State University System of Florida
Hinkley Center for Solid and Hazardous Waste Management

PROGRESS REPORT 4

January 16, 2024

TITLE: PFAS in biosolids: Partitioning during wastewater treatment and leaching from Florida biosolids

COMPLETION DATE: February 29, 2024 (new completion date)

Project was extended.

PRINCIPAL INVESTIGATORS:

- Berrin Tansel, Ph.D., P.E., Professor, Civil and Environmental Engineering Department, Florida International University
- Yelena Katsenovich, Senior Research Scientist, Applied Research Center (ARC), Florida International University
- Natalia Soares Quinete, Assistant Professor, Environmental and Bioanalytical Chemistry, Florida International University

During the first quarter of this project, the following activities have been performed:

During this period, the following activities have been performed:

1. Test methods
   Continued leaching experiments, PFAS analyses (expanded for 40 PFAS compounds), and biosolids characterization for (TS, organic content, and protein) have been finalized.

2. Biosolids characterization tests (Dr. Katsenovich)
   - Biosolids were analyzed for total solids, volatile solids, fixed solids fractions.

3. Biosolids PFAS leaching experiments (Dr. Katsenovich)
   One undergraduate student, Zariah Nasir, continues working with Dr. Katsenovich. Continued:

   - Conducting leaching experiments to evaluate the release of PFAS from biosolids under site-specific conditions.
   - Protein analyses according to procedures by preparing 5% surfactant Triton X-100 and drying samples for subsequent grinding and weighting 0.5 g in each triplicate. The protein analysis will be conducted via Pierce™ BCA Protein Assay Kit obtained from Fisher Scientific.

Initiating the PFAS leaching experiments began with determining the moisture content. Samples from both treatment plants were carefully placed in aluminum dishes and subjected to drying in an oven at 100^{o} C until stable weight was obtained. Subsequently, the dried samples were transferred to a desiccator for cooling before recording weight. In a sequential process, each of the dried samples underwent incineration in a furnace at 550°C for a duration of 2 hours. Following incineration, the samples were carefully transferred to a desiccator for the purpose of cooling (Figure 1A). The resulting solid concentrations obtained from this process formed the basis for the subsequent leaching phase.
Throughout these procedures, samples were collected from the digester, thickener, and centrifuge stages in duplicates, while dried biosolid samples were gathered in triplicates. To ensure precise handling, 50 mL polypropylene tubes were employed, and 5 g of each sample were combined with 50 mL of distilled water. This careful preparation facilitated the leaching process, allowing for a detailed study of the leaching of PFAS into the aqueous phase. Controlled agitation was achieved using an end-over-end tube revolver, maintained at a constant speed of 10 rpm. Samples were collected at specific time intervals (1 day, 3 days, 7 days, 14 days, and 30 days) for subsequent analysis.
Following each designated sampling point, the samples underwent centrifugation at 4,500 RPM for 30 minutes. The resulting supernatant was then carefully extracted and transferred into separate clean 50 mL polypropylene tubes (Figure 1B). Meanwhile, the solid residue was preserved by storing it in a freezer.
In addition, selective 1 mL subsamples were collected and stored under controlled refrigeration for further analysis utilizing an ICP-OES machine. Disposable tubes were employed, containing 200 uL of the sample and 3.8 mL of 1% Nitric Acid. A dilution factor was calculated for each sample. The tubes were subsequently subjected to analysis using the ICP-OES instrument (Perkin Elmer Optima 7300 DV), providing data for elements such as phosphorus, calcium, iron, aluminum, and magnesium. Throughout the analysis, careful monitoring of analytical recoveries was conducted to ensure that they remained within acceptable ranges.

