SUITABILITY OF ELUTRIATION FOR THE

EXTRACTION OF MICROPLASTICS FROM

NATURAL SOILS

By

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Abstract: Microplastics are a class of emerging contaminant which pose serious risks to the health and safety of terrestrial and marine ecosystems. Much research has been conducted regarding the prevalence of microplastics in marine environments; however, the vast majority of microplastics wash into the ocean via freshwater systems where research into the spatial and temporal distribution of microplastics is severely lacking. The lack of research into this field is largely due to the absence of any standardized methodology for the extraction of microplastics from a broad range of soils. Though many processes are available for the separation of plastics from certain constituents of soil, the complex and heterogeneous nature of both soils and plastics makes this process complicated and time-consuming, and not all soils require the same treatments. As such, no widely-accepted methodology is available for the extraction of microplastics from soils, which makes comparisons between studies difficult. This study aims to evaluate if elutriation, a method commonly employed in marine microplastic extraction, is effective for the separation of plastics in a broad range of soils. Five soils of varying composition were collected from freshwater and terrestrial ecosystems in Oklahoma, and were subjected to microplastic extraction with and without elutriation, followed by identical digestion and flotation steps. The number of extracted particles was compared, and linear regression was performed to identify trends between relative particle extraction and soil composition. Multiple strong correlations were observed, indicating that elutriation can be used to increase particle extraction in sandy and silty soils, while clayey and organic-rich soils showed greater particle extraction without elutriation. This suggests that soil composition is indicative of which extraction methodology results in greater yield. The information gathered here will provide other groups with a rapid and inexpensive method for determining the optimal procedure for microplastic extraction from soils, paving the way for a standardized extraction methodology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERATURE

1.1 General information about microplastics

Microplastics are broadly defined as plastic materials with a diameter of less than 5mm, while

plastic particles smaller than 1μm are often considered nanoplastics, though the precise lower

limit is not widely agreed-upon (Birch et al., 2020; Koelmans et al., 2019). Microplastics are a

heterogeneous mixture of particles with varying shape, color, density, chemical composition, and

other characteristics (Hidalgo-Ruz et al., 2012; Koelmans et al., 2019). The densities of most

plastics are dependent on the specific polymer, additives, and stage of degradation, and generally

falls in the range of 0.8-1.4 kg/m^3, though heavier polymers do exist (Hidalgo-Ruz et al., 2012).

Microplastics enter the environment through a variety of sources and broadly fall into the

categories of primary and secondary microplastics. Primary microplastics are plastic materials

that have been purposely manufactured with a size <5mm (e.g. industrial pellets and plastic beads

from exfoliating products) and comprise roughly 9% of the land-based output of microplastics

(Birch et al., 2020; Hidalgo-Ruz et al., 2012). Secondary microplastics on the other hand are

plastic particles formed from the breakdown of larger plastic debris, and include fibers and

fragments from containers, nets, and fabrics (Hidalgo-Ruz et al., 2012). Secondary sources are the

major contributor of microplastics in the environment, as most

1

microplastics are generally fragmented or fibrous in nature, indicating prior breakdown from plastic debris or fabric (Birch et al., 2020; Hidalgo-Ruz et al., 2012).

1.2 Sources and fate of microplastics

Land-based sources account for 80% of the microplastic input into the environment, and urban centers are especially important sources as tire wear and synthetic textiles are major contributors to plastic pollution in the environment (Birch et al., 2020). In addition, exfoliating products and synthetic fibers from clothing are often released into nearby rivers and streams in the effluents of wastewater treatment plants, or are directly applied to land as biosolids (Kay et al., 2018). From this point, plastics are broken down via mechanical weathering and UV degradation as long as they remain suspended in the water column (Koelmans et al., 2019). Once entrained in sediment, however, these plastics are shielded from UV radiation and mechanical abrasion, so it is thought that sediments act as a long-term sink for microplastics (Guo et al., 2020; Quinn et al., 2017). The deposition of microplastics in sediment can be quite substantial, as microplastics can comprise up to 3.3% of mass in highly contaminated beach sediments (Quinn et al., 2017). Furthermore, 94% of marine plastic pollution resides in the seabed, which equates to around 70kg/km^2 (Birch et al., 2020). Once in the environment, microplastics undergo a complex series of interactions which change the physical and chemical properties of the polymers.

1.3 Weathering of microplastics

There are four major mechanisms of degradation of microplastics. The first is mechanical degradation, or macroscopic damage due to externally-applied shear forces (Yousif & Haddad, 2013). The second mechanism is chemical degradation, which includes the interaction of plastics with chemical reagents. In the special case where the chemical reagent is biological in nature, such as an enzyme, the degradation is often referred to a biodegradation (Yousif & Haddad, 2013). The third mechanism is thermal degradation, where chemical changes are brought about

by elevated temperatures without the effects of a chemical reagent (Yousif & Haddad, 2013). The final mechanism of degradation is radiolytic degradation, which is caused by the initiation of free-radical interactions by radiation. In the special case where the radiation is delivered in the form of photons, and generally falling in the infrared to ultraviolet (UV) spectrum, the degradation is often referred to as photodegradation (Yousif & Haddad, 2013). UV degradation is of special concern as the high-energy wavelengths reaching the surface of Earth are often powerful enough to cause significant degradation to most plastics, resulting in yellowing, embrittlement, and a general decrease in the mechanical properties of most polymers (Yousif & Haddad, 2013).

