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Method Development for the Extraction and Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Wastewater and Biosolids

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METHOD DEVELOPMENT FOR THE EXTRACTION AND ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN WASTEWATER AND BIOSOLIDS

by

Victoria Krull

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ABSTRACT

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by

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Utah State University, 2024

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Through the everyday use of household products per- and polyfluoroalkyl substances (PFAS) enter wastewater treatment plants and are present in wastewater effluent and biosolids. These substances present numerous health risks to humans and animals, including immune system suppression, and developmental effects.

In this study methods for PFAS extraction and analysis are compared to determine the most reliable and accurate method for wastewater and biosolids sample processing. PFAS quantitation is achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS). Two LC methods were compared based on EPA Methods 533 and 1633. Method 533 produced reliable results with the most consistency.

Biosolids extraction method was optimized by comparing a sonication method and an automated method performed with an Energized Dispersive Guided Extraction (EDGE) system. The sonication method showed higher percent recoveries of spiked extraction standards compared with the EDGE system.

Both wastewater and biosolids samples require clean-up and concentration using solid phase extraction (SPE). Since 2018 the United States Environmental Protection Agency

(EPA) has published Methods 537.1, 533, and 1633 for the determination of PFAS in water, and other environmental media. SPE methods for biosolids and wastewater were developed at the Utah Water Research Lab and compared to EPA Method 1633. Method 1633 showed higher percent recovery of the extraction standards for biosolids extraction. For wastewater, the methods were statistically the same but EPA Method 1633 is the recommended method due to its development for wastewater samples.

From these results it is recommended that for extraction of biosolids the sonication method based on EPA Method 1633 be used. For SPE cleanup it is recommended that EPA Method 1633 be followed for both wastewater and biosolids. For analysis EPA Method 533 is recommended for both wastewater and biosolids samples. These methods have shown the best recoveries of extraction standards and the most consistency in chromatography.

(127 pages)

PUBLIC ABSTRACT

Method Development for the Extraction and Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Wastewater and Biosolids

Victoria Krull

Per and poly-fluorinated alkyl substances (PFAS) are manufactured chemicals that have water and grease repellant properties and do not readily biodegrade. PFAS are present in many consumer products and end up in waste systems and the environment. Studies have shown that PFAS can have adverse health effects on humans and animals.

There are many challenges to processing and analyzing samples containing PFAS including: adsorption of PFAS to equipment, contamination from equipment containing PFAS, and the high sensitivity needed to analyze for PFAS in the parts per trillion detection range. The United States Environmental Protection Agency (EPA) has been developing methods to analyze for PFAS in environmental samples, starting with drinking water and moving towards more complex environmental matrixes. Testing of these methods with available laboratory equipment must occur to optimize a lab's capacity to quantify PFAS.

To quantify them, PFAS must first be extracted from a sample and then analyzed using liquid chromatography paired with mass spectrometry. This study compares extraction and analytical methods for the quantification of PFAS in biosolids and wastewater. Analytical methods based on EPA Methods 533 and 1633 were compared and the results from Method 533 were more precise and accurate than Method 1633. An automated and manual extraction method were compared for the extraction of PFAS from

biosolids and the manual method was determined to produce better recovery of PFAS. Finally, two methods of sample clean up and concentration for wastewater and biosolids samples were compared between methods developed at the Utah Water Research Lab and EPA Method 1633. Method 1633 was found to have higher percent recoveries of PFAS for biosolids and wastewater.

From these results it is recommended that for extraction of biosolids EPA Method 1633 be used. For SPE cleanup it is recommended that EPA Method 1633 be followed for both wastewater and biosolids. For analysis EPA Method 533 is recommended for both wastewater and biosolids samples. These methods have shown the best recoveries of extraction standards and the most consistency in results.

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Lastly, I'd like to thank my family and friends for supporting me through my graduate studies and research.

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1 Introduction

Per and poly-fluorinated alkyl substances (PFAS) are a group of thousands of synthetic chemical compounds, each consisting of a fully (per) or partially (poly) fluorinated carbon chain connected to a functional group. The hydrophobic, fluorinated tail and hydrophilic, functional groups give PFAS hydrophobic and oleophobic properties, respectively. The carbon-fluorine bonds make the compounds chemically stable, effective surfactants, and difficult to degrade. Due to these unique properties, PFAS have been used extensively in industry and consumer products (Wang et al. 2017).

Since the 1940s PFAS have also been used commercially and industrially in fire retardants, metal plating, and surfactants. They are also found in consumer products, including non-stick cookware, Teflon tape, water repellent products, and food packaging (Glüge et al. 2020). During production and usage, PFAS can be released into the environment and have been observed in water, air, sediment, and organisms (Ahrens et al. 2011, Gellrich et al. 2013, Munoz et al. 2022).

PFAS have been found to cause adverse health effects in humans, including liver and kidney disease, immune system suppression, and increased cholesterol levels (DeLuca et al. 2021). In response to these concerns some international regulations have emerged including the phase out of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) production under the Stockholm Convention (Stockholm Convention 2008, 2019; Falandysz 2022).

The phase out of PFOS and PFOA has led to the creation of PFAS alternatives that provide similar surfactant qualities but with shorter chain length. These alternatives are

expected to be less hazardous to the environment and humans. However, recent research has found these alternatives have similar recalcitrance to PFOA and PFOS and higher mobility due to their shorter carbon chain lengths (Wang et al. 2015; Gomis et al. 2018). These PFAS alternatives, along with previously produced PFAS, are ubiquitous and accumulate in areas of waste discharge like landfills and wastewater treatment plants (Cousins et al. 2022).

Studies have reported high levels of PFAS in wastewater treatment plants (WWTPs) throughout the world (Lenka et al. 2021). Conventional WWTPs have low removal efficiency of PFAS and have rarely monitored for PFAS as there are currently no finalized regulatory standards in the United States for wastewater. As PFAS pass through the treatment plant they have been observed to transform and adsorb to solids. Longer chain compounds have shown higher affinity to adsorb to solids while shorter chain compounds are less likely to adsorb to solids and instead remain in solution (Gallen et al. 2018). This causes biosolids to act as a sink for long chain PFAS and effluent to contain higher levels of short chain PFAS. During treatment long chain compounds can also break down to shorter chain compounds leading to higher concentrations of short chain PFAS in the effluent than the influent (Zhao et al. 2013, Kozik 2024).

There is still much to be understood about the fate, transport, and behavior of PFAS in the environment. To achieve greater understanding, effective methods for PFAS extraction and analysis from diverse environmental samples must be developed. The EPA developed methods for the determination of PFAS in drinking water beginning in 2009 with Method 537 which was modified to Method 537.1 in 2018 and included 18 target PFAS. Both of these methods used styrene-divinylbenzene as the sorbent material in the

SPE cartridges which was not sufficient for the extraction of smaller, more polar and more acidic PFAS compounds (Shoemaker and Tettenhorst 2020). In 2019 the EPA published Method 533 which measured 25 PFAS in drinking water and incorporated isotope dilution to correct for incomplete analyte recovery from complex matrices. This method utilizes a weak anion exchange sorbent for SPE which provided better extraction of short chain and more polar PFAS (Wendelken and Rosenblum 2018).

Until 2024 the EPA had no published method for the determination of PFAS in environmental samples other than drinking water. In the interim the UWRL (Kozik) developed methods for the extraction and analysis of PFAS in wastewater, biosolids, soil, sediment, and plants based on EPA Method 533 and ISO Method 25101(ISO 2009, Wendelken and Rosenblum 2019). In 2024 the EPA finalized Method 1633 for the extraction and analysis of PFAS in wastewater, soil, biosolids, and tissues (EPA 2024b). In response the UWRL applied EPA Method 1633 and compared it with the existing methods for PFAS extraction from wastewater and biosolids.

While these EPA methods are helpful in providing standardized regulatory methods they can be difficult to understand and may not be executable or practical for a research laboratory. The rate of publication of EPA Methods for PFAS quantitation has not met the rate of growing public concern and the need for research. This led to the UWRL developing methods for PFAS extraction and analysis for environmental samples not included in EPA methods. As the EPA publishes new methods, these methods need to be compared against the UWRL methods to determine the optimum methods for accuracy and feasibility of PFAS extraction and analysis at the UWRL.

For method comparison between the UWRL and EPA methods, a Bardenpho WWTP with magnetite addition in Utah was sampled for wastewater and biosolids. The effluent from this plant is used as irrigation water for local farmers and the biosolids are composted and currently landfilled or sold to large farms with plans to sell to the public. To evaluate these methods, comparisons were made between biosolids extraction methods, solid phase extraction methods for clean-up and concentration of wastewater and biosolids extracts, and several analytical methods for quantitation of PFAS in these generated samples. The results of these comparisons were used to finalize recommended methods for PFAS extraction and analysis for wastewater and biosolids samples collected in this study.

1.1 Objectives

Objective 1: Compare extraction (sonication and EDGE), SPE, and analytical methods for PFAS in wastewater and biosolids.

Objective 2: Recommend extraction and analytical methods for the determination and quantification of PFAS in biosolids and wastewater.

2 Literature Review

2.1 PFAS Sources and Distribution

PFAS is released to the environment through point sources, such as fluoropolymer manufacturers, and non-point sources from many consumer products, and persists in ground and surface water, air, and soil (Stock et al. 2007, Ahrens et al. 2011, Gellrich et al. 2013, Brusseau et al. 2020).

Significant point sources of PFAS are aqueous film-forming foams (AFFF) used at airports, and manufacturing plants that utilize PFAS in their production processes. PFAS have also been observed at high levels in wastewater treatment plants and landfills. These sources can lead to human exposure through direct contact with PFAS contaminated irrigation water, PFAS being taken up by agricultural plants and then being ingested, and/or by ingestion of impacted ground and surface water. This last exposure route, contaminated drinking water, has been found to be a major source of direct PFAS exposure for many communities (DeSilvia et al. 2020).

Consumer products are also a major source of human PFAS exposure. The highest concentrations of PFAS were found in consumer products including apparel, carpet and textiles followed by paper and packaging. Humans can also be exposed to direct contact with PFAS through personal care products, pesticides, ski wax, and adhesives (Gluge et al. 2020).

Two of the most studied PFAS compounds, PFOA and PFOS, have been observed in water, air, soil, human blood, and urine (Olsen et al. 2007; Beesoon et al. 2012; Zhang et al. 2013b; Wang et al. 2014; Bentel et al. 2020). The pervasiveness of these compounds in the environment led to PFOA and PFOS being added to the Stockholm Convention on Persistent Organic Pollutants in 2008 and 2019, respectively, after which the production of these compounds was significantly reduced (UNEP 2009; Dixon-Anderson et al. 2018; Stockholm Convention 2008, 2019). However, these changes have not eliminated PFAS production but have transitioned production from long-chain PFAS to shorter chained PFAS. Perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) have been developed as alternatives to PFOA and PFOS (Wang et al. 2015). These

compounds contain ether C-O bonds and were speculated to have less bioaccumulation potential than longer-chain compounds they replaced (Bentel et al. 2020). The most prominent ether alternative is 2,3,3,3- Tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA), known commercially as GenX.

In the Netherlands, grass samples were taken around a fluoropolymer plant. The samples had higher levels of GenX than PFOA, reflecting the shift from PFOA to GenX contamination in recent years (Brandsma et al. 2019). Little is known about the toxicity of GenX alternatives. A study done with rats consuming doses of GenX indicated that toxicity of GenX is comparable to PFOA and is heavily driven by kinetics (Gomis et al. 2018). Short chain alternatives still pose a risk to the environment and human health. The risk is elevated by the lack of research around the toxicity and transport pathways of these PFAS alternatives.

While shifts have occurred in PFAS manufacturing, PFAS are still pervasive in the environment with over 95% of Americans having measurable amounts of PFAS in their blood serum (Kato et al. 2011). Whether from direct emission of PFAS during product manufacturing, product use, and/or product disposal, or indirect sources such as the biotic or abiotic formation of PFAS from precursor compounds, sources of PFAS are numerous and global (Lewis et al. 2015).

2.2 PFAS Properties and Adsorption Behavior

PFAS compounds are characterized by the length of their carbon chain and functional groups. PFAS carbon chains can range in length from four to 18 molecules, with long-chain PFAS being characterized by compounds having six or more carbons (Wang et al.

2015, 2017). The length of the carbon chain influences the compound's behavior in the environment, its bioaccumulation, and its toxicity (OECD 2022). PFAS with smaller carbon chains are more soluble and have weaker sorption to environmental media due to their high polarity (Arp et al. 2006, O'Connor et al. 2022). As PFAS compounds increase in chain length, vapor pressure decreases, and volatilization is hindered causing long chain compounds to be more present in sediments and solids than in air (Rayne et al. 2009). This behavior leads to an increase in the value of the sorption coefficient (K_d) with increasing PFAS chain length. An adsorption study by Cai et al. (2022), corroborates that the sorption of PFAS to soil increases with increased chain length and higher soil organic matter (Fig.1).

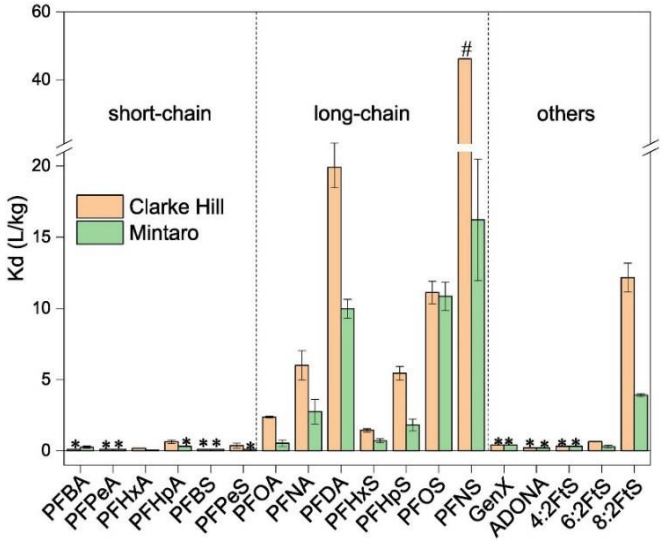


Fig. 1. Sorption coefficients (K_d) of emerging and legacy PFAS in two soils, Clarke Hill: organic matter 4.9%, Mintaro: organic matter 2.6%. Minimum Kd values were used for compounds with very low to negligible sorption, which was marked with the symbol(*). Error bars correspond to standard deviations (n =3). (Cai et al. 2022)

Chain length also influences the health risk PFAS presents to humans. Generally, shorter chain PFAS have a shorter elimination half-life in humans due to their greater water solubility (Zhang et al. 2013b).

The two prominent functional groups characterizing PFAS are carboxylic and sulfonic acids. Both are hydrophilic and due to their low acid dissociation constants (K_a), are generally found in the environment in the anionic state. Sulfonic acids have a higher hydrophobic nature than carboxylic acids and have been observed to show a higher affinity to sludge and sediment (Zhang et al. 2013a; Liu et al 2019).

Adsorption and biodegradation are the main processes responsible for contaminant removal in WWTPs, but PFAS do not biodegrade easily making adsorption critical in PFAS fate and transport (Lenka et al. 2021). There are many factors that impact sorption of PFAS. A few of these are hydrophobic interactions, electrostatic repulsion and attraction, Van der Waals interactions, site competition, pore blockage, anion exchange capacity and partition effects (Fig. 2) (Gagliano et al. 2020; Joo et al. 2021).

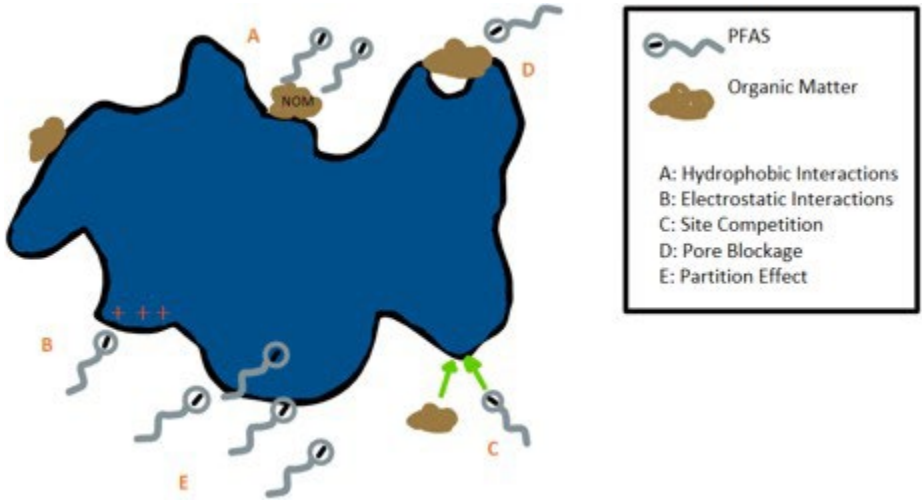


Fig. 2. PFAS Adsorption Mechanisms in Soils

The influence of these factors is controlled by the physio-chemical properties of PFAS, including the compound's chain length, functional group, and compound polarity. For PFAS with carbon chains less than five carbons, electrostatic effects driven by the polarity of the functional head are most influential in adsorption behavior. For PFAS with chains longer than five carbons, the hydrophobicity of the tail causes hydrophobic interactions to be more dominant (Zhang et al. 2013a).

Lastly, extrinsic factors like pH, sorbate properties, and the amount of organic material present influence PFAS adsorption. Sediment organic carbon and protein content are the driving sediment characteristics influencing sorption along with pH. The partitioning of PFAS to biosolids increases as pH is decreased due to the reduction of the repulsion force between anionic PFAS and negatively charged solids (Higgins and Luthy 2006; Oliver et al. 2019; Ebrahimi et al. 2021). The optimization of these factors, along

with biosolid stabilization methods, can be used to influence the leaching behavior of PFAS in wastewater treatment biosolids.