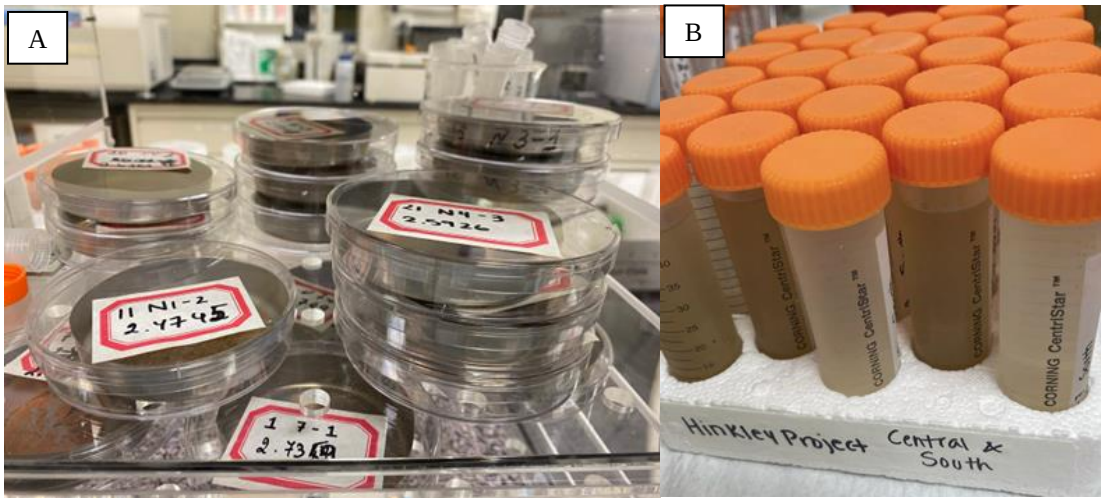

Figure 1. A) Dried samples in triplicates after incineration in a furnace at 550°C for 2 hours transferred to a desiccator for cooling and analyzed for total solids, volatile solids, fixed solids fractions; B) Extracted supernatant transferred into separate clean 50 mL polypropylene tubes for PFAS analysis.

4. Biosolids and leachate PFAS analyses (Dr. Quinete)

   One undergraduate student, Maria Mendoza Manzano and one graduate student, Joshua Ocheje, in the Ph.D. program in the Chemistry and Biochemistry department are working with Dr. Quinete.

   Continued:

   - Method development for assessment of 40 PFAS on biosolids samples
   - Extraction and LC-MS/MS analysis of PFAS in biosolids used for the leachate experiments.
   - Analyzing biosolids leachate samples for PFAS content and component profile; determine the prevalent PFAS compounds.

   Sample preparation/extraction steps: To avoid cross-contamination, all containers, bottles and tubing used during extraction and sample preparation were rinsed twice with solvents of different polarities: methylene chloride, hexane, acetone, methanol. All the solvents used in this analysis are HPLC grade and previously evaluated for potential PFAS contamination. Leachate samples were processed through solid phase extraction (SPE) using Strata-XL AW (500 mg/3 mL) cartridges on a semi-automated SPE equipment for extraction and preconcentration of PFAS (Figure 2) . In short, cartridges were successively pre-conditioned with 12 mL of 0.3% ammonia (NH_4) in methanol, 12 mL of methanol and equilibrated with 5 mL of water, before loading of 45 mL biosolid leachate sample spiked with 50 µL of the labeled extraction standard (MPFAC-HIF-ES) mixture (2.5 ng mL^{-1}). Samples were loaded (Figure 2A) into the cartridges under vacuum and after all has passed, cartridges are left to dry for about 1-2 hours. In the second stage (Figure 2B), cartridges are eluted with 10 mL of 0.3% NH_4 in methanol, which is further evaporated to dryness under a gentle nitrogen flow in a heated water bath at 40 °C (Figure 3), then reconstituted to a 450 µL volume with 95:5% (vol/vol) 2mM ammonium formate/methanol. Reconstituted samples are transferred into LC polypropylene vials and 50 µL of 2.5 ng mL^{-1} labeled internal standard mixture (MPFAC-HIF-IS) are added before injection. Samples are kept refrigerated until LC-MS/MS analysis.
   For quality control purposes, blanks, spiked blanks, and duplicate analysis were processed for each experiment through the same procedure as the samples. Blank samples consisted of 45 mL LC-MS grade water spiked with the labeled extraction standard (MPFAC-HIF-ES) mixture (2.5 ng mL^{-1}), while spiked blanks were prepared with 45 mL LC-MS grade water spiked with 250 µL of 2.5 ng mL^{-1} of native standard mixture (containing 40 PFAS from PFAC-MXF, PFAC-MXG,

