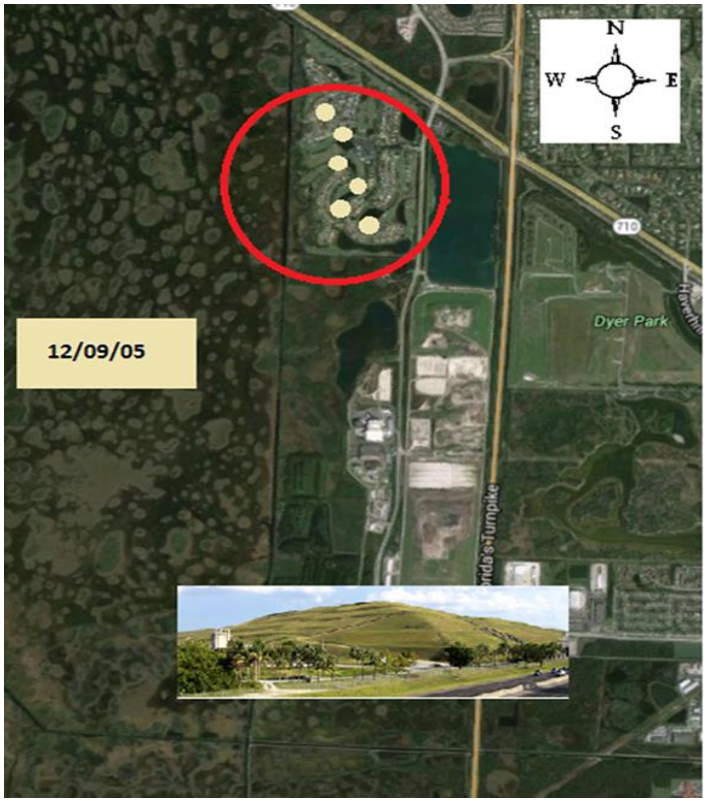

Figure 14. Location of odor complaints on the 9th of December 2005

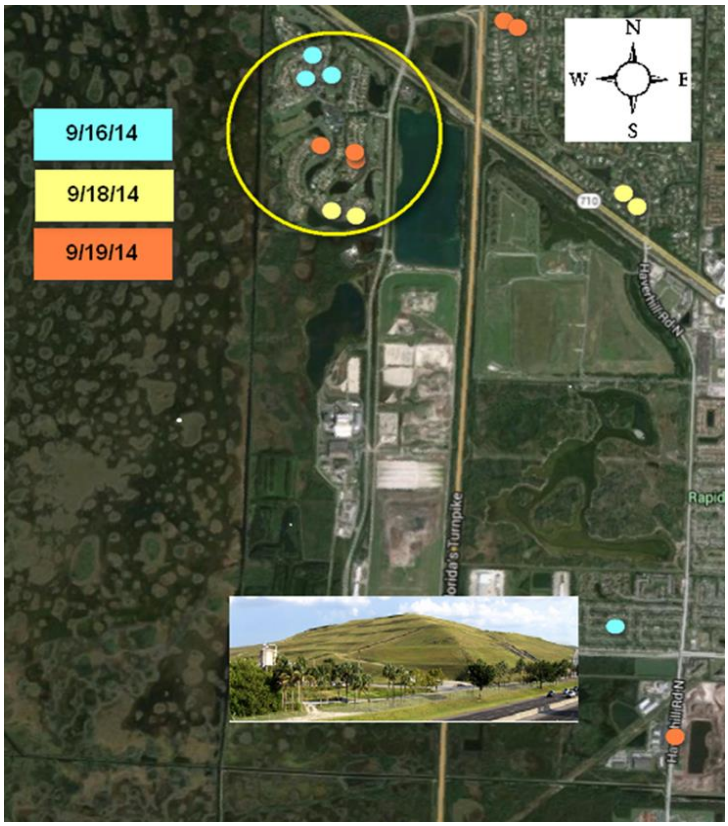

Figure 15. Location of odor complaints on the 16th, 18th and 19th of September 2014