Irradiation by UV light causes a host of damaging effects on plastic polymers, including changes in morphology, chemical composition, and sorption behavior. In terms of morphology, UV radiation induces roughening and cracking of plastic surfaces on the microscopic scale (G. Liu et al., 2019). The chemical changes induced by UV degradation are also significant, as free-radical interactions initiated by reaction with UV radiation, especially when in the presence of gaseous oxygen result in chain scission, cross-linking, and increased surface crystallinity and hardness (Hüffer et al., 2018; G. Liu et al., 2019; Wu et al., 2020); however, the most profound changes occur due to the addition of functional groups to the surface of the microplastics. The addition of functional groups, especially those with double-bonded oxygen atoms, such as ketones, aldehydes, and carboxylic acids, alter the surface properties of the microplastics, as these highly electronegative groups introduce a charge to the surface of the microplastic (O'Connor et al., 2016; Wu et al., 2020; Yu et al., 2020). In general, UV-aged microplastics have greater surface area and are less hydrophobic, which increases environmental mobility as well as contaminant binding (J. Liu et al., 2019).

1.4 Microplastics as an environmental contaminant

Microplastic contamination poses an environmental risk as plastic particles of all sizes pose a risk to organisms at all trophic levels. Large plastic particles may be retained in the gastrointestinal tracts of animals and create blockages, leading to hindered growth or death of the animal (Wright & Kelly, 2017). Small microplastics are of particular concern because of their potential of being consumed by aquatic organisms, and plastics <200nm have the potential to cross epithelial linings of organisms, where they can then induce toxicity to many organs, particularly the liver (Wang et al., 2020; Wright & Kelly, 2017). Ingested UV-aged particles induce even greater toxic effects, causing severe apoptosis and necrosis of liver tissues (Wang et al., 2020). Smaller particles present in soil may be taken up by and build up in plant tissues (Azeem et al., 2021; Zhang et al., 2022). Accumulation of plastic particles has been demonstrated to exert toxicity in plants, delaying germination, inhibiting photosynthesis, and inducing oxidative damage (Zhang et al., 2022). Furthermore, these plants may enter the food chain, subjecting higher-order organisms to concentrated sources of micro- and nanoplastics. Furthermore, microplastics have been observed in the digestive tracts of spotted seal pups (*Phoca largha*), and these microplastics showed signs of recent degradation from digestion (Wang et al., 2021). Specifically, plastic particles observed in the intestines of deceased spotted seal pups were found to be more numerous and smaller in sized compared to those found in the stomach (Wang et al., 2021). The degradation of microplastics in the digestive systems of animals is a major environmental concern as degradation of polymers during digestion would create a pathway for desorption of sorbed contaminates and leaching of plasticizers from the newly-exposed plastic surfaces. Particle size reduction is also concerning as nano-sized plastics can be absorbed across the intestinal epithelium where they can then exert toxicity to the animal (Wright & Kelly, 2017).

Due to the potential environmental impacts of plastic accumulation in soils, it is important to study the composition of these polymers. Despite the ubiquity of microplastics in Earth's

ecosystems, the spatial and temporal distribution of microplastics, which would normally be used to calculate exposure risks, remain largely unkown (Birch et al., 2020). This gap in modern knowledge of these pollutants is largely due to the lack of any standardized sampling, extraction, or quantification protocols with regards to microplastics (Birch et al., 2020). Therefore, a general protocol for the isolation of microplastics from soils is necessary in order to assess the environmental implications of current and future microplastic contamination. Such a protocol must be capable of separating microplastics to reasonably high purity from a complex matrix of sand, silt, clay, and organic matter, while inducing minimal alterations to the physical and chemical properties of the microplastics.

1.5 Characterization and quantification of microplastics

Many tools and technologies are available for the identification of plastic polymers in extracted samples; however, no standardized methodologies have been universally accepted. One of the greatest challenges to the identification of microplastics is the heterogeneous nature of these materials. Microplastics by nature are a complex blend of many different polymers, each with different physical and chemical properties (Koelmans et al., 2019). Additionally, polymers are composed of repeating organic monomers mostly comprised of elements such as carbon, oxygen, hydrogen, and nitrogen, making elemental analytical techniques unhelpful.