PFAC-MXH, PFAC-MXI and PFAC-MXJ) and 50 µL the labeled extraction standard (MPFAC-HIF-ES) mixture (2.5 ng mL^{-1}). List of PFAS being analyzed is shown in Table 1.

Sample analysis: After SPE, 100 µL of samples were injected and analyzed by an Agilent 1290 Infinity II LC interfaced to an Agilent 6470 triple quadrupole LC-MS/MS system equipped with Agilent Jet Stream electrospray ionization (ESI) source in negative mode. The LC system was modified with PFAS free tubing and a delay column (Hypersil GOLD aQ C18, 20 × 2.1 mm, 12 µm) was placed between the mobile phase mixer and the sample injector. A Hypersil GOLD pentafluorophenyl (PFP) column (150 mm × 2.1 mm, 3 µm) with a PFP guard column (Hypersil Gold PFP 5 µm drop-in guards) was used as analytical column for PFAS separation and maintained at a temperature of 50 °C using 95:5 2mM ammonium acetate:methanol and methanol as mobile phases in a flow rate of 0.4 mL/min. Sample acquisition was performed using a multiple-reaction monitoring (MRM) method in negative mode for the simultaneous quantification of multiple PFAS, which included when available two transitions per compound for quantitative and identification (qualitative) purposes.

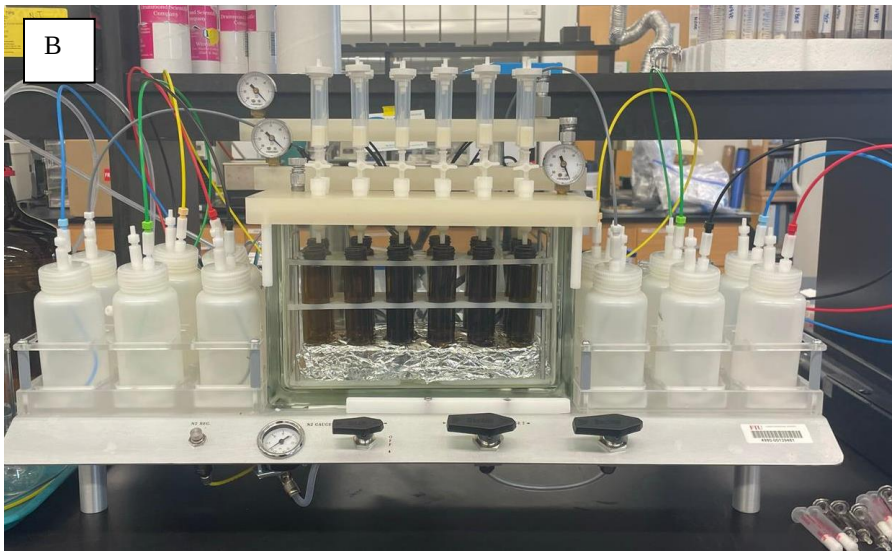

Figure 2: Semi-automated SPE equipment showing (A) stage 1- leachate samples being loaded in the cartridges and (B) stage 2- samples being eluted into 60 mL glass amber vials.

Figure 3: Nitrogen evaporation of the leachate samples after elution

Table 1. List of analyzed PFAS.