2.3 PFAS Toxicity and Regulations

Concern over the toxicity of PFAS has increased significantly over the last decade as PFAS have been found to increase risks of some cancers and reduce immune system response in humans (EPA UCMR 2024). This has led to increased monitoring and regulations. For example, the EPA Unregulated Contaminant Monitoring Rule (UCMR) has expanded the number of PFAS being monitored from six compounds in 2012 to 29 compounds in 2024 (EPA UCMR 2024).

PFAS have been detected in humans across the world (Kannan et al. 2004; Cousins et al. 2022). The most common compounds detected in humans are PFOS, PFOA, and Perfluorohexanesulfonic acid (PFHxS) (Zhang et al. 2013b). Humans are slow eliminators of PFAS, with elimination half-lives of 3.8, 5.4, and 8.5 years for PFOA, PFOS, and PFHxS, respectively (Olsen et al. 2007; Beesoon et al. 2012).

Due to the growing concern around PFAS the EPA has encouraged states to monitor PFAS in water sources. In 2020 the Utah Department of Environmental Quality (UDEQ) developed a reconnaissance plan to assess the influence of PFAS on Utahns (UDEQ 2020). The plan looked to identify potential commercial PFAS users in the state and analyze for PFAS in drinking water. The UDEQ sampled 148 public drinking water wells throughout Utah, PFAS concentrations were below the method detection limit (MDL). The MDL was generally 2.5 ng/L or lower for individual PFAS compounds with a few compounds having an MDL of 20 ng/L. The UDEQ plans to continuously update the

PFAS risk assessment for Utah by expanding sampling locations and conducting seasonal monitoring (UDEQ 2023). This will be necessary with the announcement of the legally enforceable EPA PFAS drinking water standards of 4.0 ng/L for PFOA and PFOS, 10 ng/L for PFHxS, PFNA, and HFPO-DA, or a cumulative hazard index of 1 for mixtures containing f of PFHxS, PFNA, HFPO-DA, and PFBS (EPA 2024c). This regulation will require Utah to continue sampling for PFAS and gain increased analytical sensitivity to lower MDLs from 20 ng/L to below 4 ng/L.

The health effects of individual PFAS compounds are difficult to quantify as there are thousands of PFAS compounds and many variables influencing human exposure and exposure response. Most assessments of human exposure to PFAS have focused on a few prominent PFAS but have failed to account for the shifts to short chain alternatives and the dose-additive behavior that has been observed in PFAS mixtures (De Silva et al. 2021; EPA 2024c).

2.4 Exposure Pathways

There are multiple pathways for human exposure to PFAS including ingestion, inhalation, and dermal exposure. Ingestion can occur through dietary sources, household dust, drinking water and hand-to-mouth exposure through nail biting, smoking, and finger foods (Vestergren and Cousins 2009; D'Hollander et al. 2010; Poothong et al. 2019). Ingestion of PFAS is an increasing concern as biomagnification of PFAS has been observed in plants, fish, and waterfowl (Houde et al. 2011; Brown et al. 2020). In New York, it was found that PFOS was biomagnified in fish by a factor of nine, illustrating the exponential accumulation of PFAS through trophic levels (Sinclair et al. 2006). PFAS

have also been seen to accumulate in agricultural crops through PFAS-impacted irrigation water, biosolids land application, and proximity of agricultural fields to PFAS production facilities (Brown et al. 2020).

Ingestion of PFAS contaminated drinking water is a major source of direct PFAS exposure. PFAS have been found in surface water, drinking water, sewage, and groundwater as well as in snow and rain (Gellrich et al. 2013). Kozik (2024) found PFAS in reclaimed irrigation water and rain water in northern Utah's Cache Valley, with elevated concentrations of carboxylic acids compared to sulfonic acids (Fig. 3). These data show the pervasiveness of PFAS even in communities without large businesses that manufacture products known to contain PFAS. The UDEQ identified a few industries in Cache Valley that could potentially be using PFAS, including electroplating companies and the airport, but none of these potential sources have been proven to contribute PFAS to the environment (UDEQ 2020).

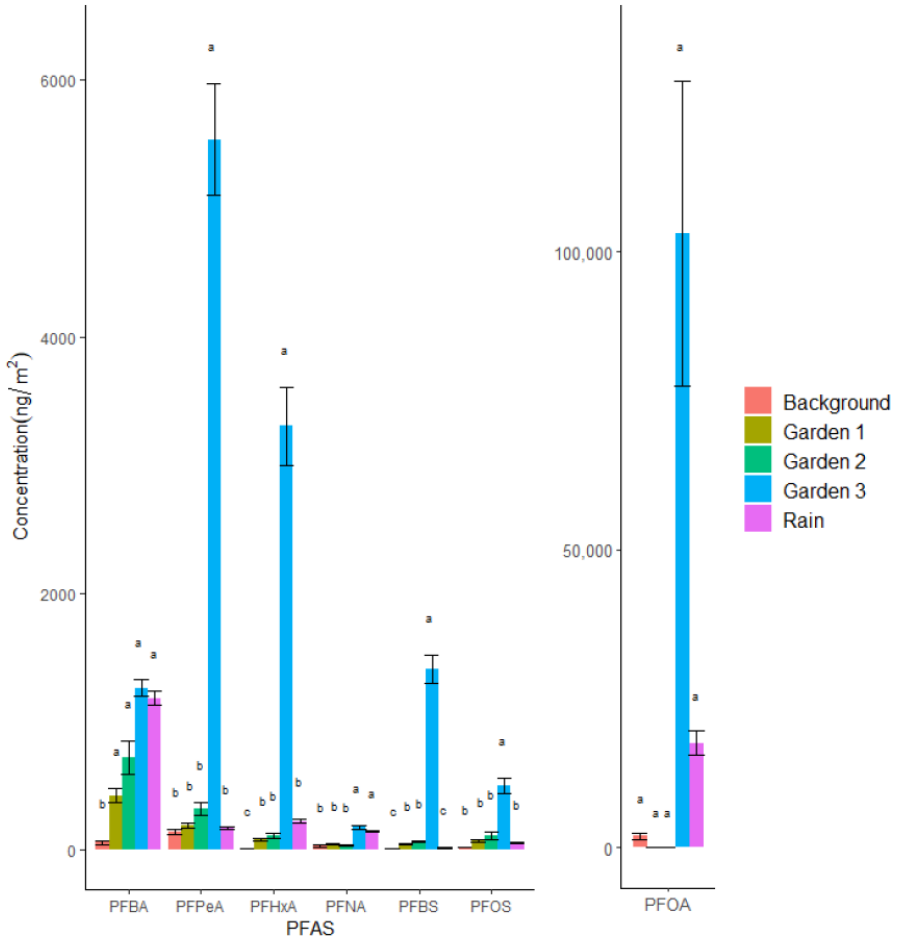


Fig. 3. Annual PFAS loading in Cache Valley from garden spigots and rain per square meter of irrigated land, using the plant consumption data from Hill et al. (2011), with Tukey HSD Groups (Kozik 2024).

A main source globally of PFAS in wells and groundwater is AFFF, often used at airports and military bases. Hu et al. (2016) found a 20% increase in detectable PFAS at water supplies that were in the same watershed as military sites. Throughout the U.S., drinking water supplies for 6 million residents were impacted with PFAS at levels at or above 70 ng/L for PFOA and PFOS advisory limits based on UCMR3 (Hu et al. 2016).

With the EPA drinking water limit of 4 ng/L for PFOA and PFOS many more sites will be added to this list (EPA 2024c).

The health risk from inhalation is particle size dependent. Particles <10 microns can penetrate deep into the lungs and lead to respiratory problems (Cooper and Alley 1996). Unlike soils and water, the atmosphere does not accumulate PFAS long-term but provides a mechanism for long-range transport of PFAS laden particles (D'Ambro 2021).

Indoor spaces generally have higher concentrations of airborne PFAS than outdoor spaces due to PFAS in stain resistant carpets, paints, furniture, and household cleaners (Schlummer et al. 2013). Additionally, most North Americans spend approximately 90% of their time indoors (De Silvia et al. 2021) making indoor exposures a more likely occurrence than outdoor exposure. Toddlers and babies have the potential for higher exposure to PFAS due to their proximity to the carpet and higher hand-to-mouth exposure (Zheng et al. 2020).

2.5 PFAS in Wastewater

PFAS can enter the wastewater stream through domestic and industrial discharges, landfill leachates, stormwater, and agricultural runoff (O'Connor et al. 2022). PFAS in wastewater can impact soil, drinking water, plants, and humans through the land application of biosolids, use of reclaimed wastewater, and potable reuse.

Due to the hydrophobicity of long chain PFAS compounds, they readily sorb to solids in the wastewater. In contrast, short chain compounds remain in the wastewater (Zhang et al. 2013a; Liu et al. 2019). PFAS compounds between C4 to C8 carbon chain, i.e., PFOA, PFOS, PFHxS, PFBA, and PFHxA, are the most prevalent PFAS compounds found in

domestic wastewater. PFHxS is prevalent due to its wide use in stain and water repellant products (The Danish Environmental Protection Agency 2015; O'Connor et al. 2022).

The analysis of PFAS concentrations throughout wastewater treatment plants has shown that most treatment plants have low PFAS removal efficiency. Additionally, some PFAS compound concentrations in the effluent are often higher than the influent. This indicates transformation of PFAS precursors generally to perfluoralkyl acids (Lenka et al. 2021; Helmer et al. 2022). Kozik (2024) found elevated sulfonic PFAS concentrations in the effluent from a membrane bioreactor WWTP in northern Utah (Fig. 4).

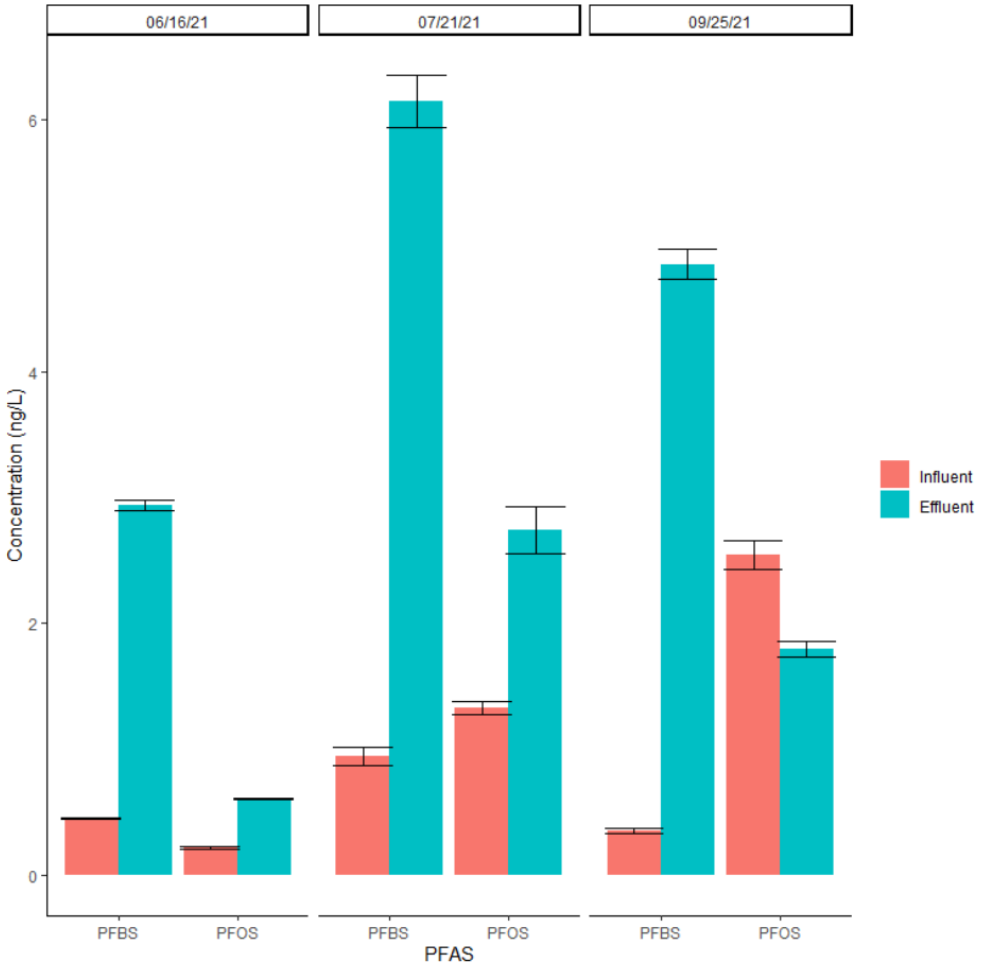


Fig. 4. Change in PFBS and PFOS sulfonic concentrations through a WWTP in northern Utah. Error bars indicate 95% confidence intervals (Kozik 2024).

Kozik also analyzed for carboxylic compounds and found that PFPeA consistently had higher concentrations in the influent than effluent (Fig. 5). This is unexpected as PFPeA is a degradation product of PFOA and other longer chain PFAS and would be

expected to appear in larger concentrations in the effluent (Wang et al 2011; Fang et al. 2023).

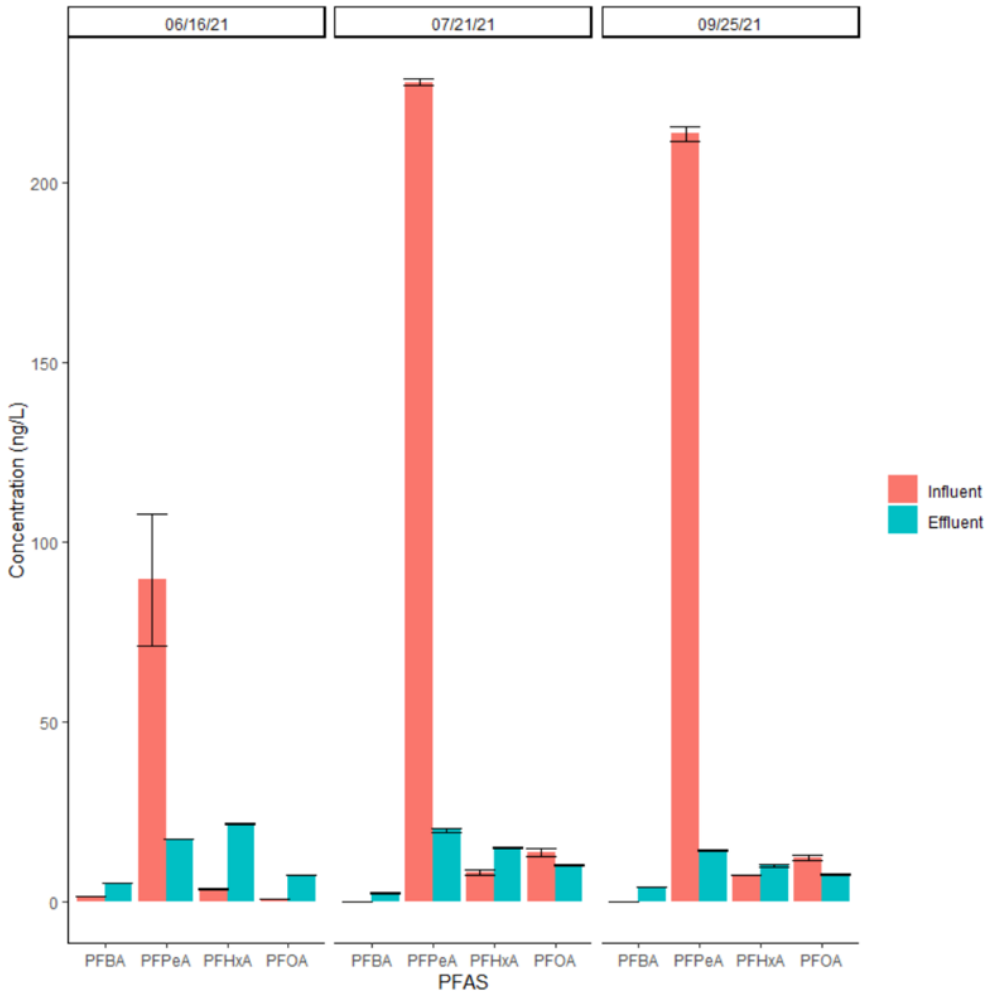


Fig. 5. Change in selected carboxylic acid PFAS concentrations through a WWTP in northern Utah. Error bars indicate 95% confidence intervals (Kozik 2024).

Kozik's research highlights that PFAS is present in even rural community wastewater treatment plants and confirms that there are significant differences in PFAS concentrations between the influent and effluent.

Transformation can occur through oxidation, photolysis, and hydrolysis, and under anaerobic and aerobic conditions (Buck et al. 2011; Allred et al. 2015). This transformation occurs because long-chain poly-fluoroalkyl substances containing carbon-hydrogen, carbon-oxygen, and carbon-nitrogen bonds can be broken down to per-fluoroalkyl substances that only contain the stronger carbon-fluorine bonds (Ji et al. 2020). Currently, the most studied classes of PFAS precursors are fluorotelomer alcohols (FTOHs) and fluorotelomer sulfonates (FTS) which have been observed to form shorter-chained PFAS in landfills and wastewater treatment plants (Fig. 6) (Schultz et al. 2006; Lenka et al. 2021; Liu et al. 2021; Zhang et al. 2022). Sources of these precursors are still relatively unknown, but recent research has revealed unexpected origins like toilet paper production (Thompson et al. 2023).