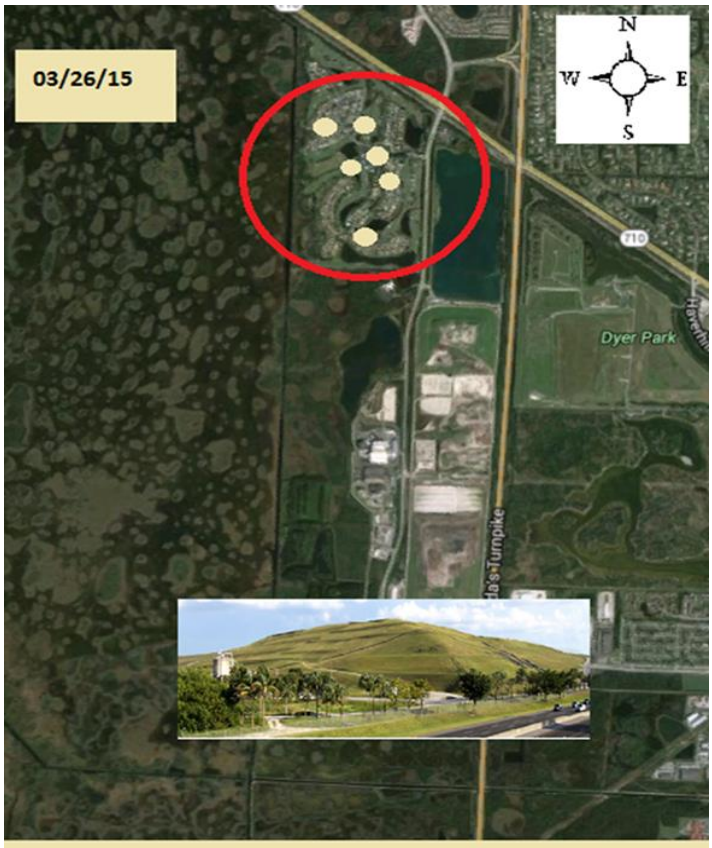

Figure 16. Location of odor complaints on the 26th of March 2015

September 16, 2014

	High	Low	Average
Temperature	88.1 °F	74.4 °F	81.1 °F
Dew Point	75.9 °F	72.8 °F	74.4 °F
Humidity	96%	63%	81%
Precipitation	0 in	--	--

	High	Low	Average
Wind Speed	8 mph	--	1 mph
Wind Gust	10 mph	--	--
Wind Direction	--	--	SE
Pressure	30.03 in	29.89 in	--

September 18, 2014

	High	Low	Average
Temperature	92.4 °F	73.1 °F	79.6 °F
Dew Point	76.7 °F	71 °F	73.7 °F
Humidity	95%	58%	83%
Precipitation	0 in	--	--

	High	Low	Average
Wind Speed	13 mph	--	3 mph
Wind Gust	13 mph	--	--
Wind Direction	--	--	South
Pressure	29.9 in	29.83 in	--

September 19, 2014

	High	Low	Average
Temperature	88.7 °F	71.2 °F	77.2 °F
Dew Point	77.6 °F	69.7 °F	74.4 °F
Humidity	98%	67%	92%
Precipitation	0.54 in	--	--

	High	Low	Average
Wind Speed	14 mph	--	2 mph
Wind Gust	14 mph	--	--
Wind Direction	--	--	ENE
Pressure	29.92 in	29.84 in	--

March 26, 2015

	High	Low	Average
Temperature	84 °F	71.5 °F	76.6 °F
Dew Point	74.7 °F	67.1 °F	70.8 °F
Humidity	94%	68%	83%
Precipitation	0.07 in	--	--

	High	Low	Average
Wind Speed	22 mph	--	5 mph
Wind Gust	22 mph	--	--
Wind Direction	--	--	South
Pressure	30.04 in	29.84 in	--