Abbreviation	Compound Name	Molecular Formula	Molecular Weight
Perfluoroalkyl carboxylic acids			
PFBA	Perfluorobutanoic acid	$C_4HF_7O_2$	214.04
PFPeA	Perfluoropentanoic acid	$C_5HF_9O_2$	264.05
PFHxA	Perfluorohexanoic acid	$C_6HF_{11}O_2$	314.05
PFHpA	Perfluoroheptanoic acid	$C_7HF_{13}O_2$	364.06
PFOA	Perfluorooctanoic acid	$C_8HF_{15}O_2$	414.07
PFNA	Perfluorononanoic acid	$C_9HF_{17}O_2$	464.08
PFDA	Perfluorodecanoic acid	$C_{10}HF_{19}O_2$	514.08
PFUdA	Perfluoroundecanoic acid	$C_{11}HF_{21}O_2$	564.09
PFDoA	Perfluorododecanoic acid	$C_{12}HF_{23}O_2$	614.10
PFTrDA	Perfluorotridecanoic acid	$C_{13}HF_{25}O_2$	664.11
PFTeDA	Perfluorotetradecanoic acid	$C_{14}HF_{27}O_2$	714.11
Perfluoroalkyl sulfonic acids			
PFBS	Perfluorobutanesulfonate	$C_4HF_9O_3S$	300.10
PFPeS	Perfluoropentanesulfonate	$C_5HF_{11}O_3S$	350.11
PFHxS	Perfluorohexanesulfonate	$C_6HF_{13}O_3S$	400.11
PFHpS	Perfluoroheptanesulfonate	$C_7HF_{15}O_3S$	450.12
PFOS	Perfluorooctanesulfonate	$C_8HF_{17}O_3S$	500.13
PFNS	Perfluorononanesulfonate	$C_9HF_{19}O_3S$	550.14
PFDS	Perfluorodecanesulfonate	$C_{10}HF_{21}O_3S$	600.14
PFDoS	Perfluorododecanesulfonate	$C_{12}HF_5O_3S$	700.16
Fluorotelomer sulfonic acids			
4-2 FTS	1H,1H,2H,2H-perfluorohexanesulfonate	$C_6H_5F_9O_3S$	328.15
6-2FTS	1H,1H,2H,2H-perfluorooctanesulfonate	$C_8H_5F_{13}O_3S$	428.17
8-2 FTS	1H,1H,2H,2H-perfluorodecanesulfonate	$C_{10}H_5F_{17}O_3S$	528.18
Perfluorooctane sulfonamides			
FOSA	Perfluorooctanesulfonamide	$C_8H_2F_{17}NO_2S$	499.15
NMeFOSA	N-methyl perfluorooctanesulfonamide	$C_9H_4F_{17}NO_2S$	513.17
NEtFOSA	N-ethyl perfluorooctanesulfonamide	$C_{12}H_{10}F_{17}NO_3S$	571.25
Perfluorooctane sulfonamidoacetic acids			

N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	$C_{11}H_6F_{17}NO_4S$	571.21
N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	$C_{12}H_8F_{17}NO_4S$	585.23
Perfluorooctane sulfonamide ethanols			
NMeFOSE	N-methyl perfluorooctanesulfonamidoethanol	$C_{11}H_4F_{21}NO_3S$	629.19
NEtFOSE	N-ethyl perfluorooctanesulfonamidoethanol	$C_{12}H_6F_{21}NO_3S$	643.21
Per- and Polyfluoroether carboxylic acids			
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	$C_6HF_{11}O_3$	330.05
ADONA	4,8-Dioxa-3H-perfluorononanoic acid	$C_{10}H_{11}N_4NaO_5S$	322.27
PFMPA	Perfluoro-3-methoxypropanoic acid	$C_4HF_7O_3$	230.04
PFMBA	Perfluoro-4-methoxybutanoic acid	$C_5HF_9O_3$	280.04
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	$C_5HF_9O_4$	296.04
Ether sulfonic acids			
9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	$C_8HClF_{16}O_4S$	532.58
11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	$C_{10}HClF_{20}O_4S$	632.60
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	$C_4HF_9O_4S$	316.10
Fluorotelomer carboxylic acids			
FPrPA or 3:3FTCA	3-Perfluoropropyl propanoic acid	$C_6H_5F_7O_2$	242.09
FPePA or 5:3FTCA	3-Perfluoropentyl propanoic acid	$C_8H_5F_{11}O_2$	342.11
FHpPA or 7:3FTCA	3-Perfluoroheptyl propanoic acid	$C_{10}H_3F_{17}O_2$	478.10