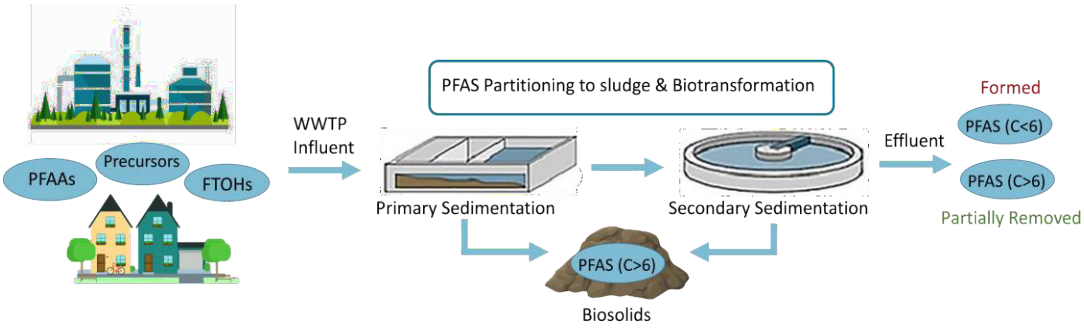


Fig. 6. Fate and transport of PFAS in wastewater treatment plants

Treatment trains and operational parameters can also influence PFAS concentrations in WWTPs. Previous research is inconclusive as to the effect of wastewater treatment processes, without added PFAS removal techniques, may have on PFAS concentration

trends and removal. Schultz et al. (2006) concluded that there are no observable trends in PFAS formation across similar treatment processes, so the influence of treatment is indeterminate. Guerra et al. (2014) countered that PFAS formation varied based on treatment type, with higher PFAS generation occurring during biological treatment steps compared to physical treatment processes. While it is unclear if treatment processes directly influence PFAS fate it is known that regulating operational parameters and physio-chemical properties can reduce the concentrations of PFAS in the effluent due to increased partitioning to biosolids (Zhang et al. 2013a; Ebrahimi et al. 2021). Additionally, adjusting parameters like temperature and retention time can influence PFAS transformation and adsorption in the treatment process (Guerra et al. 2014).

2.6 PFAS in Biosolids

The treatment of wastewater generates substantial amounts of biomass. The disposal of this waste creates an environmental challenge due to the quantity of solids and their high nutrient and organic content. Common disposal options are land application, disposal in a landfill, and incineration. The increasing cost of incineration and disposal in landfills, and restrictions on ocean disposal makes land application a favorable disposal option (Lu et al. 2012).

When sludge from wastewater treatment plants is treated to local, state, and federal standards for land application it is defined as biosolids (Lu et al. 2012). Biosolids are nutrient rich and high in organic matter which can improve the chemical and biological properties of soils and stimulate microbial activity (Haynes et al. 2009). Due to the cost

effectiveness and beneficial use of biosolids nearly 43% of biosolids produced in the US are land applied (EPA 2021).

While land application has been readily adopted as a beneficial disposal option, concerns have been growing recently over the environmental impact of this practice. Land applied biosolids have been observed to contain organic pollutants. These contaminants can accumulate in soil, leach into groundwater, and bioaccumulate in plants grown in biosolid amended soils (Ghisi et al. 2019; Munoz et al. 2022).

Kozik (2024) quantified PFAS concentrations in fields that were amended with biosolids 6 months prior (new field soil), 3 years prior (old field soil), and a background field that had no biosolids application (Fig. 7). He found that the highest concentrations of PFAS were found in the biosolids followed by the new field soil, the old field soil, and the lowest levels were in the background soil. Kozik also found PFAS in field grasses grown in the old and new field soil treated with biosolids (Fig. 8).This indicates that PFAS is being lost to the environment over time either through leaching into the groundwater, through biodegradation, or via plant uptake.

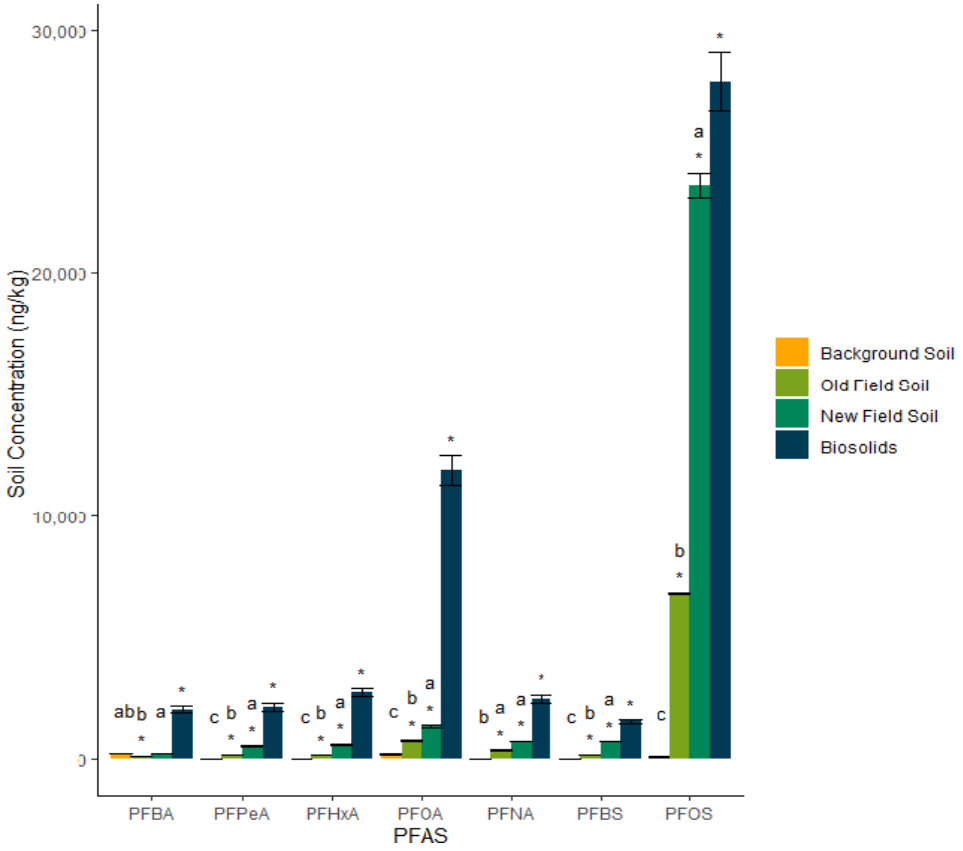


Fig. 7. Concentrations of PFAS compounds in background hayfield, biosolids amended field soils, and biosolids. Error bars indicate 95% confidence intervals. * indicates a significant difference from the mean of the control field Dunnett's test $p<0.05$. Letters are Tukey's HSD groups (Kozik 2024).

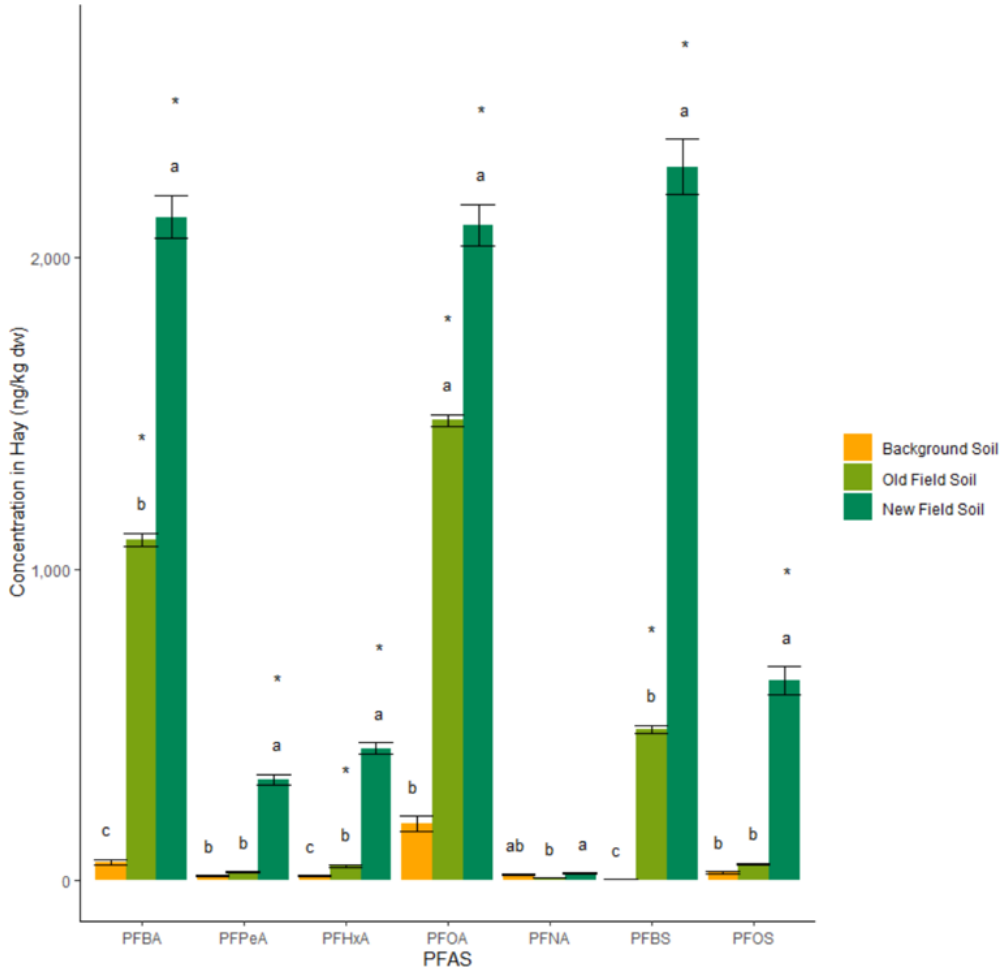


Fig. 8. Concentrations of PFAS compounds in control grass and biosolid amended field grasses. Error bars indicate 95% confidence intervals. * Indicates a significant difference from the mean of the control grass sample Dunnett's test $p<0.05$. Letters are Tukey HSD groups (Kozik 2024).

The concentrations of PFAS in the grasses were highest in the field with recently applied biosolids showing that plant uptake influences PFAS concentrations in plants. Significant concentrations of PFAS were also observed in the plants grown in soil that had biosolids applied 3 years prior, revealing the persistence of PFAS in soils and their continued uptake into plants over time as plant harvesting takes place.

Currently in the United States there are no federal regulations for PFAS concentration in biosolids. Increased levels of PFAS due to biosolid land application has led to contamination of ground and surface water (DeSilva et al. 2020). In Maine, land applied biosolids contaminated drinking water wells with PFAS resulting in a ban on biosolid land application in 2022. Alternatively, Maine is disposing of biosolids to landfills or shipping them out of state (Miller 2020, IATP 2020, Hogue 2022). This drastic legislation has caused increased concern from other states about the continued use of biosolid land application and potential PFAS contamination. Continuing bans on biosolid land application would result in increased disposal in landfills which increases costs to communities and reduces usable landfill space for municipal solid waste.

PFOS has been observed as the main form of PFAS in both biosolids and biosolids-amended soil (Sepulvado et al. 2011; Brusseau et al. 2020; Pepper et al. 2021). PFOS can originate from a direct source or through biotransformation. PFOS precursors adsorb to sludge and can transform to PFOS during biosolids treatment (Zhang et al. 2017). PFAS precursors that were observed in biosolids were not detected in the amended soil indicating rapid biotransformation or leaching after land application (Pepper et al. 2021).

Samples of biosolids from a membrane bioreactor plant in Utah were obtained and analyzed for seven carboxylic and seven sulfonic PFAS in 2021 (Tables 1 and 2) (Kozik 2024). The mass of PFAS associated with biosolids on an annual basis was calculated and combined with data from the plant influent and effluent to determine the percent removal of PFAS through biosolids wasting (Tables 3 and 4).

Table 1: Carboxylic acid PFAS compound concentrations found in biosolids generated from the membrane bioreactor WWTP (Kozik 2024)

Compound	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA
Mean (ng/kg)	2,030	2,120	2,740	239	11,900	2,450	11,800
95% Confidence interval	483	724	578	117	2,340	723	2,270

Table 2: Sulfonic acid PFAS compound concentrations found in biosolids generated from the membrane bioreactor WWTP (Kozik 2024)

Compound	PFBS	PFPeS	PFHxS	PFHpS	PFOS	PFNS	PFDS
Mean (ng/kg)	1,539	32	250	131	27,900	653	5,820
95% Confidence interval	298	23	167	54	4,690	472	4,180

Table 3: Percent removal through biosolids wasting for carboxylic acids PFAS compounds that are removed through the membrane bioreactor treatment plant, with the annual influent mass of PFAS in each flow stream (Kozik 2024)

Compound	PFBA	PFPeA	PFHxA	PFOA	PFNA	PFDA
Removal from Water (g/yr)	-4.9	231	-132	0.81	0.81	0.498
Mass in Biosolids(g/yr)	0.26	0.27	0.35	1.5	0.31	1.5
Percent of Mass Associated with Biosolids	4.3	1.1	1.5	11.1	41.5	67.4
Percent Influent Mass in Biosolids	34	0.11	3.7	11.7	25.0	123
Percent Influent Mass in Effluent	750	9.7	242	93.8	35.3	60

Table 4: Percent removal through biosolids wasting for sulfonic acids PFAS compounds that are removed through the membrane bioreactor treatment plant, with the annual influent mass of PFAS in each flow stream (Kozik 2024)

Compound	PFBS	PFPeS	PFHxS	PFOS	PFDS
Removal from Water (g/yr)	-5.9	6.8	0.93	-0.51	0.33
Mass in Biosolids(g/yr)	0.20	0.004	0.031	3.6	0.74
Percent of Mass Associated with Biosolids	2.8	0.06	2.1	58.8	85.9
Percent Influent Mass in Biosolids	23	0.06	2.1	179	164
Percent Influent Mass in Effluent	790	2.9	37.4	126	27

From these results, an increase of PFAS in the biosolids is observed for the longer chain compounds, specifically PFDA, PFOS and PFDS. This could be due to the increased hydrophobicity of these longer chain compounds, favoring adsorption to biosolids, and the transformation of even longer carbon chain PFAS precursors to these compounds. The last rows in Tables 3 and 4 show the percentage of PFAS in the influent that is accounted for in the effluent. For PFBA, PFHxA, PFBS, and PFOS the percentage is over 100 indicating that these more water-soluble compounds are also being generated from the transformation of longer carbon chain precursors during the treatment process.

2.8 PFAS Methods

Analytical methods are still developing for the determination of PFAS in environmental samples. There are many challenges to processing and analyzing samples containing PFAS including: adsorption of PFAS to sampling and experimental equipment, contamination from equipment containing PFAS, and the high sensitivity needed to analyze for PFAS in the parts per trillion detection range.

Currently the EPA has published three multi-laboratory validated methods for PFAS analysis in drinking water, Method 537 which was updated to Method 537.1, Method 533, and Method 1633 for non-potable water and other environmental samples (Wendelken and Rosenblum 2019, Shoemaker and Tettenhorst 2020, EPA 2024b) (Table 5).

These EPA methods can be difficult to understand and apply, and require high levels of quality control and instrument sensitivity. This may not be practical for a research laboratory that is working with diverse environmental samples or unique research applications. The need for in-house method development was revealed as the UWRL worked to understand the fate and transport of PFAS in the air, soil, plants, compost, and wastewater. The rate of publication of EPA Methods is not matching the growing research needs around PFAS.

The Utah Water Research Laboratory (UWRL) started working on PFAS method development in 2019 and modified EPA Method 537.1 to analyze for PFAS in irrigation water. In 2020 a method was developed at the UWRL combining the SPE techniques described in analytical notes from Waters™ based on ISO Method 25101 and the analysis and QA/QC procedures from EPA Method 533 (ISO 2009, Wendelken and Rosenblum 2019, Rosnack et al. 2020). This method was used for extracting and analyzing PFAS from wastewater samples and included eight more analytes than EPA Method 537.1. Additionally, this method utilized isotope dilution for more robust QA/QC along with WAX SPE to enhance the extraction of short chain PFAS. This method was defined as UWRL-SPE(W) and was the method primarily used for extraction of PFAS from wastewater at the UWRL from 2020 to 2023 (Section 4.1.2).

Until 2024, the EPA had no published finalized method for the extraction of PFAS from environmental matrices other than drinking water. Starting in 2020 the UWRL worked to develop Method UWRL-Ex., a method for the extraction of PFAS from solid samples (Section 4.2.2.2). Solid samples are generally extracted through the use of Soxhlet, sonication, or automated extraction. Method UWRL-Ex uses sonication of a dried solid sample in methanol for extraction. In addition to the extraction method UWRL-SPE(S) was developed as a method for cleanup and concentration of the solid extract. This SPE method was based on the analytical notes from Waters™ and ISO Method 25101(ISO 2009, Rosnack et al. 2020). The details of this method are reported in Section 4.2.2.4.

Automated extraction of PFAS from biosolids using an EDGE instrument was also developed at the UWRL. The EDGE methods are described in Section 4.2.3. After solid samples were extracted with the EDGE the extract went through concentration and cleanup following UWRL-SPE(S) and was analyzed on the LC-MS/MS following EPA Method 533. The utilization of automated extraction methods for PFAS extraction from solids is further discussed in the results section.

In 2023 the EPA published drafts of Method 1633 and the UWRL developed new methods for PFAS extraction and analysis of wastewater and biosolids based on this method. The details of these methods are reported in Sections 4.1.1 and 4.2.1. EPA Method 1633 includes a more robust extraction procedure for solids, and incorporates qualifier MRM's for analytes and extraction standards. At the UWRL, beginning in 2023, solid samples were extracted, cleaned up, and concentrated following EPA Method 1633. The settings for the LC were either based on EPA Method 533 or 1633 depending on the

goals of the analysis. The MRMs and settings for the MS/MS were based on EPA Method 533 along with all target analytes, extraction standards, and internal standards. This was due to the availability of the standards for EPA Method 533 at the UWRL. Tables 5 and 6 summarize these various EPA and UWRL PFAS methods, respectively.