Figure 17. Meteorological conditions on the dates

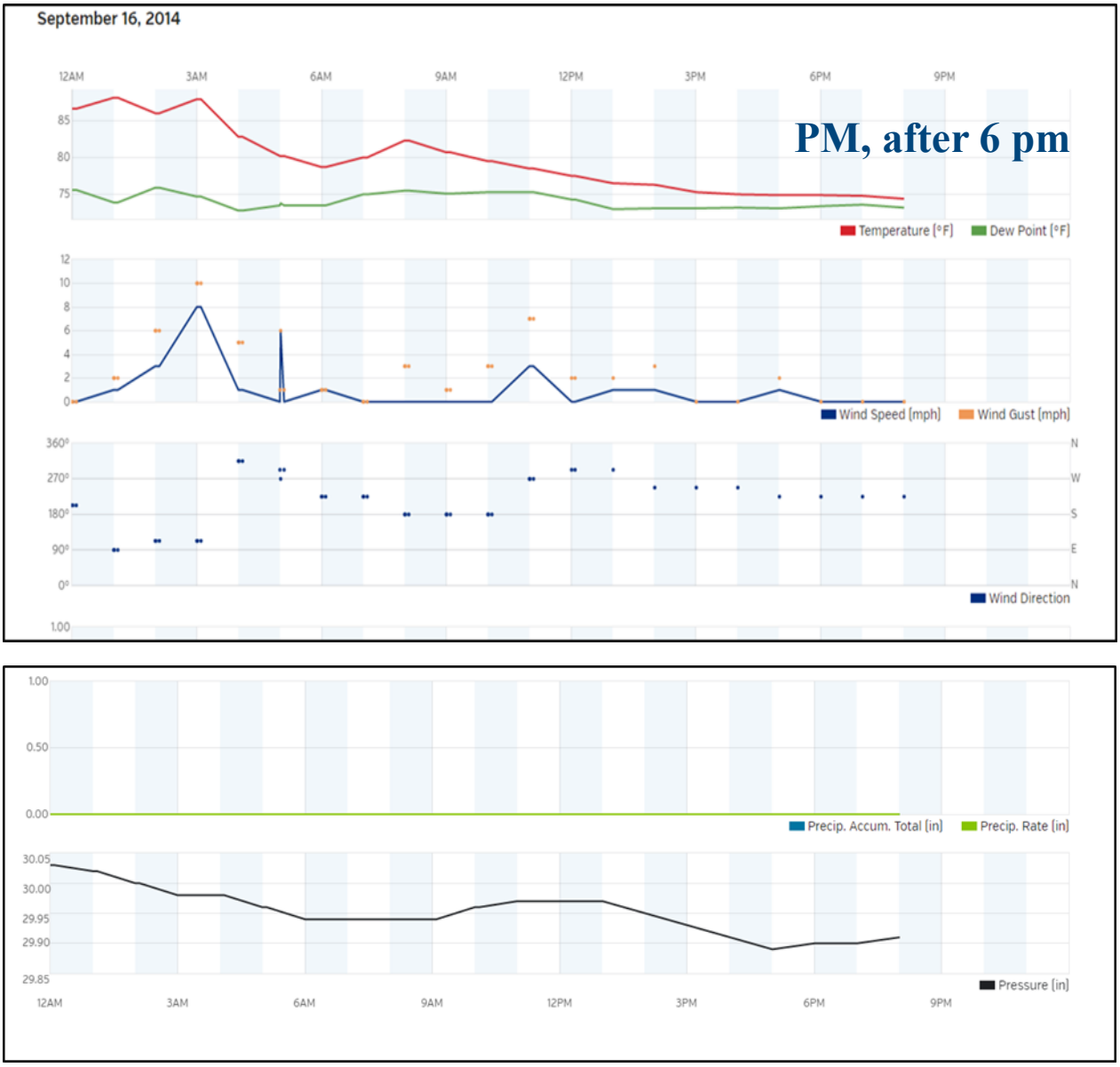

Figure 18. Graphical representation of meteorological conditions on the 09/16/14 (Odor complaints occurred in the afternoon- after 6 pm)