Results:

Preliminary results reported in Progress Report 3 were reviewed.

- Method detection limits calculated based on the lowest level of the calibration curve (that produced a S/N> 3 and accuracy between 70-130%) and considering a 90 times concentration factor ranged from 0.02 to 22 ng/L.
- Recoveries ranged for most of the compounds from 40 to 160%
- We have identified predominant PFAS in biosolid leachate in South Florida: PFOS, FPePA, PFBA, PFHxA and 6-2 FTS.
- Distinct PFAS composition between South District and Central District plants (Figure 4), but overall predominance of long-chain PFAS over emerging short-chain PFAS (Figure 5)

- Leaching experiments have led to the highest PFAS concentrations after 1 day (Figure 6)
- PFOS were detected in 82% of the samples analyzed and PFOA in 95% (N=80) with concentrations up to 109 ng/L and 25 ng/L, respectively.

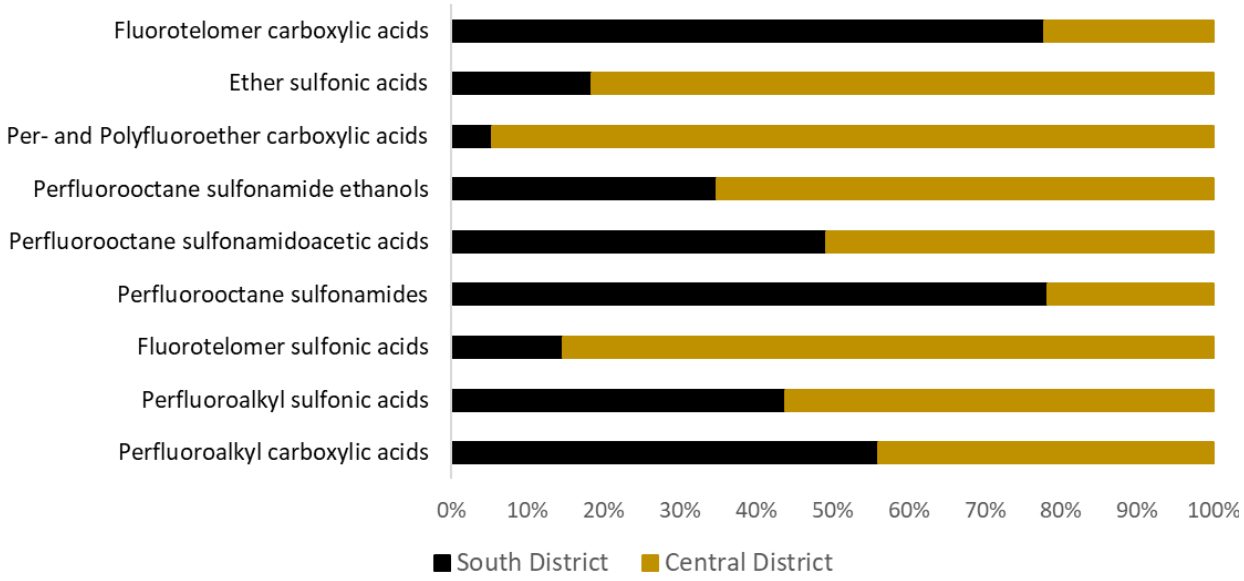

Figure 4. PFAS Average Composition in Biosolid Leachate by class

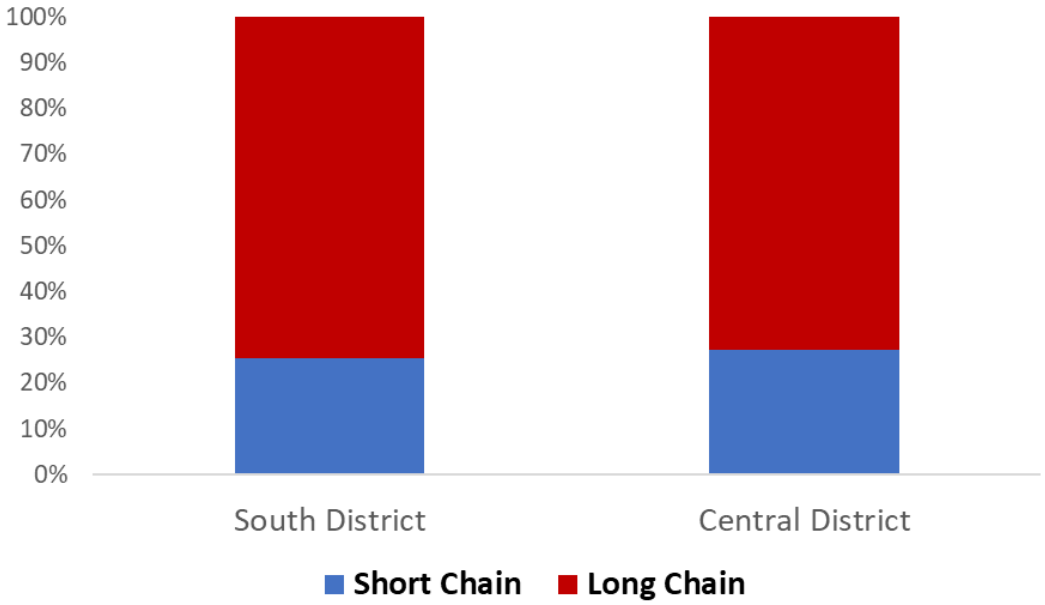

Figure 5: PFAS composition in biosolid leachate in terms of chain length

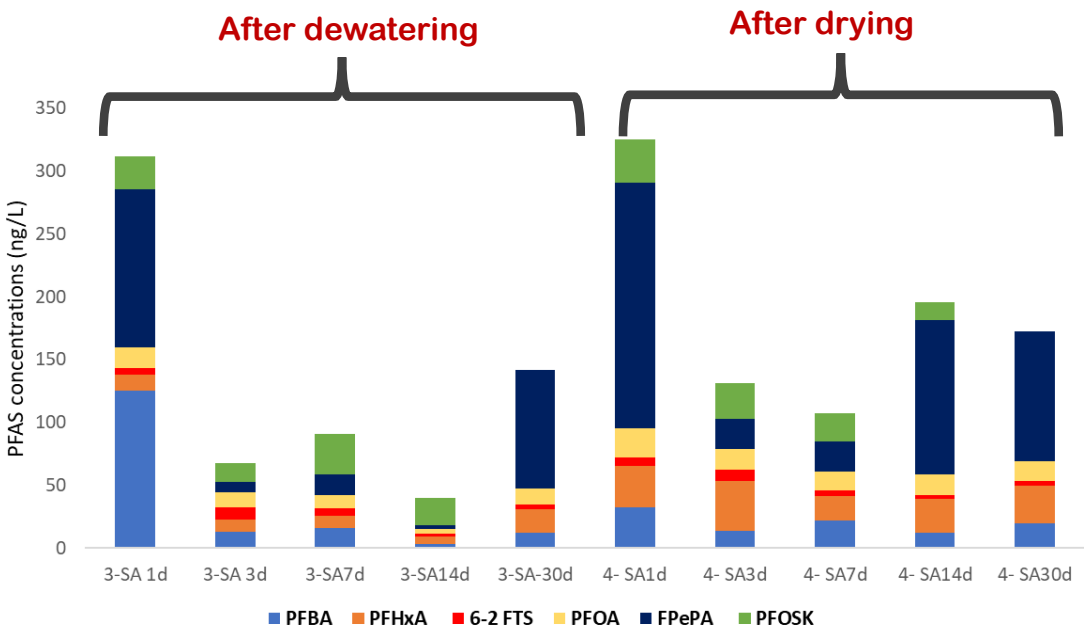

Figure 6: Predominant PFAS leaching from biosolids after dewatering and drying processes from South District plant (1 day to up to 1 month)

In the analytical part, what we have accomplished so far:

- Optimization of LC-MS conditions and MS parameters (MRM transitions) for the analysis of 40 PFAS