Table 5: EPA Methods for Determination of PFAS (EPA 2024a)

Media	Method	Description
Drinking water	537.1 (2018/2020) Determination of Selected PFAS in Drinking Water by SPE and LC/MS/MS	EPA method for measuring 18 PFAS in drinking water including HFPO-DA
	533 (2019) Determination of PFAS in Drinking Water by Isotope Dilution Anion Exchange SPE and LC/MS/MS	EPA method for measuring 25 PFAS in drinking water
Non-potable water and other environmental media	1633 (2024)	EPA method for measuring 40 PFAS in wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue.

Table 6: UWRL and EPA 1633 PFAS Methods

Name	Extraction	SPE	LC Analysis	QqQ Analysis
CEM Method	EDGE extraction Table 8. Section 4.2.3	UWRL-SPE(S) Section 4.2.2	EPA Method 533 Table A5	UWRL 533 Tables A1-3
Calvin's Method	EDGE extraction Table 9. Section 4.2.3	UWRL-SPE(S) Section 4.2.2	EPA Method 533 Table A5	UWRL 533 Tables A1-3
UWRL PFAS Methods Solids	UWRL-Ex Section 4.2.2.2	UWRL-SPE(S) Section 4.2.2	EPA Method 533 Table A5	UWRL 533 Tables A1-3
UWRL PFAS Method Liquids		UWRL-SPE(W) Section 4.1.2	EPA Method 533 Table A5	UWRL533 Tables A1-3
EPA Method 1633	EPA Method 1633 Section 4.2.1.1-4.2.1.3	EPA Method 1633 Section 4.2.1.4	EPA Method 1633 Table A4	UWRL 533 Tables A1-3

3 Materials and Methods

3.1 Sampling Sites

Samples were taken from a wastewater treatment plant in Utah that utilizes a modified Bardenpho method along with magnetite addition for treatment. The plant's effluent is used by local farmers for irrigation water and the produced biosolids are composted and currently landfilled or sold to large farms with plans to sell to the public.

The Bardenpho plant sampled in this study treats approximately 18 MGD of wastewater from a group of municipalities with a total population of approximately 100,000 residents along with local businesses and large industries, the majority of which are non-potential PFAS sources (Carollo 2015). The plant utilizes the Bardenpho process for biological nutrient removal (Figs. 9 and 10).

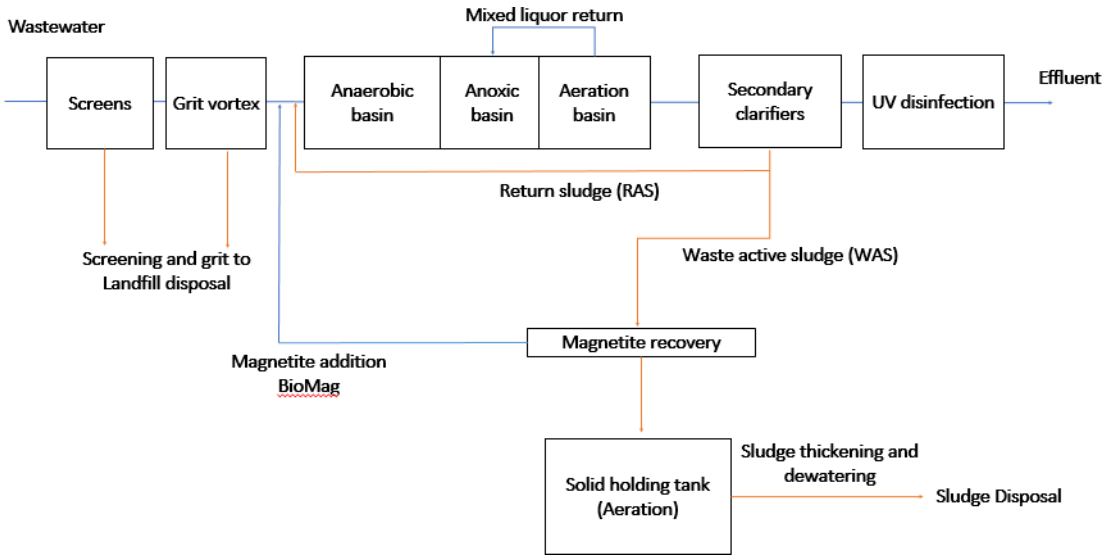


Fig. 9. Schematic of the Bardenpho Plant

Fig. 10. Bioretention basins at the Bardenpho Plant

This plant employs a Biomag® process which infuses magnetite particles into biological floc, increasing plant capacity by achieving faster settling in the secondary clarifiers (Carollo 2018). Shear mills and magnetic drums are used to separate magnetite from the waste activated sludge (WAS) (Fig. 11). The plant recovers 90-95% of the magnetite (Logan City 2022) for reuse in the process.

Fig. 11. Magnetic recovery drum

The residual magnetite remains in the biosolids. The plant produces 60 tons of biosolids a day that is mixed with green waste in a 4:6 ratio by volume. The mixture is placed in windrows and turned five times during a 15-day period while maintaining a temperature of 131°F (55°C). Meeting these requirements, along with testing for pathogens and heavy metals, produces Class A biosolids that can be applied as compost for farms, gardens, and lawns. The treated effluent is discharged to man-made wetlands for polishing during the non-irrigation season and used as irrigation water for local farmers during the irrigation season.

3.2 Sampling Procedure

At the Bardenpho plant, triplicate samples were taken of the influent, effluent, and from the anaerobic, anoxic, and aerobic basins with a 1L HDPE plastic bottle attached to a pole. The sample in the 1L bottle were poured into a 250 mL HDPE bottle for samples being extracted with UWRL-SPE(W) or a 500 mL HDPE bottle for samples being

extracted with EPA Method 1633. The influent was sampled after grit removal and before alum addition. The effluent was sampled after UV disinfection.

Biosolids samples were collected after the rotary press in quart Ziploc bags. Samples being extracted by UWRL-Ex. were air dried. Once dried, the solids samples were ground with a mortar and pestle and sieved through a 2 mm sieve prior to extraction and analysis. Samples being extracted with EPA Method 1633 were not dried and were stored at 4°C before extraction.

Trip blanks filled with DDW were taken on each sampling trip to assess potential contamination that may have occurred in the field during sampling or from the sampling bottles. Two field blanks were also taken on each sampling trip to assess contamination from the sampling equipment. The field blanks consisted of rinsing the sampling equipment with 250 mL DDW after sampling from the anoxic tank and another after sampling the effluent. All samples were stored at 4°C until extraction. Samples were extracted within 28 days from collection and analyzed within 28 days of extraction in accordance with EPA Method 1633 (EPA 2024b).

4 Analytical Methods

4.1 Extraction of Liquid Samples

4.1.1 Extraction of Liquid Samples based on EPA Method 1633

4.1.1.1 Conditioning

Water's Oasis WAX SPE cartridges (polymeric reversed-phase, weak anion exchange for PFAS (6cc 150 mg 30µm) (Appendix C) were conditioned by flushing 15 mL of 1% methanolic ammonium hydroxide followed by 5 mL of 0.3 M formic acid without vacuum. Cartridge reservoirs were filled with DDW to keep resin wet during sample loading.

4.1.1.2 Sample Processing

Influent samples and samples from the anoxic, anaerobic, and aeration tanks were homogenized by inverting the sample three to four times and allowing the sample to settle. The sample volumes were determined by weighing the full sample bottle and the empty sample bottle to 0.1 g.

Fifty µL of the extraction standard (ES) were spiked into the samples and QC samples, in the original sampling bottles, for a final ES concentration of 200 ng/L prior to analysis. If needed, the samples were adjusted with 50% formic acid or 3% aqueous ammonium hydroxide to ensure a pH of 6.5 ± 0.5.

4.1.1.3 Solid Phase Extraction

Clean salinized glass wool was packed to half the height of the WAX SPE cartridge barrel and the WAX cartridges conditioned following the steps in Section 4.1.1.1.

Aqueous samples were poured from sampling bottles into 250 mL HDPE reservoir bottles attached to WAX cartridges (Fig. 12).

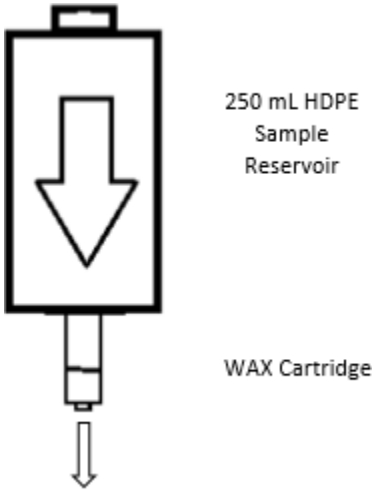


Fig. 12. Diagram of the reservoir system used for the WAX SPE

The vacuum was adjusted so that the sample passed through the cartridge at 5 mL/min. The empty sampling bottles were retained for further rinsing.

For samples with suspended material, when the WAX cartridges clogged a second pre-conditioned cartridges was loaded with the remaining sample. After the sample passed through the cartridges and was eluted following the procedure described below the two eluates were combined and concentrated to 5 mL using the TurboVap.

After the complete sample passed through the cartridge the walls of the reservoir bottle were rinsed twice with 5 mL of reagent water. The reservoir was then rinsed with 5

mL of 1:1 0.1M formic acid/methanol. The rinses were pulled through the cartridge under vacuum.

The cartridge was dried for 15 minutes, and clean 15 mL collection tubes were placed in the SPE manifold. The sample bottles were rinsed with 5 mL of 1% methanolic ammonium hydroxide and the rinse transferred to the reservoir using a glass pipette. The rinse was pulled through the cartridge under vacuum into the collection tubes. Twenty-five µL of concentrated acetic acid was added to the collection tube and vortexed followed by the addition of 10 mg of carbon. The collection tubes were hand shaken for less than 5 minutes, immediately vortexed, and centrifuged at 2,800 rpm for 10 minutes.

Fifty µL of the internal standard were spiked into clean 15 mL centrifuge tubes for a final concentration of 200ng/L. The supernatant from the extract was filtered using a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe into the centrifuge tubes containing the internal standard.

Two hundred µL of the extract were pipetted into a micro-vial for LC-MS/MS analysis. The remaining extract was labeled and stored at 4°C.

4.1.2 Extraction of Liquid Samples, Method UWRL-SPE(W)

4.1.2.1 Conditioning

Water's Oasis WAX SPE cartridges for PFAS (6 cc 150 mg 30 µm) were conditioned by flushing 5mL of methanol followed by 5 mL of DDW through the cartridge. Cartridge reservoirs were filled with DDW to keep resin wet during sample loading.

4.1.2.2 Sample Loading

Aqueous samples were poured from sampling bottles into reservoir bottles attached to the cartridges (Fig. 12). Samples were drawn through the cartridges at a rate of 2 drops per second under 10 inHg.

4.1.2.3 Flushing

After the sample has passed through the cartridge the cartridge was dried under 15 inHg of vacuum for 3 minutes. The sample bottles were rinsed with 10 mL of 25 mM sodium acetate and the rinse poured into the corresponding reservoir bottle. The rinse was drawn through the cartridge under 10 inHg vacuum. Finally, the cartridges were dried for 5 minutes at 15 inHg vacuum.

4.1.2.4 Elution

After drying, 15 mL polypropylene centrifuge tubes were placed under the cartridges to collect the eluate. The sample bottles were rinsed with 5 mL of 2% ammonium hydroxide in methanol. Approximately 2 mL of the rinse was drawn through the cartridge at 5 inHg of vacuum and collected in the centrifuge tubes. The flow path was closed, allowing the remaining 3 mL to saturate the cartridge for 3 minutes. The sample bottles were rinsed once more with 5 mL of 2% ammonium hydroxide in methanol and poured into the cartridge reservoir. The elution solvent was allowed to flow through the cartridge under gravity by disconnecting the vacuum.

4.1.2.5 Turbovap

Caliper ZA7516 Turbovap drying tubes were washed with soap and water and rinsed three times with methanol and dried. The eluted samples were poured into the Turbovap tubes and placed into a Caliper Life Sciences Turbovap 2 and evaporated under a flow of nitrogen gas. The bath temperature was set to 60°C and the pressure to 0.9 bar. The samples were reconstituted using 1 mL methanol in the Turbovap tube and then transferred to a 1.5 mL centrifuge tube prior to analysis. Ten µL of the IS was spiked into the 1 mL extract. Two hundred µL of the extract were pipetted into a micro-vial for LC-MS/MS analysis. The remaining extract was labeled and stored at 0-4°C.

4.2 Extraction of Solid Samples

4.2.1 Extraction of Solid Samples based on EPA Method 1633

4.2.1.1 Sample Processing

Biosolid samples were mixed with a stainless steel spoon to homogenize the sample. Five to 10 g of sample were weighed to three significant figures. The weights were recorded for percent solids calculations. The samples were dried in an oven at 110 °C and cooled in a desiccator. The weight of the dried samples was recorded, and the percent solids calculated using Equation 1.

$$\% \text{ solids} = \frac{weight\ of\ sample\ after\ drying\ (g)}{weight\ of\ sample\ before\ drying\ (g)} \times 100 \quad (1)$$

An aliquot of undried sample was weighed out into a 50 mL centrifuge tube. The aliquot was equivalent to 0.5g dry weight biosolids, calculated from Equation 1. The aliquot was spiked with 50 µL of the extraction standard for a final concentration of 200

ng/L prior to analysis. The centrifuge tube was vortexed to disperse the extraction standard and allowed to equilibrate for 30 minutes.

4.2.1.2 Extraction

Ten mL of 0.3% methanolic ammonium hydroxide were added to each centrifuge tube. The centrifuge tubes were vortexed until the sample was dispersed. The sample was shaken for 30 minutes on a reciprocal shaker table at low speed followed by centrifugation at 2800 rpm for 10 minutes. The supernatant was decanted into a clean 50 mL centrifuge tube.

Fifteen mL of 0.3% methanolic ammonium hydroxide were added to the remaining sample in each centrifuge tube. The centrifuge tubes were vortexed until the sample was dispersed. The sample was shaken for 30 minutes on a reciprocal shaker table at low speed followed by centrifugation at 2800 rpm for 10 minutes. The supernatant was decanted into the centrifuge tube with the supernatant from the first extraction.

Five mL of 0.3% methanolic ammonium hydroxide were added to the remaining sample in each centrifuge tube. The centrifuge tubes were shaken by hand and centrifuged at 2800 rpm for 10 minutes and the supernatant decanted into the centrifuge tube holding the first and second extractions.

4.2.1.3 Carbon Addition and Evaporative Drying

Ten mg of carbon were added to the combined extract. The centrifuge tube was mixed by hand for less than 5 minutes and centrifuged at 2800 rpm for 10 minutes. The extract from the centrifuge tube was decanted into a Turbovap tube. The extract was

diluted to approximately 35 mL with reagent water. If a sample was already more than 35 mL volume, then no water was added.

The water content of the sample was determined using the following equation:

$$\text{Water content in sample (g)} = \frac{Sample\ weight\ (g) x\ Moisture\ (\%)}{100} \quad (2)$$

Each sample was concentrated in Caliper ZA7516 Turbovap drying tubes that were washed with soap and water and rinsed three times with methanol and dried. The Turbovap tubes were placed into a Caliper Life Sciences Turbovap 2 to be evaporated under a flow of nitrogen gas to the volume determined using Table 7. The bath temperature was set to 60°C and the pressure to 0.9 bar.

Table 7. Final concentrated volume based on the water content in a sample

Water Content in Sample	Concentrated Final Volume
<5 g	7 mL
5-8 g	8 mL
8-9 g	9 mL
9-10 g	10 mL

Extracts were concentrated in the Turbovap for 25 minutes then vortexed if the sample is <20 mL or mixed by pipette if the volume was > 20 mL. After mixing the extract continued to be concentrated for 10 minutes and then mixed again if the volume was above the desired volume.

Forty to 50 mL of reagent water were added to the extract and vortexed. The pH was checked to determine if the pH was 6.5 ± 0.5 and adjusted with 50% formic acid or 30% ammonium hydroxide if needed.

4.2.1.4 Solid Phase Extraction

The SPE methods for aqueous and biosolid samples following EPA Method 1633 are similar. The only differences are that biosolid samples go through carbon cleanup before SPE and aqueous samples undergo carbon cleanup before analysis. Additionally, the volume of the sample before SPE is approximately 40-50 mL for biosolids and 500 mL for aqueous samples.

Clean salinized glass wool was packed to half the height of the WAX SPE cartridge barrel and the WAX cartridges conditioned following Section 4.1.1.1.

The liquid extract from the biosolid samples, described in Section 4.1.2.2, was poured into 250 mL HDPE reservoir bottles attached to the cartridges. The vacuum was adjusted so that the sample passed through the cartridge at 5 mL/min. The empty sampling bottles were retained for further rinsing.

After the complete sample passed through the cartridge the walls of the reservoir bottle were rinsed twice with 5 mL of reagent water. The reservoir was then rinsed with 5 mL of 1:1 0.1 M formic acid/methanol. The rinses were pulled through the cartridge under vacuum.

The cartridge was dried for 15 minutes, and clean 15 mL collection tubes placed in the SPE manifold. These collection tubes were spiked with 50 µL of internal standard. The sample bottles were rinsed with 5 mL of 1% methanolic ammonium hydroxide and

the rinse transferred to the reservoir using a glass pipette. The rinse was pulled through the cartridge under vacuum into the collection tubes.

Twenty-five µL of concentrated acetic acid were added to the extract and swirled to mix. Fifty µL of the internal standard were spiked into clean 15 mL centrifuge tubes and the extract added to the tube. The extract was filtered using a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5 mL polypropylene syringe. Two hundred µL of sample were pipetted into a micro-vial for LC-MS/MS analysis. The remaining sample was labeled and stored at 0-4°C.

4.2.2 Extraction of Solids Samples, UWRL Methods

4.2.2.1 Sample Processing

Samples were air dried under a fume hood for 12 hours. The samples were ground using a mortar and pestle and sieved through a 2 mm sieve.