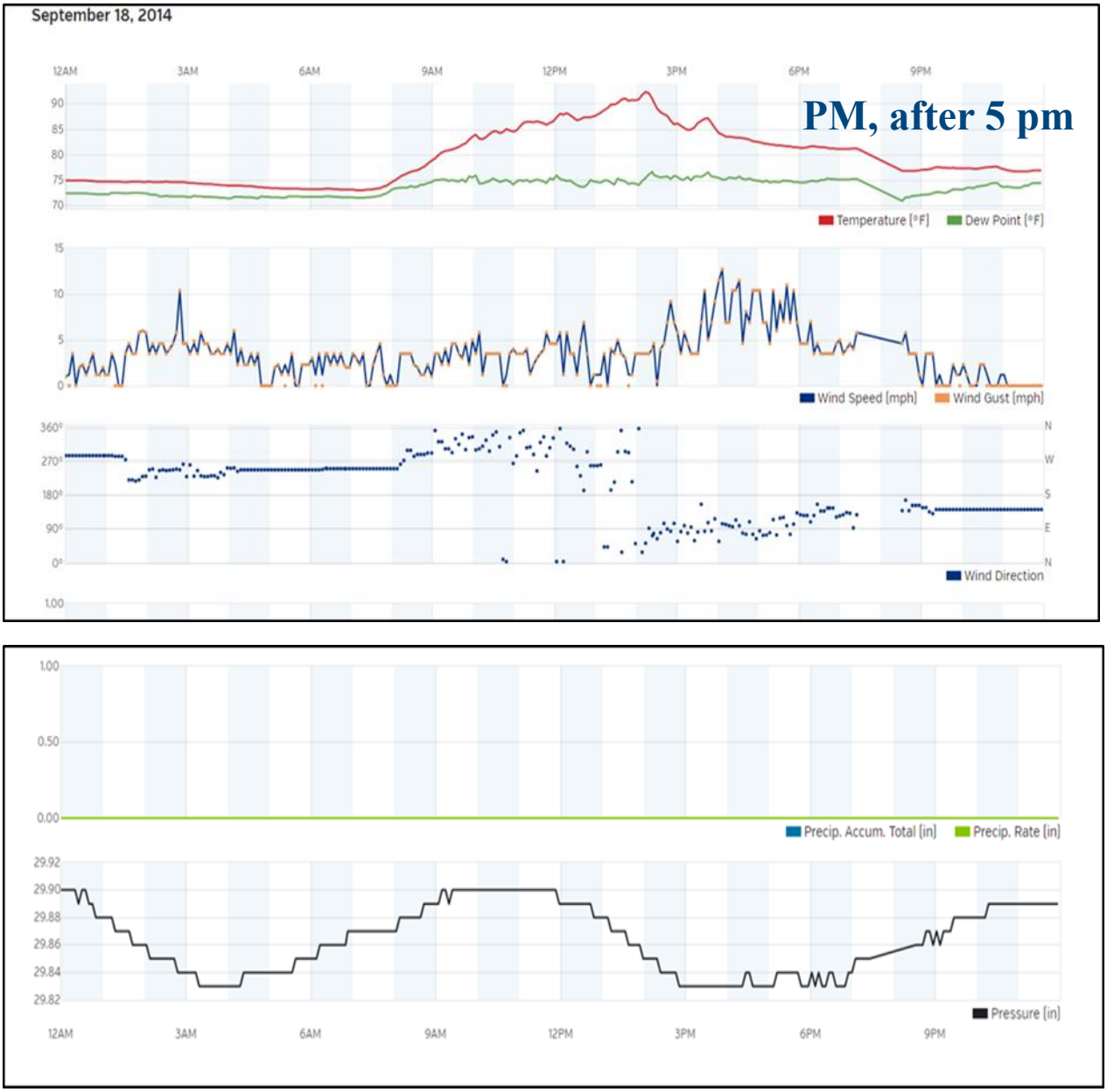

Figure 19. Graphical representation of meteorological conditions on the 09/18/14 (Odor complaints occurred in the afternoon- after 5 pm)