- Extraction and LC-MS/MS analysis of a total of 80 biosolids leachate samples from the leaching experiments, plus blanks and spiked blank samples.
- Dilution and re-run of samples with exceeding the calibration curve concentration.
- All leachate data is completed.
- Method development and validation for assessment of 40 PFAS on biosolids (solid) samples
- Extraction and LC-MS/MS analysis of PFAS in biosolids used for the leachate experiments.

Next steps:

- Currently processing biosolid data using the MassHunter QQQ Quantitation analysis software for quantitation of PFAS in the solid part (mass balance).

5. Regular weekly progress meetings are taking place with the co-PIs and students.

6. The project web page has been updated at the following address:
   biosolids.fiu.edu

7. Literature review on PFAS is on-going.

8. Manuscript preparation

Two manuscripts are being prepared for journal submission. These manuscripts focus on:

i. Characterization of PFAS in biosolids
ii. Leaching potential of PFAS from biosolids

9. Conference participation

Two presentations have been made to the following conferences:

(1) Development of a sensitive method for determination of per and polyfluoroalkyl substances (PFAS) in biosolids leachates

Authors: Joshua Ocheje, Yelena Katsenovich, Berrin Tansel, Natalia Quinete

Conference: SETAC North America 44th Annual Meeting, 12 – 16 November 2023, Louisville, Kentucky, USA.

(2) Characterization of per- and polyfluoroalkyl substances (PFAS) in biosolids

Authors: Berrin Tansel, Yelena Katsenovich, Natalia Quinete, Joshua Ocheje, Zariah Nasir
Symposium: 2023 FWEA Biosolids Symposium, September 14, 2023 St. Petersburg College, Clearwater Florida

Planned activities for the remainder of the project timeline:

- Continue PFAS analyses in biosolids samples and samples of leachate from biosolids.
- Continue investigating PFAS related data and information as well as biosolids land application practices and potential runoff and partitioning data for biosolids related organic matter.
- Develop manuscripts for submittal to journals:
  - Time dependent solubilization and the release characteristics of the PFAS homologues from biosolids.
  - Scientific understanding of PFAS originating from biosolids as a source in the environment, potential exposure pathways for human health and ecological effects.
  - Recommendations for appropriate testing and land application practices of biosolids in Florida.

Months	Planned Activities	Status
December	• Weekly project update meetings • Finalize partitioning and leaching analyses of different types of PFAS • PFAS analysis for solid fractions of biosolids	• Continuing • Continuing • Continuing
January	• In-depth analyses of data from test results • Weekly project update meetings • Manuscript preparation • Preparation of final report • Draft final report	• Continuing • Continuing • Continuing • Continuing • Continuing
February	• Weekly project update meeting • TAG Meeting (Feb 1, 2024) • Manuscript preparation • Finalize project report	• Continuing • Scheduled • Continuing • Planned