4.2.2.2 Extraction: Method UWRL-Ex.

One gram of sample was weighed out and placed in a 15 mL centrifuge tube. The sample was spiked with 10 µL of ES, for a final concentration of 200 ng/L prior to analysis, and 7 mL of methanol were added to the centrifuge tube. The sample was then sonicated for 30 minutes followed by centrifugation at 3,000 rpm. The supernatant was poured off into a clean 15 mL centrifuge tube and an additional 3 mL of methanol were added to the sample. The sample was sonicated for 30 minutes and centrifuged for 20 minutes at 3,000 rpm. The supernatants were combined and diluted in 250 mL bottles of

deionized water before solid phase extraction and the pH of the sample was adjusted to below 3 using acetic acid.

4.2.2.3 Conditioning

Water's Oasis WAX SPE cartridges for PFAS (6cc, 150 mg, 30 µm) were conditioned by flushing 10 mL of 2% methanolic ammonium hydroxide followed by 5 mL of methanol and 5 mL DDW through the cartridge. Cartridge reservoirs were filled with DDW to keep resin wet during sample loading.

4.2.2.4 Solid Phase Extraction: Method UWRL-SPE(S)

Samples were passed through the SPE cartridge at a rate of 2 drops per second. When the sample had passed through the cartridge the cartridge was dried by drawing ambient air through the cartridge for 5 minutes. Four mL of 25 mM sodium acetate buffer were drawn through the cartridge and the cartridge was dried for an additional 5 minutes. The vacuum was turned off and a 15 mL centrifuge tube was placed under each cartridge. The sample bottles were rinsed with 5 mL of 2% methanolic ammonium hydroxide and 2 mL of the rinse were drawn through the cartridge under 5 inHg of vacuum. The remaining 3 mL of solvent were retained in the cartridge for 5 minutes and then passed through the cartridge under gravity. The sample bottles were rinsed again with 5 mL of 2% methanolic ammonium hydroxide and the rinse passed through the cartridge under gravity.

4.2.2.5 Turbovap

Caliper ZA7516 Turbovap drying tubes were washed with soap and water and rinsed three times with methanol and dried. The eluted samples were poured into the Turbovap tubes and placed into a Caliper Life Sciences Turbovap 2 and evaporated to dryness under a flow of nitrogen gas. The bath temperature was set to 60°C and the pressure to 0.9 bar. The samples were reconstituted using 1 mL methanol in the Turbovap tube and then transferred to a 1.5 mL centrifuge tube prior to analysis. Ten µL of IS were spiked into the 1 mL extract for a final concentration of 200 ng/L. Two hundred µL of the extract were pipetted into a micro-vial for LC-MS/MS analysis. The remaining extract was labeled and stored at 0-4°C.

4.2.3 Extraction of Solids Samples using EDGE

Biosolids samples were extracted using the EDGE to determine if an automated extraction method would result in high recoveries of the extraction standards. Along with biosolid samples, filters were spiked with ES and run concurrently on the EDGE as a method blank. The filters followed the same SPE and analysis procedure as the biosolids samples.

4.2.3.1 Drying

Biosolid samples taken from the Bardenpho plant on 11/17/2022 were placed in a fume hood to be air-dried for at least 12 hours and up to 2 days. The dried samples were ground with a mortar and pestle and sieved through a 2-mm sieve. One gram of the sieved sample was spiked with 10 µL of the 20,000 ng/L extraction standard.

4.2.3.2 Extraction

Solid samples were extracted using the energized dispersive guided extraction (EDGE) system. The EDGE combines pressurized liquid extraction and dispersive solid phase extraction (Kinross et al. 2020). All tubing in the EDGE was replaced with polyethylene tubing to reduce PFAS contamination (Appendix C). Q-cups used to hold solid samples in the EDGE were washed with soap and water and rinsed with methanol prior to each use.

The HDPE reservoir and sampling bottles were washed with soap and water, dried, and rinsed three times with methanol. Water's Oasis WAX cartridges were cleaned before conditioning by flushing each cartridge with 5 mL Optima grade methanol followed by 5 mL of DDW. Samples were placed into aluminum Q-cups with a CEM S1 Q-disk and placed in the EDGE. The EDGE method is based on the CEM method notes (Table 8) for extraction of PFAS from soil (CEM 2020).

An alternative EDGE method recommended by USU PhD student Calvin Luu (Table 9) was also tried to test if the use of Ethyl Acetate would result in higher recovery of PFAS surrogates from biosolids.

Table 8. CEM EDGE Method for extraction of PFAS from solid samples

Heating Program				
Cycle	Solvent	Solvent Top Add (mL)	Temp (°C)	Hold (mm:ss)
1	Methanol/water (80:20) with 0.3% ammonium hydroxide	10	65	3:00
2	Methanol/water (80:20) with 0.3% ammonium hydroxide	10	65	4:00
Wash Program				
Cycle	Solvent	Wash Volume (mL)	Temp (°C)	Hold (mm:ss)
1	methanol	10		00:03

Table 9. Calvin Luu Method for extraction of PFAS from solid samples

Heating Program				
Cycle	Solvent	Solvent Top Add (mL)	Temp (°C)	Hold (mm:ss)
1	Ethyl Acetate	10	100	3:00
2	Methanol	10	65	4:00
Wash Program				
Cycle	Solvent	Wash Volume (mL)	Temp (°C)	Hold (mm:ss)
1	water	30	100	00:15

4.2.3.3 SPE and Evaporative Drying

The extract from the EDGE was decanted into a 250 mL bottle of DDW that has been acidified to a pH below 3 with acetic acid. The sample was then loaded onto the WAX cartridge using the procedure described for the extraction of liquid samples. After the sample was run through the WAX cartridge and the SPE procedure concluded, the samples were evaporated to dryness in the Turbovap. Drying and reconstitution followed

the same procedure described in Subsection 4.1.2.5 of the extraction of liquid samples section.

4.3 Chemicals

4.3.1 Standards and Surrogates

PFAS standards were purchased from Wellington Laboratories (Appendix C). The standard is made of an extraction standard mixture, an internal standard mixture, and the native PFAS analyte solution. The PFAS extraction standard mixture including nine mass-labeled perfluoroalkylcarboxylic acids (C_4-C_{12}), three mass-labelled perfluoroalkanesulfonates (C_4, C_6, and C_8), three mass-labelled fluorotelomer sulfonates (4:2, 6:2, and 8:2) and mass-labelled GenX (M3HFPO-DA). The mass-labelled PFAS internal standard mixture consists of two mass-labelled perfluoroalkylcarboxylic acids (M3PFBA and M2PFOA) and a mass-labelled perfluoroalkylsulfonate (M4PFOS) (Table A1-3). The native PFAS analytes in the standards are given in Table 10. The standards were stored at -18°C and made into 20,000 ng/L working standard solutions by diluting 1.2 ml of the 500 ng/mL stock into 30 mL of methanol. The working standard was further diluted in methanol to the analytical standards of 100, 200, 400, 600, 800, 1,000, and 2,000 ng/L.

4.3.2 Chemicals and Reagents

4.3.2.1 Acetonitrile

LCMS grade or equivalent acetonitrile from Fisher was used as the mobile phase for LC/MS analysis when running EPA Method 1633 (Appendix C).

Table 10. PFAS analytes

Analyte	Abbreviation
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF3ONS
4,8-Dioxa-3H-perfluorononanoic acid	ADONA
Hexafluoropropylene oxide dimer acid	HFPO-DA
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA
Perfluorobutanoic acid	PFBA
Perfluorobutanesulfonic acid	PFBS
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS
Perfluorodecanoic acid	PFDA
Perfluorododecanoic acid	PFDoA
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA
Perfluoroheptanesulfonic acid	PFHpS
Perfluoroheptanoic acid	PFHpA
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS
Perfluorohexanesulfonic acid	PFHxS
Perfluorohexanoic acid	PFHxA
Perfluoro-3-methoxypropanoic acid	PFMPA
Perfluoro-4-methoxybutanoic acid	PFMBA
Perfluorononanoic acid	PFNA
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS
Perfluorooctanesulfonic acid	PFOS
Perfluorooctanoic acid	PFOA
Perfluoropentanoic acid	PFPeA
Perfluoropentanesulfonic acid	PFPeS
Perfluoroundecanoic acid	PFUnA

4.3.2.2 Acetic Acid

LCMS grade or equivalent acetic acid from Fisher was used during solid phase extraction to promote microbial stability while storing extracted samples (Appendix C).

4.3.2.3 Ammonium Acetate

A 2 mM Ammonium Acetate in 95:5 vol:vol water/acetonitrile solution was used as the mobile phase for the LC/MS when running EPA Method 1633. The solution was prepared using 0.154 g ammonium acetate in 950 mL of DDW and 50 mL of acetonitrile. This solution was replaced every 2 months.

A 20 mM Ammonium Acetate solution was used as the mobile phase for the LC/MS when running EPA Method 533. The solution was prepared using 0.77 g ammonium acetate in 0.5 L of DDW. This solution was replaced every time the LC/MS was run.

4.3.2.4 Sodium Acetate Buffer

Acetate Buffer was made by adding 410 mg of sodium acetate to 200 mL of DDW. The buffer was adjusted to a pH of 4 by adding acetic acid and/or sodium hydroxide as required.

4.3.2.5 Formic Acid

A 0.3 M Formic acid solution was prepared by dissolving formic acid (13.8 g) in reagent water (1 L). The solution was stored at room temperature and replaced after 2 years.

A 50% v/v Formic acid solution was prepared by mixing 50 mL formic acid with 50 mL reagent water. The solution was stored at room temperature and replaced after 2 years.

A 0.1 M formic acid/methanol solution was prepared by mixing equal volumes of methanol and 0.1 M formic acid. The solution was stored at room temperature and replaced after 2 years.

4.3.2.6 Methanol

LCMS grade or equivalent methanol from Fisher was used (Appendix C).

4.3.2.7 Methanolic Ammonium Hydroxide

Methanolic ammonium hydroxide (0.3%) was made up by adding ammonium hydroxide (1 mL, 30%) to methanol (99 mL). The solution was stored at room temperature and replaced after 1 month.

Methanolic ammonium hydroxide (1%) was made up by adding ammonium hydroxide (3.3 mL, 30%) to methanol (97 mL). The solution was stored at room temperature and replaced after 1 month.

Methanolic ammonium hydroxide (2%) was made up by adding ammonium hydroxide (6.6 mL, 30%) to methanol (93.4 mL). The solution was stored at room temperature and replaced after 1 month.

4.4 Quality Control

4.4.1 Standard Curve

The standard curve for PFAS analytes and extraction standards (200 ng/L) in liquid samples had seven standard levels at 100, 200, 400, 600, 800, 1,000, and 2,000 ng/L. A minimum of five contiguous calibration standards are required for valid analysis. The lowest calibration standard must meet a signal-to-noise ratio of 3:1 and be at a concentration less than or equal to the Limit of Quantification (LOQ). The results for each standard should be within ± 50% of the true value to pass UWRL QA/QC limits.

4.4.2 Minimum Reporting Limit (MRL)

The minimum reporting limit (MRL) is the minimum concentration that can be reported by a laboratory as a quantified value for a method analyte. To determine the MRLs a minimum of seven samples, DDW blanks spiked with the method analytes, extraction standards, and internal standards, were processed through all steps of the method. The mean and standard deviation of these samples were calculated for each analyte.

The Half Range for the Prediction Interval of Results (HR_{PIR}) was calculated using the following equation:

$$HR_{PIR} = 3.963S \quad (3)$$

where, S = the standard deviation and 3.963 is a constant value for seven replicates.

The Upper and Lower Limits for the Prediction Interval of Results (PIR = Mean ± HR_{PIR}) are calculated using the following equations:

$$\text{Upper PIR Limit} = \frac{Mean+HRPIR}{Fortified\ Concentration} \text{ x } 100 \quad (4)$$

$$\text{Lower PIR Limit} = \frac{Mean-HRPIR}{Fortified\ Concentration} \text{ x } 100 \quad (5)$$

The MRL is determined when the Upper PIR Limit is less than or equal to 150% and the Lower PIR Limit is greater than or equal to 50%. If these criteria are not met then the MRL has been set to low and must be confirmed again at a higher concentration (Wendelken and Rosenblum 2018).

Table C2 shows the MRL values for some PFAS analytes. During the time MRL determination experiments were being conducted there were instrument troubles that led to poor chromatography and low ES and IS recovery. This caused some of the data to be unusable and MRLs could not be calculated for all compounds.

4.4.3 Instrument Blank and CCVs

The instrument blank for EPA Method 1633 consists of a solvent mix of methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid (ammonium hydroxide (3.3 mL, 30%), reagent water (1.7 mL) and acetic acid (0.625 mL) to methanol (92 mL).) The solvent mix was spiked with the extraction standards and the internal standards at a concentration of 200 ng/L. The instrument blank is run at the beginning of the analytical sequence, after the analysis of the highest calibration standard and with each Continuing Calibration Verification (CCV) sample.

The instrument blank for UWRL 533 consisted of methanol spiked with internal and extraction standards at 200 ng/L. An instrument blank was run following each CCV.

The CCV samples were analyzed after the standard curve and at the end of the analysis batch. A CCV was also analyzed after every 10 samples. The CCV had a concentration of 200 ng/L of the target analytes, the extraction standards, and the internal standards. The recovery of the native analytes must be within 50-150% to pass UWRL QA/QC limits.

4.4.4 Method Blank

A method blank is analyzed with each sample batch to determine if interferences are being introduced by the laboratory environment, extraction apparatus, glassware, or

reagents (Wendelken and Rosenblum 2019). A method blank was run with each sample extraction batch and was processed and extracted following the same procedure as the samples. The method blank was analyzed after the analysis of the instrument blank and prior to the analysis of samples. The method blank was prepared with a similar matrix to the sample matrix. If any PFAS is found in the blank at: 1) a concentration greater than the MRL for the analyte, 2) at a concentration greater than one-third the regulatory compliance limit, or 3) at a concentration greater than one-tenth the concentration in a sample in the extraction batch, whichever is greatest, analysis of samples was halted, and the problem corrected (EPA 2022a).

4.4.5 Isotope Dilution

Isotope Dilution is an analytical technique that uses the ratio of the area of the native analyte to the area of the isotopically labeled extraction standards to calculate the concentration of each native analyte. The isotopically labeled extraction standard is added to the sample at a known concentration and carried through the analytical procedure. This technique is used to account for potential loss of analytes during sample preparation.

4.4.6 Internal Standards

The internal standards are used to calculate the relative concentration of the extraction standards based on instrument performance. For this study the acceptance criteria for the internal standards were set at a percent deviation from the average of the standard curve of ± 50%. Three internal standards listed in the standards and surrogates section and Table A1 were spiked into samples and standards at a concentration of 200 ng/L for

M2PFOA and M3PFBA and 600 ng/L of M4PFOS before being analyzed with the LC/MS QqQ.

During analysis only M2PFOA and M4PFOS were used as internal standards due to the inconsistent chromatography of M3PFBA at the beginning of the analytical run. All carboxylic acids had M2PFOA as the internal standard for analyte concentration calculations and M4PFOS was used as the internal standard for all sulfonic acids.

4.4.7 Extraction Standards

The extraction standards are used to calculate the recovery of the PFAS analytes through the sample extraction and concentration steps. The 16 extraction standards described in the standards and surrogates section and Table A2 were added at a concentration of 200 ng/L to the samples prior to extraction, except for the fluorotelomer sulfonates which had concentrations of 800 ng/L (Wendelken and Rosenblum 2019). The percent recovery of these extraction standards is calculated by taking the ratio of the measured concentration of the extraction standard, after adjustment based on the internal standard recovery, to the expected concentration of 200 ng/L or 800 ng/L and multiplying by 100. The acceptance criteria for the extraction standards was set at a recovery of 50-150% for this study. If the percent recovery criteria were not met samples were diluted and/or smaller amounts of biosolids were extracted for analysis.

4.4.8 Limit of Quantification (LOQ)

The limit of quantification (LOQ) is the smallest concentration that produces a quantitative result with known and recorded precision and bias. The LOQ is set at or above the concentration of the lowest initial calibration standard.

4.4.9 Ongoing Precision and Recovery Standard (OPR)

The OPR is a method blank spiked with a known concentration of analyte. The OPR is analyzed identically to the samples. The purpose of the OPR is to ensure that the analyte concentrations remain within the limits of the method for precision and recovery. The OPR samples were prepared with reagent water at the same volume as the samples. One OPR sample was spiked with native analytes at 2x the LOQ. A second OPR sample was spiked at the concentration of the mid-level calibration standard.

The percent recovery of the native compounds was calculated using Equation 6.

$$\text{Recovery (\%)} = \frac{Concentration\ of\ analyte\ found(\frac{ng}{mL})}{Concentration\ of\ spiked\ analyte\ (\frac{n}{mL})} \text{ x } 100 \quad (6)$$

The percent recoveries were compared to the OPR limits given in Table A6 in Appendix A. If compounds did not meet the acceptance criteria, then samples were re-extracted and re-analyzed.

4.4.10 Field Blank (FB)

The field blank (FB) is used to ensure that PFAS measured in the field samples were not inadvertently introduced during sample collection and handling. The FB is processed in the same way as the field samples. The FB is 250 mL of DDW that is used to rinse the sampling container in the field. Analysis of the FB is only required if a field sample

contains a method analyte or analytes at, or above, the MDL. If a method analyte found in the field sample is present in the FB at a concentration greater than one-third of the MDL, then the results for that analyte are invalid for all samples associated with the failed FB (Wendelken and Rosenblum 2019).

4.4.11 Trip Blank

The trip blank is a 250 mL HDPE bottle of DDW that is filled at the lab and taken to the field. The trip blank is used to determine if sources independent of the field sampling procedure are a source of PFAS contamination. The trip blank was extracted and analyzed with the same procedures as the aqueous samples.