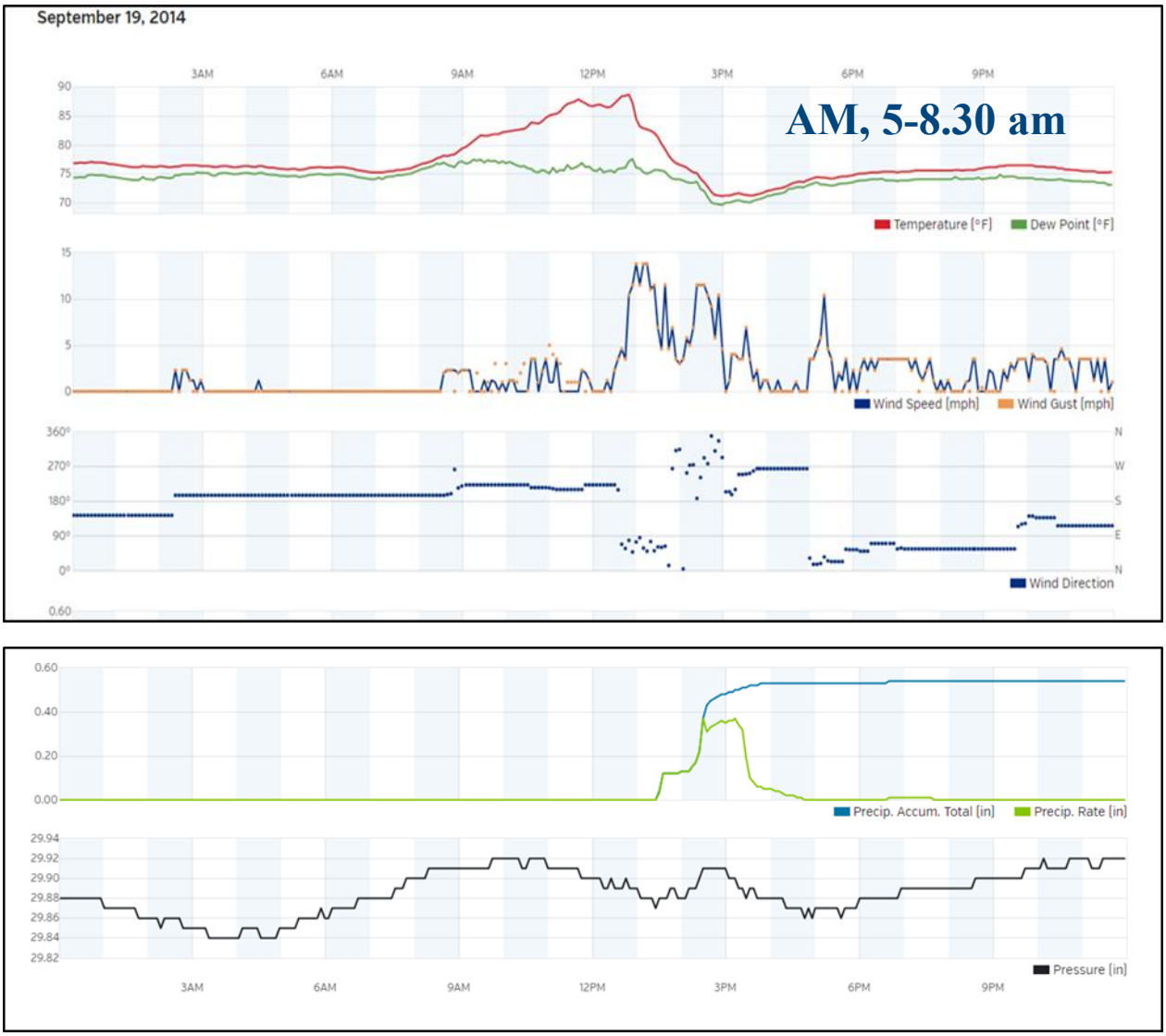

Figure 20. Graphical representation of meteorological conditions on the 09/19/14 (Odor complaints occurred in the morning- 5-8.30 am)