4.5 Mass Spectroscopy and Chromatography

4.5.1 Mass Spectroscopy

An Agilent 6490 HPLC-MS QqQ was used for detecting and quantifying PFAS in the study samples. The settings that were used for the mass spectrometer are referenced in Tables A4 and A5. All multiple reaction monitor (MRM) settings and retention times were from EPA Methods 1633, 533, and Agilent application notes and are referenced in Appendix A (Wendelken and Rosenblum 2019, Pierri et al. 2020, EPA 2024b). EPA Method 533 does not include qualifier MRMs, the method UWRL 533 is a modification of EPA Method 533 that includes qualifiers for PFAS analytes. These qualifier MRM's were based on Agilent application notes.

4.5.2 Autosampler Volume

Three hundred µL conical polypropylene autosampler vials were used to hold samples for analysis (Appendix C). Approximately 200 µL of sample were added to each vial for analysis.

4.5.3 Delay Column

A delay column was added to the liquid chromatography stack between the binary pumps and autosampler. The delay column is used to increase the retention time of any background interference in the solvent so it enters the analytical column after the latest retention time window of the compounds of interest. This differentiates the background interference from target PFAS in the samples. The delay column used was a 4.6 x 30 mm InfinityLab PFC Delay Column with a maximum pressure limit of 1200 bar.

4.5.4 Analytical Column

A 1.8-µm Zorbex RRHD Eclipse Plus C18, 2.1 x 50 mm (Appendix C), analytical column with a 1.8-µm Zorbex RRHD Eclipse Plus C18, 2.1 x 5 mm (Appendix C), guard column was used for chromatography. The columns were heated to 50°C and have a maximum pressure limit of 1200 bar.

4.5.5 Mobile Phases

The mobile phases for chromatography were 2 mM ammonium acetate in 95% water and 5% acetonitrile and Optima grade acetonitrile for EPA Method 1633. For EPA Method 533 20 mM ammonium acetate in water and Optima grade methanol were used. The ammonium acetate acts as a buffer and signal enhancer. The ammonium acetate

solution was remade every 2 months for Method 1633 or every 2 days for Method 533 and stored at room temperature.

When the solvents were changed from EPA Method 533 to EPA Method 1633 high concentrations of PFOA were detected. It is unknown why changing solvents led to an increase of background PFOA. The outlet capillary on the column was disconnected and the pump turned on to flush the column between solvent changes. The column was flushed for approximately 30 minutes, reconnected, and when EPA Method 1633 was rerun there was no background PFOA detected when column flushing was carried out.

4.6 Statistical Methods

Microsoft Excel and Statistical Analysis System (SAS) software were used for all statistical analyses. Aqueous samples were collected in triplicate and each sample was extracted independently. Solid samples were collected as one sample and split into three smaller aliquots for extraction. To stabilize variance and insure a more normal distribution of the data Box-Cox power transformations using the lambda with the lowest residual were applied. ANOVAs were used to determine significant differences between methods for extraction, SPE, and analysis at the $p < 0.05$ level. If there was significant difference, a Tukey Honest Significant Difference or Tukey-Kramer post-hoc analysis was performed to determine which groups were significantly different. Tukey's was chosen over other post hoc tests because it has a higher power to detect significant differences between multiple groups and controls the probability of making a Type One error, i.e., rejecting the null hypothesis when it is true (Lee and Lee 2018). T-tests were used to compare the average recovery of methods to the ideal recovery of 100%. R-squared values for the LC/MS QqQ calibration curves were calculated using Excel. The

LC/MS QqQ analysis software, Mass Hunter version B.09.00, calculates the correlation coefficient not the regression coefficient and erroneously reports it as R-squared.

5 Results and Discussion

Extracting and analyzing for PFAS in environmental samples is a challenging task due to complex sample matrices and the unique chemistry of PFAS. Reliable PFAS analysis depends on proper sample cleanup and concentration along with strict quality control procedures. The development of the best practices for PFAS extraction and analysis is still on going with EPA's development of new methods for diverse environmental samples and the introduction of lower regulatory standards (EPA 2024b, EPA 2024c). At the UWRL, development of PFAS extraction and analysis methods has been ongoing over the last 5 years. Comparison tests have been done to evaluate the effectiveness of PFAS identification and quantification using different methods of extraction, the influence of different solvents, and the accuracy of different analytical methods based on EPA guidelines.

For the comparisons of EDGE methods no QA/QC parameters were applied. The data for all replicates of all compounds was included and used in the statistical analysis. This was due to the low recoveries of the extraction standards from the biosolids using EDGE. For the comparisons of the sonication and EDGE methods, SPE, and analysis methods the QA/QC criteria of 50-150% recovery of the extraction standard was applied. If this criterion was not met, then that value was removed from the statistical analysis. All data required an internal standard recovery of 50-150% to be used in statistical analysis.

5.1 LC Analysis Method Comparison

Two LC methods were compared, EPA Methods 533 and 1633, based on the regression equations (R^2 values) for standards curves generated by each method for each

compound of interest. Standard curves were generated for each method on three separate dates. The main difference between the methods is the use of methanol or acetonitrile as the carrier solvent. Both methods followed the MRMs given in EPA Method 533 with additional qualifier MRMs for the analytes based on Agilent application notes (Appendix A). The details of the two methods are given in Section 4.5 and Appendix A.

The QA/QC criteria of 50-150% extraction standard recovery was applied to all the data along with the minimum requirement of five standards meeting the criteria for the standard curve to be usable. If two or more of the triplicate standard curves did not meet these criteria, then the compound could not be included in the statistical analysis. Table 11 shows the results of the comparison.

Table 11. PFAS standard curve compounds that did not pass QA/QC

Compounds	EPA 533 compounds that did not pass QA/QC	EPA 1633 compounds that did not pass QA/QC
PFBA		x
PFMPA		x
PFPeA		
PFBS		
PFMBA		
PFEESA		
NFDHA		x
4:2FTS		x
PFHxA		
PFPeS		x
PFHpA		
PFHxS		x
ADONA		
6:2FTS		x
PFOA		
PFHpS		
PFNA		
PFOS		
9Cl-PF3ONS		
8:2FTS		x
PFDA		
PFUnA		
11Cl-PFOUdS		x
PFDoA	x	

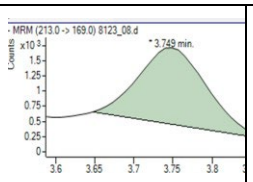
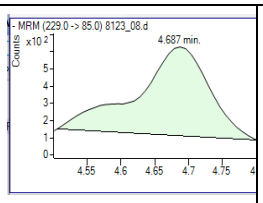
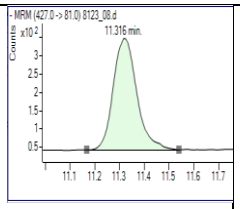
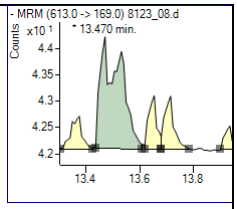
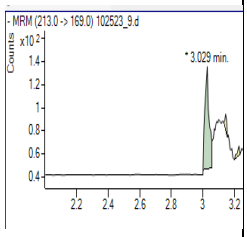
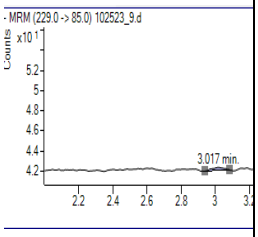
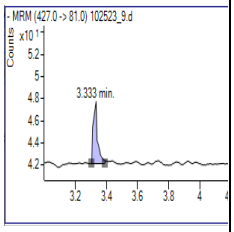
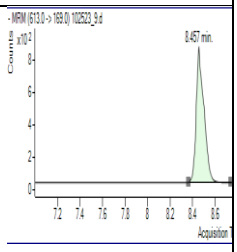
EPA Method 533 only had one compound that did not meet the QA/QC criteria while EPA Method 1633 had nine compounds that did not meet QA/QC requirements. In the following extraction and concentration methods comparisons, EPA Method 533 was used as the LC method for analysis. Because PFDoA did not meet the QA/QC criteria for EPA

Method 533 its corresponding extraction standard M2PFDoA was taken out of all further analyses.

Another indicator of the quality of analysis is the clarity of the compound chromatograms. Several chromatograms of compounds that did not pass QA/QC criteria were evaluated for the quality of the chromatography between the two analysis methods (Table 12). Compounds that elute near the beginning or end of the analytical run more commonly have poorer peak clarity. For PFBA, PFMPA, and 6:2FTS the chromatography for Method 533 showed clearer peaks early in the run and had R^2 values greater than 0.98 as compared to results using Method 1633 which had R^2 values less than 0.6. PFDoA had a retention time near the end of the run and had clearer chromatography and a R^2 closer to one with EPA Method 1633.

Table 12. PFAS analytical methods comparison between EPA Methods 533 and 1633

Compound	PFBA	PFMPA	6:2-FTS	PFDoA
R^2 Method 533	0.994	0.985	0.989	0.272
R^2 Method 1633	0.236	0.515	0.285	0.967

Method 533				
Method 1633				

From these results EPA Method 533 shows more consistency than EPA Method 1633 with regards to the R^2 values, passing the QA/QC criteria, and the quality of chromatography. The only exception is PFDoA which was more accurately analyzed with EPA Method 1633. The poor recovery of PFDoA may have occurred from having the wrong retention time for PFDoA for EPA Method 533 or because it was eluting at the end of the analytical run. To improve the chromatography of PFDoA, a larger retention time window could be used, the run extended, and additional qualifiers for the PFDoA ES added to ensure that the measured peak is the correct compound. Additionally, EPA Method 1633 may show better chromatography with continued optimization of the method. During the time of this comparison the compound mixture of analytes, ES, IS and the compound MRMs were following EPA Method 533, if this method was updated to EPA Method 1633, which includes additional PFAS compounds and ES qualifiers, the analytical results may be improved. A complication with the application of EPA Method 1633 is the use of acetonitrile as an LC solvent. At the UWRL when switching from methanol to acetonitrile the chromatography appeared to worsen and background

contamination was more prevalent. With the current status of method development at the UWRL, EPA Method 533 is recommended for overall accuracy and consistency across the wide range of PFAS compounds of interest in this study.

5.2 PFAS Extraction

5.2.1 EDGE Extraction Methods Comparison

After sample collection, the next step in PFAS analysis is PFAS extraction. Due to the complex matrix of biosolids and their high concentration of organic matter, extraction solvents can influence recoveries of PFAS from these materials. A test was conducted to compare two methods for EDGE extraction with the main difference being the extraction solvent. The method for extraction of PFAS from solids from the manufacturers of the EDGE, CEM, was compared with a method developed by a Chemistry PhD student at Utah State University, Calvin Luu. The CEM method uses methanol as the primary extraction solvent while Calvin's method uses methanol and ethyl acetate. The full methods are described in Tables 8 and 9.

A 3-way ANOVA was conducted to compare the effect of the two methods, the compounds, and the mass of biosolids extracted, on the percent recovery of ES from the biosolids. All data were included in the ANOVA with no QA/QC parameters applied due to the low ES percent recoveries from EDGE extraction. The data were transformed using a Box Cox transformation with a lambda of 0.3 and a 3-way ANOVA was run. There was a significant difference between the methods at the $p < 0.05$ level for the two methods ($F(df = 1) = 398.45$, $p=0.00$), the compounds ($F(df = 13) = 49.87$), the amount ($F(df = 1) = 5.97$), the method x compound interaction ($F(df = 13) = 21.55$), and the method x

amount interaction (F(df = 1) = 18.19 (Table B1). When comparing compounds, Tukey's HSD showed that M2-8:2FTS, M2-6:2FTS, M2-4:2FTS, and M4PFBA were significantly different. This can be observed in Figure 13 as the recovery of the FTS compounds are over 200% for the CEM method. These compounds were removed from the data because they skewed the average recovery of the CEM method. With these compounds removed the CEM method had a mean recovery of 62% compared to a mean of 38% for Calvin's method for ES recovery from biosolids. A t-test indicated that both methods had mean percent recoveries that were significantly different from 100% (Table B2).

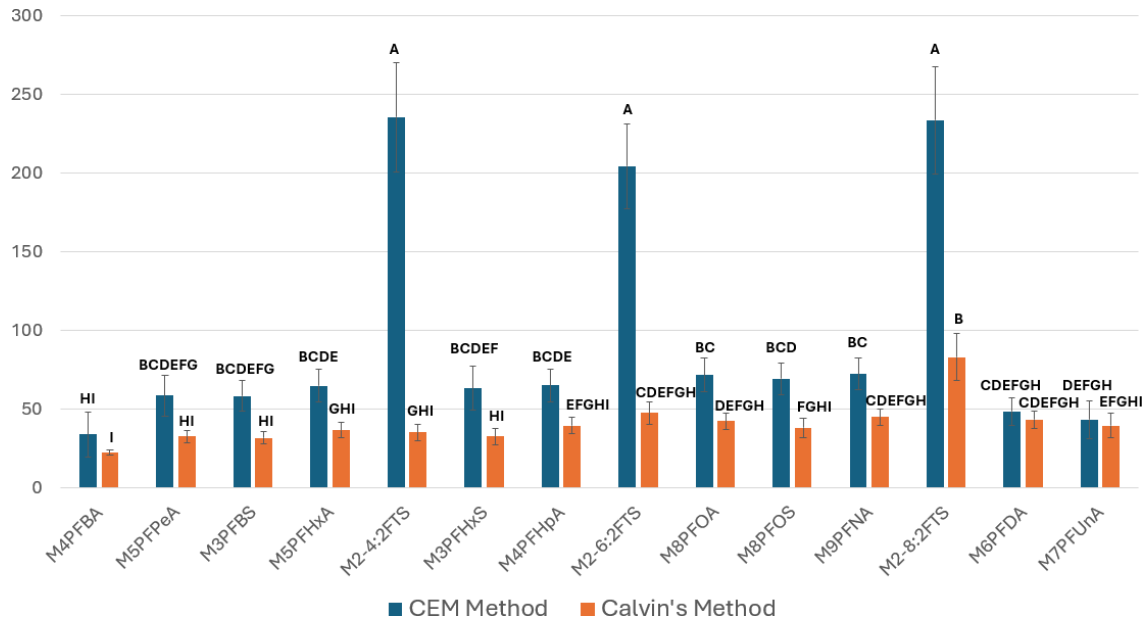


Fig. 13. EDGE Methods comparison for ES percent recovery from biosolids. (Error bars indicate 95% confidence interval of sextuplet measurements. Letters are Tukey HSD groups)

These results show that the CEM method has higher extraction standard recovery than Calvin's method, although both are significantly less than 100%. The CEM method

shows unexpectedly high recoveries of the FTS compounds. When extracting with the EDGE using the CEM method, it is advisable to check the percent recoveries of the extraction standards for the FTS compounds as they may underestimate the reported analyte concentrations. Additional quality control parameters for the accepted percent recovery of the extraction standard should be applied if the analyte concentrations are being considered.

The fluorotelomer sulfonates showed high recoveries in biosolid samples with the CEM method but not in the spiked filters that were run concurrently with the biosolids samples (Fig. 14). These results suggest that the biosolids matrix may be interfering with the ES recovery and causing an additive effect to the recovery of the FTS compounds.

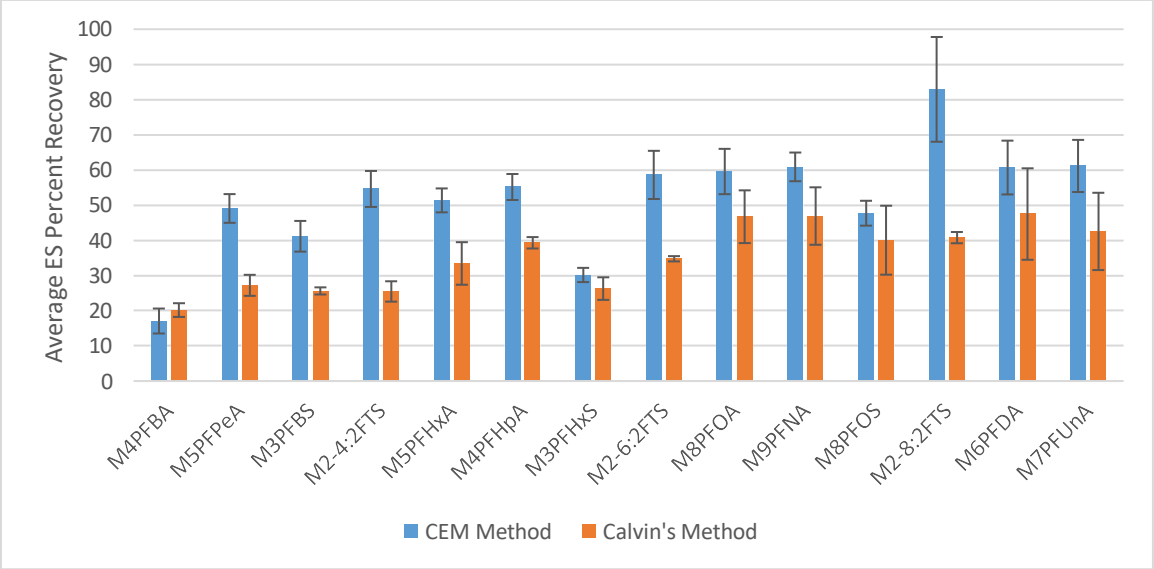


Fig. 14. ES percent recovery for Calvin's EDGE Method from spiked filter samples. (Error bars indicate 95% confidence interval of duplicate measurements of Calvin's method and quintuple measurements of the CEM method)

More experiments on extracting fluorotelomer sulfonates from biosolids with automated extraction should be done to understand the abnormally high recoveries of these compounds from biosolids using the CEM EDGE extraction method.

From these results the CEM method shows a higher average percent recovery of the ES from biosolids than Calvin's method. Calvin's method could be improved upon by including pH adjustment to ensure that the PFAS compounds are being completely released from the biosolids. If the EDGE is being utilized for extraction of PFAS from solids it is recommended to use the CEM method.