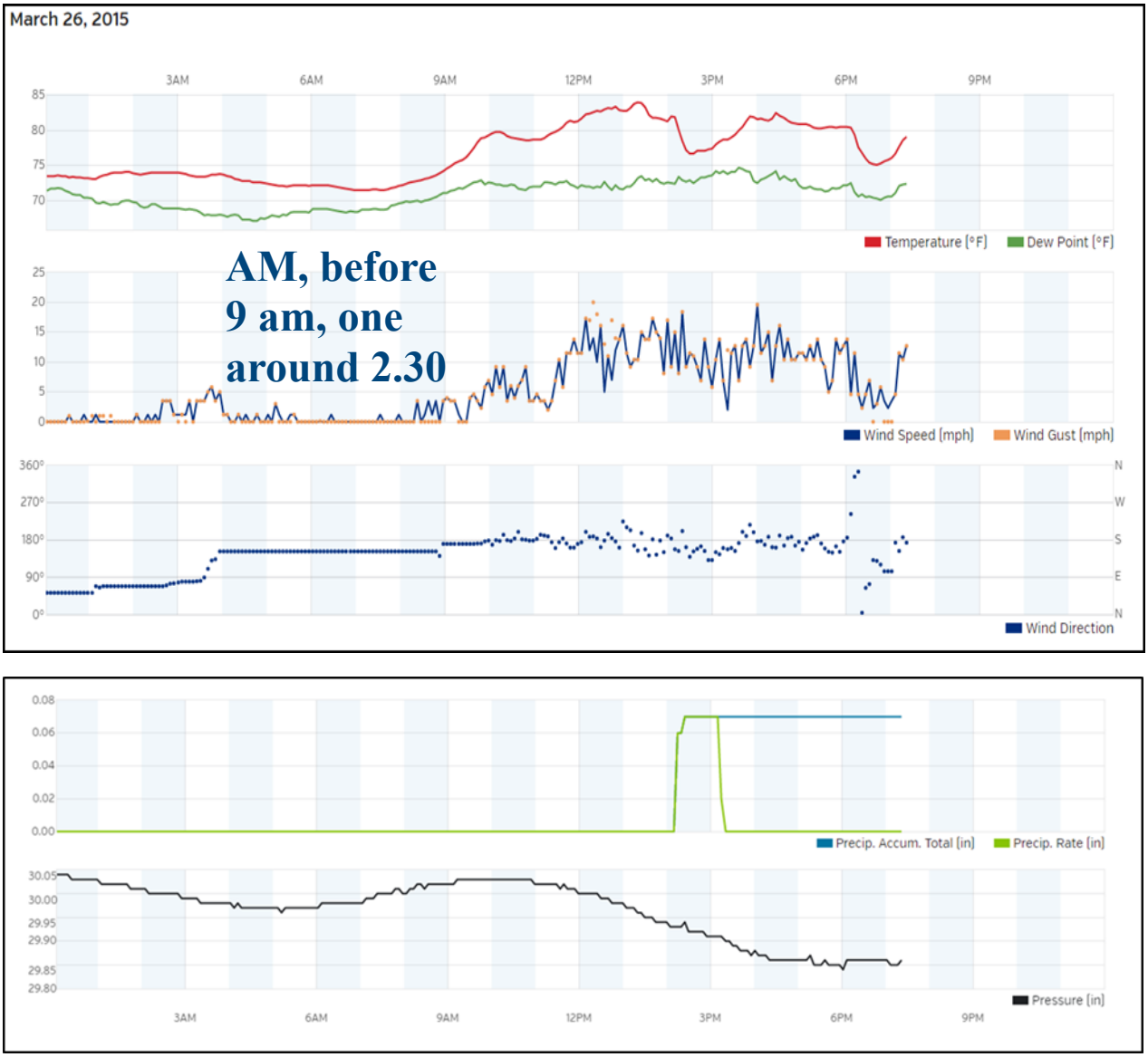

Figure 21. Graphical representation of meteorological conditions on the 03/26/15 (Most of the odor complaints occurred in the morning- before 9 am; one occurred around 2.30 pm)

- **TASK 4. Perform protein sensitivity experiments**. The reactor was built using two one-way check valves, flexible air line tubing and a 250mL HDPE sample bottle. It is planned to bubble hydrogen sulfide, mercaptans, and/or dimethyl sulfide gas through odorant binding protein (OBPIIa) suspended in liquid phase. Within the vessel, the protein will be exposed to a steady stream of pressurized gas from a tank with flow rate carefully controlled with a regulator. After the gas exposure period, an aliquot of liquid sample will be transferred by pipette to a cuvette for analysis by fluorometry to quantify binding and establish a relationship between concentration and fluorescence.