5.1.2 Sonication versus EDGE

The UWRL-Ex. method uses sonication to extract PFAS from solids. Sonication is a straightforward, but more time intensive manual method compared to utilizing automated extraction instruments like the EDGE. To compare the extraction efficiencies of a manual versus automated extraction technique an experiment was conducted which compared the recovery of spiked PFAS extraction standards on sand samples between sonication and EDGE. Triplicate sample replicates of 1 g of sand were spiked with 10 µL of the extraction standard and extracted with the EDGE and four sample replicates of 1 g of sand spiked with 10 µL of the ES were extracted with sonication. The sonication extraction procedure is described in Section 4.2.2. Samples extracted with the EDGE followed the CEM extraction program shown in Table 8.

After extraction, both sonication and EDGE extracts underwent SPE following the procedure described in Section 4.1.2. The extracts were then analyzed with the LC/MS QqQ following Method 533 described in Table A5.

The QAQC criteria of 50-150% extraction standard recovery was applied to the data. For the sonication method 18 samples (18/56=32%) did not pass QA/QC criteria, while four samples (4/42 = 10%) did not pass for the EDGE method. If two or more of the triplicate sample replicates or three or more of the four sample replicates did not meet these criteria, then the compound was not included in the statistical analysis. The ES recovery of M4PFBA was above 150% for all sample replicates extracted with sonication. Due to this result all M4PFBA sample replicates were removed for both methods. Similarly, the ES recovery of M5PFPeA was below 50% for two of the triplicate sample replicates extracted with the EDGE so M5PFPeA was also removed from the statistical analysis. The samples that passed were analyzed with a 2-way ANOVA comparing the ES percent recoveries of the two methods, with compound and method as factors. A Box Cox transformation with a lambda of -1.0 was performed on the data and a 2-way ANOVA performed with the transformed data. The results indicated that the methods ($F(df = 1) = 93.92,\ p = 0.00$) were significantly different but not the compounds ($F(df = 11) = 1.12,\ p = 0.37$) or the interaction ($F(df = 11) = 0.72,\ p = 0.71$) (Table B3). After conducting t-tests comparing the results of each method to the ideal recovery value of 100% the results showed that sonication had an average recovery that was statistically equal to 100% while the EDGE average recovery was below 100% (Table B4).

Overall, sonication showed higher percent recoveries of the ES than the EDGE method (Fig. 15) and was statistically equal to 100% recovery when QA/QC criteria were applied to final analytical results.

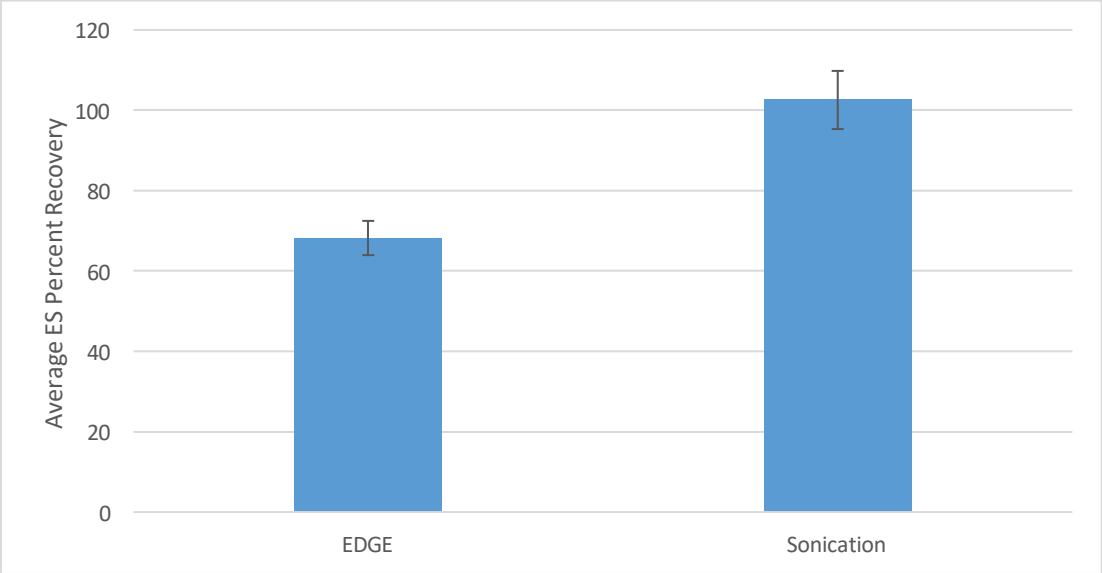


Fig. 15. PFAS ES percent recovery from sonication versus EDGE extraction with QA/QC criteria applied. (Error bars indicate 95% confidence interval of 38 measurements per method)

When determining whether to use EDGE or sonication for extraction data quality objectives and lab capability should be considered. While the sonication method showed average recoveries equal to 100% it is a much more labor and time intensive process than using the EDGE. Additionally, more samples did not meet the QA/QC criteria for the sonication method compared to the EDGE. While the ES recoveries from the EDGE were lower they were more consistent than from the sonication method. If the data quality objectives allow for a lower percent recovery then the EDGE method may be the most efficient. For PFAS extraction of solids at the UWRL, the sonication method is recommended as it is analogous to the extraction methods given in EPA Method 1633 and has better extraction recovery efficiency than the EDGE method.

5.2 Extraction and SPE Method Comparison

5.2.1 Biosolids Extraction and SPE Method Comparison

Biosolids from the Bardenpho plant were collected on 02/24/23 and extracted following EPA Method 1633 described in Section 4.2.1. Additional biosolids were collected on 02/08/24 and extracted following methods UWRL-Ex. and UWRL-SPE(S) described in Section 4.1.2. Both sets of samples were analyzed following EPA Method 533 on the LC/MS QqQ (Section 4.5, Table A5). This comparison was completed to determine which extraction/concentration method has the highest percent recoveries of ES for biosolids samples.

The results show unexpectedly high recoveries of the FTS compounds for samples extracted with EPA Method 1633 with recoveries near 200% for 4:2FTS and 6:2FTS and large variance among sample replicates.

The QAQC parameters of 50-150% recovery of the extraction standard was applied to the data and all recoveries that did not meet this criterion were removed. Applying this criterion removed eight samples that were extracted/concentrated with EPA Method 1633 and four samples that were extracted/concentrated with the UWRL methods. The ES recovery of M2-4:2FTS for the triplicate sample replicates extracted with the UWRL methods all had percent recoveries under 50%. The percent recoveries of the FTS compounds for the triplicate sample replicates that were extracted with EPA Method 1633 all had two or more replicates that didn’t meet the 50-150% recovery criteria. Due to these results the FTS compounds were removed from the statistical analysis. A Box-Cox transformation was performed with a lambda of 0 on the data and a two-way

ANOVA performed with the transformed data. The results showed significant difference between the methods ($F(df = 1) = 138.52$, $p = 0.00$), among compounds ($F(df = 10) = 3.19$, $p = 0.00$), and for method x compound interactions ($F(df = 10) = 2.37$, $p = 0.03$) (Table B5). A t-test was conducted with the data that met the QA/QC criteria and Method 1633 had an average recovery of 101% and the UWRL methods had an average recovery of 69%. Method 1633 had an average recovery statistically equal to 100% (Table B6).

From this comparison EPA Method 1633 shows ES percent recoveries equal to 100% compared to UWRL Methods for the extraction/concentration of PFAS in biosolids. When the QA/QC criteria is not applied, EPA Method 1633 shows high recoveries of the fluorotelomer sulfonates which was similar to the results shown in Fig. 13. This is an unexpected result and may point to solvent interactions with the biosolids that is resulting in high recoveries of FTS.

For the EDGE extraction comparison using the CEM method, methanol is the primary solvent which had high recoveries of these compounds (Fig. 13). The biosolids extraction/concentration comparison shows that procedures following EPA Method 1633 had higher recoveries of these compounds than the UWRL methods, although both methods use methanol for extraction. These results indicate that there may be other unknown interactions that are occurring to cause these high recoveries of FTS compounds.

For the extraction/concentration of PFAS from biosolids it is recommended to use EPA Method 1633 as it has average ES percent recoveries statistically equal to 100% when QAQC criteria is applied. This method is also the EPA's only validated method for biosolid extraction. If using EPA Method 1633, the user should be mindful of the

likelihood of high recoveries of FTS compounds and may want to analyze for those compounds separately and ensure QAQC criteria are applied.

5.2.2 Wastewater SPE Method Comparison

Influent and effluent samples were collected from the Bardenpho plant on 11/17/22 and 03/02/24. The samples collected in 2022 were extracted with the UWRL-SPE(W) Method following the procedures described in Section 4.1.2 and Table A5. The samples collected in 2024 were extracted following EPA Method 1633 (Section 4.1.1). Both sets of samples were analyzed following EPA Method 533. This comparison was conducted to determine which method produced the highest recovery of ES from wastewater samples.

QA/QC criteria of 50-150% recovery of the extraction standards were applied to the results. For EPA Method 1633 15 sample replicates did not pass the QA/QC criteria and for Method UWRL-SPE(W) 16 sample replicates did not pass. Only M4PFBA extracted with the UWRL-SPE(W) method had over four of the sextuplet sample replicates that did not meet the QA/QC criteria. Due to this, M4PFBA was removed from the statistical analysis. A three-way ANOVA was performed with factors of sampling location, method, and compound. A Box Cox transformation with a lambda of 2.0 was performed on the data followed by a 3-way ANOVA with the transformed data. The results showed significant difference between methods ($F(df = 1) = 161.37,\ p = 0.00$), and among compounds ($F(df = 12) = 6.92,\ p = 0.00$), location ($F(df = 1) = 43.42,\ p = 0.00$), and the location x compound interaction ($F(df = 12) = 3.42,\ p = 0.00$) (Table B7).

The average percent recoveries of the ES for the two methods with QA/QC criteria applied were 85% for Method 1633 and 108% for the UWRL-SPE(W) Method. A t-test was conducted and both methods were found to be significantly different than 100% with Method UWRl-SPE(W) above 100% recovery and Method 1633 below 100% (Table B8).

During the extraction process the influent samples took over twice as long to process as the effluent samples due to the presence of suspended solids. Little is known about the influence of suspended solids on the effectiveness of the extraction process. Similar to the biosolids samples, the compounds that most often did not meet the QA/QC criteria were the FTS compounds. It is unexpected that some mass labeled PFAS compounds would regularly have significantly higher recoveries than the rest of the standards. Further research is needed on the behavior of fluorotelomer sulfonates when interacting with different solvents and environmental media. Further understanding of fluorotelomer sulfonates would be beneficial to the understanding of PFAS fate and transport in the environment as they have been observed to be precursors of shorter chain PFAS and are found in wastewater, landfill leachate, and AFFF impacted sites (Wang et al. 2011, Hamid et al. 2020, Yan et al. 2024).

Overall, the results from this comparison were not as conclusive as hoped for. Significant differences were determined not only between methods but also location and compound so the influence of the method cannot be isolated. Additionally, the average ES percent recoveries for both methods were significantly different than 100%. Consequently, both methods are viable options for PFAS extraction from wastewater. While neither method is statistically equal to 100% recovery, both methods have mean

recoveries within 10% of 100% and either method could be used for extraction. It may be beneficial for research laboratories to use EPA Method 1633 because it is a more recent method and the only EPA-validated method for wastewater. Research conducted using EPA Method 1633 is likely to be viewed as more cutting edge and accepted by the PFAS research community.

6 Conclusions

Researchers cannot understand the fate and transport of PFAS, and regulators implement regulations without robust analytical methods. There are many limitations to robust analysis; including the prevalence of PFAS contamination in solvents, laboratory supplies and instrumentation along with the low detection limits needed to analyze for PFAS in various environmental media. Due to the constantly evolving research around PFAS, new analytical methods are regularly being developed and implemented. Evaluation of these extraction and analytical methods are necessary before they are incorporated into laboratory use. The quality of data can change drastically depending on the methods used.

A theme through these comparisons was the importance of attention to detail. Each step of the extraction, cleanup, and analysis can influence the quality of the analytical results. During analysis on the LC-MS/MS contamination is common and it is critical to run instrument blanks, and regularly flush columns, pumps, and tubing. Different LC solvents can also influence the quality of chromatography, and level of PFAS contamination. Through the comparisons of analytical methods, it was observed that using methanol as the LC solvent had lower background contamination and clearer chromatography compared to using acetonitrile. Additionally, during analysis it is important to optimize the settings for the MS/MS. The incorporation of MRM qualifiers, optimizing retention times and accounting for loss of the analyte through isotope dilution can greatly increase the quality of the resulting data.

The extraction and SPE steps of the PFAS process can also present challenges to analyte recovery and data quality. Suspended solids, pH control, and solvent choices can

make the difference in successful extraction and cleanup. The optimization of these processes are critical in understanding the fate and transport of PFAS and the health risks that land applying biosolids present to a community. The implementation of the EPA PFAS regulations in the summer of 2024, will propel further need for PFAS research and high sensitivity analysis as communities throughout the United States will be required to test and treat for PFAS at low concentrations.

In this study, extraction, and analytical methods for PFAS in wastewater and biosolids based on EPA and UWRL methods were compared and statistically evaluated for their effectiveness in identification and quantification of PFAS from these complex matrices. Based on the findings of this study, the following recommendations were developed:

1. Based on the results of the comparison between standard curves for EPA Methods 533 and 1633 for analysis on the LC/MS QqQ, EPA Method 533 is recommended due to the quality of the chromatography and the R^2 of the standard curve being more reliable than EPA Method 1633.
2. Based on the results of the comparison between EDGE extraction methods for extracting PFAS from biosolids it was determined that the CEM extraction method had higher percent recoveries of the ES than Calvin's method and is the recommended method for EDGE extraction.
3. Based on the results of the comparison of the EDGE versus sonication methods for PFAS extraction from solids, it was determined that sonication resulted in higher percent recovery of the ES leading to more accurate quantitation of PFAS

analytes. For the extraction of PFAS from biosolids, methods utilizing sonication are recommended over using the EDGE.

4. Based on the results of the comparison between EPA Method 1633 and UWRL methods, UWRL-Ex. and UWRL-SPE(S), for biosolid extraction and SPE, EPA Method 1633 was determined to have ES percent recoveries equal to 100% and is the recommended method for extraction, sample cleanup, and concentration for biosolid samples.
5. Based on the results from the comparison between SPE methods based on EPA Methods 1633 and the Method UWRL-SPE(W) for PFAS extraction from wastewater, it was determined that either method can be used. It is recommend that EPA Method 1633 be used due to its relevance in emerging EPA directives.

From these comparisons, the compounds M4-PFBA, M2-4:2FTS, M2-6:2FTS, M2-8:2FTS, and M2PFDoA were regularly not meeting the QA/QC criteria of 50-150% ES recovery. This could be due to M4-PFBA and M2PFDoA eluting at the beginning and end of the analytical run which may lead to poor chromatography and a poor standard curve for PFDoA. For M2PFDoA, further work could be done to optimize the retention time and add ES qualifiers which may improve ES recovery. For M4PFBA, ensuring that the LC pumps are purged and the pressure consistent before the run is initiated along with regularly flushing the analytical column may improve the quality of the chromatography at the beginning of the run. For the FTS compounds new qualifiers for these compounds have been provided in EPA Method 1633, the addition of these qualifiers can insure that the correct peak is being analyzed and may improve ES recovery. Additional research

should be conducted on the extraction of FTS compounds from biosolids. The results from these comparisons alludes to potential matrix interference between the biosolids matrix and the FTS compounds. In contrast most of the compounds that elute in the middle of the analytical run consistently passed the QA/QC criteria. ADONA was regularly used in this study as a compound to compare other compound recoveries to as it consistently had linear standard curves and met all QA/QC requirements.

7 Engineering Significance

The further development of PFAS extraction and analytical methods will enhance the research capabilities of the UWRL. In 2023 the EPA established national drinking water standards that will require monitoring of public water supplies and is currently finalizing risk assessments for PFOA and PFOS in biosolids which will determine if PFAS regulations for biosolids are necessary (EPA 2016 and 2024c). This legislation will impact wastewater and drinking water treatment operators, municipalities, and manufacturers, emphasizing the need for further understanding of PFAS fate and transport and effective extraction and analytical methods. The understanding of PFAS in wastewater, biosolids, and compost requires robust methods for identification and quantification of PFAS in these complex media. Accurate and reliable PFAS quantitation will aid in informing land application practices, compost procedures and distribution, and irrigation practices for the communities utilizing these treatment plants to control the risk of PFAS compounds through the use of liquids and solids generated by these plants.

8 Future Work

Future work in optimizing extraction and analytical methods are needed to determine PFAS levels with the sensitivity needed to meet pending EPA regulations. One problem that can occur during solid phase extraction is the clogging of SPE syringes when samples with high suspended solids concentrations are passed through them. In Method 1633 a solution was proposed that uses multiple SPE cartridges for samples with higher amounts of suspended solids.

In the development of a robust extraction method two approaches were tried for cleanup of samples with high suspended solids. One approach is to keep the SPE manifold at a higher vacuum pressure and wait for multiple days for the sample to pass through the cartridge, collecting the solids in the cartridge. An alternative approach is to centrifuge the sample before the SPE process and separate the solids from the liquid sample for separate extraction and PFAS quantification. A comparison between these approaches for extracting samples with suspended solids is necessary to determine which method produces the highest percent recovery of PFAS standards and is most efficient for the laboratory technicians when dealing with high solids content liquid samples.

The fate of PFAS during composting is largely unknown. Tables 1 and 2 show that PFAS are present in biosolids. Class A biosolids are often sold to the public and present potential health risks due to PFAS exposure. Studies with composted biosolids are necessary to develop a greater understanding of the fate of PFAS during composting and to evaluate methods for reducing PFAS concentrations in biosolids

so the potential benefit or hazard of biosolids composting and land application can be better understood.