An ongoing literature review has revealed that OBPIIa is a small, soluble polypeptide found in the sensory organs of vertebrates and insects.[2] It is stable to temperature, organic solvents, and proteolytic digestion.[3] It is thermostable up to 60°C, so it is not denatured easily. It has a broad specificity and binding affinity that allows it to bind a wide variety of odorants, and it has an affinity for small, volatile organic compounds, detectable in the micromolar range. Odorant binding protein is viable as a biosensor of nuisance odors because it can be expressed in bacterial systems at low cost and easily purified. Different OBP mutants with different binding selectivity can be designed for different needs and could be combined to broaden the binding affinity of biosensors.[4]

Plasmid DNA containing the coding sequence for OBPIIa was obtained from Silva et al.[5] in Portugal. The researchers sent the plasmid absorbed on filter paper. The plasmid they sent had been cloned into the pET-28 expression vector with a Kanamycin drug selection marker and a Hexa-His affinity tag. The Hexa-His affinity tag indicates that a polyhistidine tag is used for affinity purification of a polyhistidine-tagged recombinant protein expressed in *E. coli*. Once expressed, OBPIIa will be separated from other proteins using an affinity-resin containing nickel ions.

To begin the process of protein synthesis, a culture of *E. coli* XL1 Blue II was grown in overnight culture to serve as an expression vector for the DNA plasmid. After receiving the DNA plasmid, the DNA was eluted from filter paper. The DNA was concentrated by ethanol precipitation, centrifugation, and removal of ethanol. Nanopure water was added to the concentrated DNA so that there would be no ions to interfere with electroporation. The DNA was added to the concentrated *E. coli* culture and electroporated. Transformants were inoculated onto Kanamycin Luria Broth Agar plates and incubated at 37°C. No transformants grew on Kanamycin LB Agar plates. It is possible that there was too little DNA on the filter paper to begin with, although that seems unlikely since electroporation is sensitive to as little as 15 µL of DNA. It may be more likely that the DNA was degraded by the time it was received by mail from Portugal. Another possibility is that the electroporation step did not work to create transformants. Alternatively, the *E.coli* culture may have been too unhealthy to survive the transformation process.

Silva et al.[5] have agreed to provide an additional plasmid DNA sample either already cloned into *E. coli* or in ethanol rather than absorbed onto filter paper. The new sample is expected by September 9, 2016. The next phase of experiments will be to try cloning and transformation again using electroporation. The adequate functioning of electroporator will be checked using stock DNA. Another possible avenue of transformation is to use $CaCl_2$ treatment instead of electroporation since ions would not be an issue. $CaCl_2$ treatment requires more initial DNA than electroporation.

Upon further exploration of possible methods by which to use OBPIIa to detect odor concentrations, a different plausible technique has emerged. A technique similar to an ELISA assay or Western blotting may be used to detect binding of a set of monoclonal antibodies that recognize the binding of different odorants. OBPIIa antibodies would

---

[2] Schiefner, A. F. (2014). *Crystal structure of the human odor binding protein, OBPIIa.* Munich, GE: Wiley Periodicals.
[3] Tegoni, M. P. (2000). *Mammalian odorant binding proteins.* Pisa, Italy: Biochimica et Biophysica Acta
[4] Pelosi, P. M. (2013). *Structure and biological applications of odorant-binding proteins.* Berlin: Appl Microbiol Biotechnol.
[5] Silva, C. M.-P. (2013). *Odorant Binding Proteins: a biotechnological tool for odour control.* Berlin: Appl Microbiol Biotechnol

be labeled with fluorescent tags, and if binding of the antibody is affected by the binding of odorants such that when bound to an odorant, the antibody loses binding affinity, fluorescence could be correlated to odorant concentration. Further literature review is underway to determine the biophysics of OBPIIa such as the epitopes of different binding sites, whether or not the protein can be crystallized, and how much of protein will be expected to remain soluble.

Upcoming Research Tasks:

- **TASK 1. Conduct literature review**. Continue to update the literature review.
- **TASK 2. Collect data on Florida-specific odor management strategies.** Continue to update the odor complaint database. Participate in odor data collection surveys with landfill personnel with permission. Review the ENVIROS system (Environmental Inquiry and Resource System) of Broward County to collect additional odor complaint data. Work with Mrs. Damaris Lugo from Broward County to get access to more odor complaint data. Meet with representatives from Waste Management Inc. of Florida again to collect their most current odor complaint data and arrange the installation of weather station.
- **TASK 3. Pattern identification and trend analysis.** Analyze data from Waste Management Inc. of Florida and other sources. Exclude meteorological parameters that do not affect number of odor complaints. Work on analysis of overnight pressure drop as an indicator of odor complaints.
- **TASK 4. Perform protein sensitivity experiments**. Procedures to clone, synthesize and express the protein are ongoing. Finalize the DNA amplification procedure and test monoclonal antibody option. Calibrate and test spectrometer to ensure proper functioning. Conduct pure gas tests.
- **TASK 5. Assess odor mitigation strategies**. Waste Management and SWA personnel have provided information about mitigation strategies and the next step is to conduct an alternative analysis.
- **TASK 6. Develop recommendations and preliminary cost analysis.**
- **TASK 7. Prepare publication materials**.

Project Metrics

1. List graduate student or postdoctoral researchers **funded** by this Hinkley Center project

Last name, first name	Rank	Department	Professor	Institution
Julia Roblyer	MSCE candidate	CEGE	Meeroff	FAU
Mateja Vidovic	MSCE Candidate	CEGE	Meeroff	FAU

2. List undergraduate student/researchers working on this Hinkley Center project

Last name, first name	Department	Professor	Institution

3. List research publications resulting from this Hinkley Center project (use format for publications as indicated in the Hinkley Center Investigators Guide).
   None yet

4. List research presentations resulting from this Hinkley Center project (use format for listing presentations as indicated in the Hinkley Center Investigators Guide).
   None yet

5. List research papers that have cited any publications (or the final report) resulting from this Hinkley Center project (use format for publications as indicated in the Hinkley Center Investigators Guide).
   None yet

6. List additional research funding that has been secured due to leveraging the research results from this Hinkley Center project (give project title, funding agency, amount of funding, award date, and award period)
   None yet

7. List submitted proposals which leverage the research results from this Hinkley Center project (give the proposal title, funding agency, requested funding, date submitted)
   "Detection of nuisance odors using fluorescently labeled odor binding proteins", EREF, $169,569, August 2016

8. List new collaborations initiated based on this Hinkley Center project

Dr. Jason Hallstrom (FAU I-SENSE Center), Dr. Binninger (FAU College of Science), Craig Ash and Jim Christiansen (Waste Management), Dick Pope (Hazen and Sawyer), Robert Bowker (Bowker and Asoociates), Philip Wolstenholme (Brown and Caldwell), Chris Hunniford (V&A Consulting Engineers), and Bruce Singleton (CDM Smith), Dr. Loic Briand, Research Director of the Center for Taste and Feeding Behaviour in Dijon, France, Artur Ribeiro, Professor of Biological Engineering at the University of Minho in Braga, Portugal, and Dr. Chelsea Smartt, Associate Professor of UF's Florida Medical Entomology Laboratory.

9. How have the results from this Hinkley Center funded project been used (***not will be used***) by the FDEP or other stakeholders in the solid waste field? Please note that the term "other stakeholders" is meant to broadly include any party or practitioner in the solid waste field. This includes county solid waste directors and their staff, municipal solid waste directors and their staff, solid waste facility design engineers, local/county/city solid waste management regulatory staff, federal solid waste regulatory staff, landfill owners and operators, waste haulers, waste to energy plant owners and operators, recyclers, composting plant owners and operators, yard waste operators, construction and demolition debris companies and organizations, county recycling coordinators, citizens and members of the academic community, etc. (1 paragraph maximum)

None yet to our knowledge; however, a progress report presentation was made to Waste Management personnel to show the results of preliminary analysis of odor complaints relationship with meteorological data. We plan to continue to work with our partners to share our results and refine how odor complaints are dealt with by the industry.

TAG Members:
Mark Eyeington, Mark Maclean, Mark Bruner, Owrang Kashef, D.V. Reddy, Craig Ash, Ravi Kadambala, Ron Schultz, Jeff Roccapriore, André McBarnette, Dan Schauer, Damaris Lugo, Amanda Krupa, Richard Meyers, Amede Dimonnay, Art Torvela, Ted Batkin, Roshan Jachuk, Fred Bloetscher