Further methods will need to be developed for the extraction and analysis of PFAS in soil, plant, and air samples. Air sampling is a particularly challenging media to capture and extract PFAS from. Method development for air sampling was initiated early in this project by using amberlite XAD-2 synthetic ion exchange resin sandwiched between polyurethane foam (PUF) disks (Fig. 16).

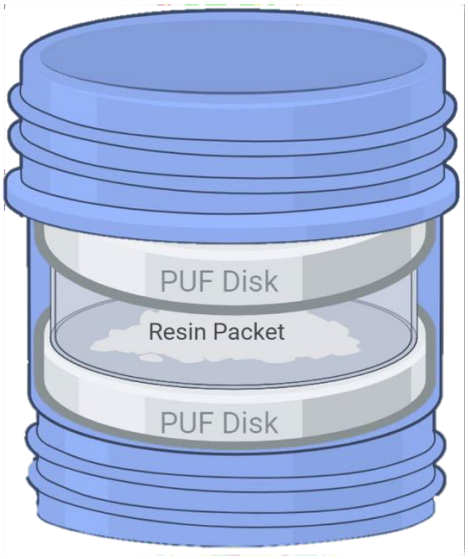


Fig. 16. PFAS air sampling housing configuration

The PUF and resin sandwich was placed in a custom made housing that attached to a Minivol active air sampler (Fig. 17). An impactor containing a quartz filter is screwed on to the top of the housing assembly (Fig. 18). The Minivols were ran for three days, this was based on papers from Wu et al. (2021), Streets and Kvale (2022), and Ahrens et al. (2023). For air sampling the objectives of the research and the

experimental set up are critical in determining sampling length. Due to the developmental state of the determination of aerosolized PFAS there are many approaches to air sampling including the use of active or passive samples, denuders, and different combinations of foam disks and resin. All these variables can influence the sampling schedule, sampler location, and the types of measurements required.

The PUFs were extracted by squeezing them in beakers filled with acetonitrile and then sonicating the PUFs, resin, and filters in acetonitrile. The extract was concentrated to dryness using the Turbovap and reconstituted with acetonitrile. The samples were analyzed with the LC/MS QqQ using acetonitrile as the carrier solvent.

Fig. 17. PFAS air sampling assembly with Minivol active sampler

Fig. 18. Impactor and housing assembly

This method had very poor recoveries of the extraction standards, which was likely due in part to the porosity of the PUFs. An additional challenge is the low concentrations of PFAS present in air samples which are difficult to detect with the LC/MS QqQ. The EPA has recently published test methods for PFAS measurement from air emissions. A new method needs to be developed based on the EPA test methods that can achieve high recoveries of the extraction standards. A reliable PFAS extraction and analytical method for air samples would enable the UWRL to expand research into air sampling with applications in quantifying PFAS emissions from manufacturers, and landfills, and understanding atmospheric PFAS fate and transport.

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APPENDICES

Appendix A

Table A1. Isotopically labeled internal standards, retention times, and MRMs for UWRL 533

Internal Standard (IS)	RT (min) 533	RT (min) 1633	MRM
$^{13}C_3$-PFBA	3.8	3.22	216 -> 172
$^{13}C_2$-PFOA	11.67	7.602	415 -> 370
$^{13}C_4$-PFOS	13.24	8.988	503 -> 80

Table A2. Extraction standards (ES): retention times, MRMs, and corresponding internal standards for UWRL 533

Extraction Standards (ES)	RT (min) 533	RT (min) 1633	MRM	Corresponding Internal Standard (IS)
$^{13}C_4$-PFBA	3.8	3.202	217 -> 172	$^{13}C_2$-PFOA
$^{13}C_5$-PFPeA	5.86	5.03	268 -> 223	$^{13}C_2$-PFOA
$^{13}C_3$-PFBS	6.314	6.05	302 -> 80	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	7.688	5.88	329 -> 309	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	7.877	6.144	318 -> 273	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	8.3	6.45	287 -> 169	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	9.988	6.92	367 -> 322	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	10.088	7.73	402 -> 80	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	11.547	7.34	429 -> 409	$^{13}C_4$-PFOS
$^{13}C_8$-PFOA	11.67	7.608	421 -> 376	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	13.207	8.189	472 -> 427	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	13.241	8.944	507 -> 80	$^{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	14.471	8.483	529 -> 509	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	14.518	8.766	519 -> 474	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	15.661	9.322	570 -> 525	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	16	9.833	615 -> 570	$^{13}C_2$-PFOA

Table A3. Method analytes, retention times, MRMs, and corresponding extraction standards for UWRL 533

Analyte	RT (min) 533	RT (min) 1633	MRM	Qualifier MRM	Corresponding Extraction Standard (ES)
PFBA	3.8	3.2	213 -> 169	NA	$^{13}C_4$-PFBA
PFMPA	4.76	4.066	229 -> 85	229-> 185	$^{13}C_4$-PFBA
PFPeA	5.86	5.028	263 -> 219	263->169	$^{13}C_5$-PFPeA
PFBS	6.3	6.053	299 -> 99	299->80	$^{13}C_3$-PFBS
PFMBA	6.46	5.45	279 -> 85	229->185	$^{13}C_5$-PFPeA
PFEESA	7.1	6.51	315 -> 135	315->69	$^{13}C_3$-PFBS
NFDHA	7.5	6.034	295 -> 201	295->85	$^{13}C_5$-PFHxA
4:2-FTS	7.66	5.876	327 -> 307	327->81	$^{13}C_2$-4:2FTS
PFHxA	7.878	6.125	313 -> 269	313->119	$^{13}C_5$-PFHxA
PFPeS	8.197	7.001	349 -> 99	349->80	$^{13}C_3$-PFHxS
HFPO-DA	8.31	6.446	285 -> 185	285->169 285->167	$^{13}C_3$-HFPO-DA
PFHpA	9.897	6.935	363 -> 319	363->169	$^{13}C_4$-PFHpA
PFHxS	10.06	7.74	399 -> 80	399->99	$^{13}C_3$-PFHxS
ADONA	10.186	7.184	377 -> 251	377->85	$^{13}C_4$-PFHpA
6:2-FTS	11.547	7.346	427 -> 81	427->407	$^{13}C_2$-6:2FTS
PFOA	11.67	7.582	413 -> 369	413->169	$^{13}C_8$-PFOA
PFHpS	11.77	8.362	449 -> 99	449->80	$^{13}C_8$-PFOS
PFNA	13.207	8.215	463 -> 419	463->219	$^{13}C_9$-PFNA
PFOS	13.242	8.969	499 -> 80	NA	$^{13}C_8$-PFOS
9Cl-PF3ONS	14.1	9.398	531 -> 351	531->83	$^{13}C_8$-PFOS
8:2-FTS	14.5	8.49	527 -> 81	527->507	$^{13}C_2$-8:2FTS
PFDA	14.518	8.779	513 -> 469	513->219	$^{13}C_6$-PFDA
PFUnA	15.662	9.309	563 -> 269	563->519	$^{13}C_7$-PFUnA
PFDoA	16	9.815	613 -> 569	613->169	$^{13}C_2$-PFDoA
11Cl-PF3OUdS	16.233	10.483	631 -> 451	631->85 631->199	$^{13}C_8$-PFOS

Table A4. Settings for Agilent 6490 LC/MS QqQ when running EPA Method 1633

LC Parameters			
Column Temp	40°C		
Injection Volume	5 µL		
Mobile Phase	A) 2mM Ammonium acetate in 95% water, 5% acetonitrile B) Acetonitrile		
Gradient flow rate	0.4 mL/min		
Gradient	Time 0 0.2 4.0 7 9 10 10.4 11.8 12.0	%B 98 98 70 45 25 5 98 98 98	Flow rate (mL/min) 0.35 0.35 0.40 0.40 0.40 0.40 0.40 0.40 0.35
MS Parameters			
Gas Temp	230°C		
Gas Flow	11 L/min		
Nebulizer	20 psi		
Sheath Gas Temp	350°C		
Sheath Gas Flow	10 L/min		

Table A5. Settings for Agilent 6490 LC/MS QqQ when running EPA Method 533

LC Parameters			
Column Temp	50°C		
Injection Volume	5 µL		
Mobile Phase	20mM Ammonium acetate in water Methanol		
Gradient flow rate	0.3 mL/min		
Gradient	Time 0 0.5 3 16 18 20	%B 5 5 40 80 80 95	Flow rate (mL/min) 0.30 0.30 0.30 0.30 0.30 0.30
MS Parameters			
Gas Temp	200°C		
Gas Flow	14 L/min		
Nebulizer	20 psi		
Sheath Gas Temp	350°C		
Sheath Gas Flow	7 L/min		

Table A6. Single-laboratory validation performance summary for target compounds and extraction standards (EPA 2024b)

ES Compound	Blank (ng/mL)	Aqueous Matrices			Solid Matrices			Tissue Matrices		
		IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)
Extraction Standard (ES)										
$^{13}C_4$-PFBA	N/A	85-91	1.6	88-108	92-99	1.6	95-109	93-97	1.0	95-105
$^{13}C_5$-PFPeA	N/A	87-95	2.4	84-111	86-106	5.3	80-110	85-108	6.0	89-103
$^{13}C_3$-PFBS	N/A	87-94	2.0	88-110	97-105	1.8	96-109	87-114	6.5	95-106
$^{13}C_2$-4:2FTS	N/A	64-106	12.1	87-137	132-135	0.6	123-145	106-221	17.6	155-291
$^{13}C_5$-PFHxA	N/A	85-92	1.9	83-108	83-101	4.8	92-106	79-111	8.5	88-98
$^{13}C_3$-HFPO-DA	N/A	89-106	4.5	88-121	98-108	2.4	83-125	87-106	4.9	81-106
$^{13}C_4$-PFHpA	N/A	78-100	6.2	83-106	87-102	4.1	90-100	88-93	1.3	80-102
$^{13}C_3$-PFHxS	N/A	83-89	1.9	85-103	92-97	1.4	92-106	92-97	1.4	91-103
$^{13}C_2$-6:2FTS	N/A	93-102	2.2	67-149	118-129	2.3	104-138	87-135	10.8	117-149
$^{13}C_8$-PFOA	N/A	77-98	6.0	84-107	89-101	3.2	92-104	91-98	1.7	86-102
$^{13}C_9$-PFNA	N/A	82-96	3.8	84-107	86-101	4.1	90-106	91-104	3.3	89-101
$^{13}C_8$-PFOS	N/A	78-92	3.9	86-110	87-107	4.9	95-109	87-93	1.6	95-103
$^{13}C_2$-8:2FTS	N/A	99-109	2.5	71-137	96-122	6.1	93-123	179-299	12.5	79-304

$^{13}C_6$-PFDA	N/A	81-98	4.7	84-106	79-101	6.0	89-109	89-104	4.0	90-104
$^{13}C_7$-PFUnA	N/A	84-100	4.4	84-109	84-104	5.4	91-116	84-118	8.4	88-109
$^{13}C_2$-PFDoA	N/A	61-103	12.9	73-101	70-93	7.1	73-106	95-125	6.8	70-108

Appendix B

Table B1. Three-way ANOVA with transformed data among EDGE methods (Factors: compound, amount, and method, response: ES% recovery)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Method	1	241.4976934	241.4976934	398.45	<.0001
Compound	13	392.9442721	30.2264825	49.87	<.0001
Method*Compound	13	169.7632789	13.0587138	21.55	<.0001
Amount	1	3.6172956	3.6172956	5.97	0.0161
Method*Amount	1	11.0239841	11.0239841	18.19	<.0001
Compound*Amount	13	5.1610713	0.3970055	0.66	0.8022
Method*Compou*Amount	13	3.5104712	0.2700362	0.45	0.9490

Table B2. One-sample T-Test for average % recovery of ES compared to 100% for ES extracted from biosolids with two EDGE methods with M4PFBA and all FTS compounds removed

Method	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
CEM		61.5832	57.5293	65.6371	15.6929	13.3018	19.1400
CL		38.0957	36.1024	40.0890	7.7161	6.5404	9.4111
Diff (1-2)	Pooled	23.4875	19.0169	27.9582	12.3654	10.9689	14.1724
Diff (1-2)	Satterthwaite	23.4875	18.9995	27.9755			

Table B3. Two-way ANOVA with transformed data among samples extracted with sonication and EDGE methods with 50-150% recovery QA/QC criteria applied (Factors: compound and method, response: ES % recovery)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Method2	1	0.00046579	0.00046579	93.92	<.0001
Compound2	11	0.00006087	0.00000553	1.12	0.3717
Method2*Compound2	11	0.00003953	0.00000359	0.72	0.7092

Table B4. One-Sample T-Test for average % recovery of ES compared to 100% for ES extracted from sand with the EDGE and sonication methods with 50-150% recovery QA/QC acceptance criteria.

Method2	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
EDGE		66.7596	63.1764	70.3427	10.4309	8.4373	13.6666
Sonication		104.7	96.6634	112.7	22.9919	18.5447	30.2637
Diff (1-2)	Pooled	-37.9261	-46.4644	-29.3878	17.7646	15.1998	21.3789
Diff (1-2)	Satterthwaite	-37.9261	-46.6218	-29.2304			

Table B5. Two-way ANOVA with transformed results for comparison of EPA extraction and SPE methods1633 vs UWRL-SPE(S) for biosolids with 50-150% recovery QA/QC criteria applied (Factors: method and compound, response: ES % Recovery)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Method2	1	2.54959875	2.54959875	138.52	<.0001
Compound2	10	0.58631684	0.05863168	3.19	0.0040
Method2*Compound2	10	0.43588802	0.04358880	2.37	0.0252

Table B6. One-Sample T-Tests for average ES % Recovery compared to 100% for biosolids extracted with EPA Methods 1633 and UWRL-SPE(S) with 50-150% recovery QA/QC criteria applied.

Method2	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
1633		101.3	96.8801	105.8	12.3976	9.9392	16.4824
UWRL-SPE(S)		68.8030	63.0041	74.6018	16.0838	12.8945	21.3831
Diff (1-2)	Pooled	32.5469	25.3708	39.7230	14.3595	12.2169	17.4205
Diff (1-2)	Satterthwaite	32.5469	25.3616	39.7322			

Table B7. Three-way ANOVA with transformed results for comparison of EPA extraction and SPE methods1633 vs UWRL-SPE(W) for wastewater with 50-150% recovery QA/QC criteria applied (Factors: method, location, and compound, response: ES % Recovery)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Method2	1	192794306.8	192794306.8	161.37	<.0001
Compound2	12	99154130.4	8262844.2	6.92	<.0001
Method2*Compound2	12	18693491.7	1557791.0	1.30	0.2326
Location2	1	51872256.4	51872256.4	43.42	<.0001
Method2*Location2	1	3944533.6	3944533.6	3.30	0.0729
Compound2*Location2	12	49053254.8	4087771.2	3.42	0.0004
Method*Compou*Locati	11	14380814.1	1307346.7	1.09	0.3765

Table B8. One-Sample T-Tests for average ES % Recovery compared to 100% for biosolids extracted with EPA Methods 1633 and UWRL-SPE(W) with 50-150% recovery QA/QC criteria applied

Method2	Method	Mean	95% CL Mean		Std Dev	95% CL Std Dev	
1633		85.2993	81.1718	89.4269	16.6576	14.2053	20.1411
UWRL-SPE(W)		107.8	104.1	111.5	15.2797	13.0595	18.4166
Diff (1-2)	Pooled	-22.5228	-28.0243	-17.0212	15.9729	14.2449	18.1818
Diff (1-2)	Satterthwaite	-22.5228	-28.0323	-17.0133			

Appendix C

Table C1. Part and ordering information for materials used in this study.

Part Description	Vendor & Hyperlink	Catalog Number	Additional Information
Polyethylene Tubing for Food and Beverage, Semi-Clear White, 1/16"ID, 1/8" OD, 25 feet long	McMaster-Carr	5384k516	Used to replace PFA tubing in EDGE to reduce PFAS contamination
OasisWAX 30µ:PFAS Analysis 6cc 150mg 300/box	Waters	186009344	SPE Cartridges used for PFAS analysis
SureSTART 2mL Polypropylene Screw Top Microvials	Thermo Fisher	6ESV9-04PP	
9mm Screw Caps	Thermo Fisher	6PSC9STB1	
Methanol LC/MS Optima 4L	Fisher Scientific	A4564	
Acetonitrile LC/MS Optima 4L	Fisher Scientific	AA47138K7	
EPA-533PAR Standards	Wellington Laboratories	533PAR1021	
EPA-533ES	Wellington Laboratories	533ES0623	
EPA-533IS	Wellington Laboratories	533IS0723	
InfinityLab PFC Delay Column, 4.6 x 30 mm	Agilent	5062-8100	Designed to delay elution of PFC compounds released from pump, solvent lines, and solvent
ZORBAX RRHD Eclipse Plus C18, 95Å, 2.1 x 50 mm, 1.8µm, 1200 bar	Agilent	959757-902	Used for EPA Methods 533 and 1633
ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200 bar	Agilent	821725-901	Used for EPA Methods 533,

			537.1, and 1633

Table C2. MRL Data

Compound	MRL (ng/L)
PFBA	-
PFMPA	0.8
PFPeA	2.4
PFBS	-
PFMBA	0.8
PFEESA	0.4
NFDHA	-
4:2FTS	-
PFHxA	0.4
PFPeS	-
PFHpA	0.4
PFHxS	1.6
ADONA	0.4
6:2FTS	-
PFOA	0.4
PFHpS	-
PFNA	0.4
PFOS	-
9Cl-PF3ONS	0.4
8:2FTS	-
PFDA	0.8
PFUnA	2.4
11Cl-PFOUdS	-
PFDoA	-