1.5.1 Visual inspection

The simplest method for identification of plastics which is commonly employed is visual inspection. This method requires only basic equipment and is by far the cheapest alternative for determining the quantity of microplastics in a sample (Lv et al., 2021). For example, microplastics could be identified in samples using a microscope with only 6x magnification (Doyle et al., 2011). This method is plagued by unreliability and human biases, however, and reports vary as to the accuracy of this method, reporting anywhere from less than 20% up to

100% (Eriksen et al., 2013; Lavers et al., 2016). The unreliability of visual identification

necessitates the validation of particle composition using other objective analytical methods, such

as spectroscopy. For example, in one study, it was found that 70% of identified microplastics

were determined by FTIR spectroscopy to be non-polymer materials (Hidalgo-Ruz et al., 2012).

In general, visual inspection is not a reliable method for the identification and quantification of

microplastics, providing inaccurate particle counts and no information on polymer composition or

mass. At best, this method may be used as preliminary information, which must be validated by

some form of analytic method such as spectroscopy.

1.5.2 Staining and Fluorescence microscopy

A simple, direct method for the counting of particles is though staining, though this method

provides no information regarding polymer identity and must be validated using other analytical

methods. Staining is essentially an attempt to automate the method of visual identification,

replacing visual light with fluorescence imagery and using a program to automatically count

particles. This method works by exploiting the lipophilic nature of most polymers, allowing a

hydrophobic dye to preferentially sorb to the surfaces of microplastics (Joana C Prata et al.,

2019). Once the sample is rinsed of excess dye and irradiated with UV light, the microplastic

particles will fluoresce in a narrow band of wavelengths, allowing the particles to be accurately

counted using an algorithm such as MP-VAT (Joana C Prata et al., 2019). This method has

multiple distinct advantages over visual inspection, namely the removal of most human bias in

counting, as the detection of particles is based mainly on the hydrophobicity of the particles. In a

study investigating the staining and fluorescent properties of 8 different common dyes, it was

determined that a 10µg/mL Nile Red solution with 254nm wavelength displayed the most

consistently-strong fluorescence among the tested polymers, though weak fluorescence was noted

for HDPE, PS, and PVC, and no fluorescence was noted for nylon (Joana C Prata et al., 2019). In

addition, no detectable change in surface chemistry was observed in the dyed polymers under

FTIR analysis (Joana Correia Prata et al., 2019). Furthermore, no fluorescence was observed for natural organic matter using any of the dyes (Joana Correia Prata et al., 2019). The selectivity of dyes for plastics is extremely important, as organic matter shares many of the same properties (e.g. density, chemical composition) with polymers, and requires the use of corrosive chemicals to remove. These steps, which have the greatest potential of any steps of the extraction procedure to induce losses and chemical changes to microplastics, could be reduced because incomplete removal of organic matter should not affect the number of particles observed, provided that no particles are obscured by excess organic matter.

Nile Red staining also has limitations, however, and one major flaw of this method involves the surface chemistry of aged microplastics. As microplastics age, UV-initiated free-radical reactions with oxygen decrease the lipophilicity of polymers over time, leading to weaker interactions between the dye and polymer surface (Joana C Prata et al., 2019; Yousif & Haddad, 2013). The decrease in sorption of the dye to aged microplastic surfaces leads to a general decrease in luminosity of the particles, which could lead to underestimation of aged plastics, particularly PE (Joana C Prata et al., 2019). Because PE comprises the largest fraction of environmental microplastics, with the majority (91%) generated through environmental weathering, the underestimation of aged PE is a major issue for this method of quantification (Birch et al., 2020). Furthermore, this method gives little information regarding mass of plastics present. A rough estimate may be calculated by using the approximate size and number of particles, but because microplastics have various shapes and densities, the results of this method are not necessarily indicative of the real mass of plastics. This method can however give a reliable estimate of the total number of particles in a sample, which can be compared with other studies which use the same methodology.

1.5.3 FTIR and Raman spectrometry

The most widespread and useful tools for the identification and analysis of polymers is Fourier

Transform Infrared (FTIR) and Raman spectroscopy (Cabernard et al., 2018; Xu et al., 2019).

Both FTIR and Raman spectroscopy use electromagnetic waves to excite the bonding electrons in

molecules, and the bending/stretching/vibrating of these bonds can be observed using a detector

(Xu et al., 2019). Once a spectrum is recorded, it can be compared against a library of possible

compounds, allowing for the identification of specific compounds and polymers. The spectra can

also be analyzed to detect specific peaks, which may indicate the presence of specific functional

groups. Both methods have distinct benefits and weaknesses. For example, Raman spectroscopy

may be influenced by fluorescence of plastics, whereas FTIR may be affected by water present in

the sample (Cabernard et al., 2018). In addition, Raman has higher spatial resolution (1μm) than

FTIR (10-20μm). In one study comparing the two methods, Raman was found to identify more

microplastics than FTIR when imagining identical samples (Cabernard et al., 2018). Raman

spectroscopy also identified more unique polymers than FTIR in the same study (Cabernard et al.,

2018). Like other methods, FTIR and Raman spectroscopy give no information regarding mass,

though these methods do have the advantage of identifying specific polymers. Due to weathering

and additives, however, identification of polymers by comparison to pure polymer spectra is

likely to result in low quality matches. The lack of a readily-available spectra library for aged

polymers and commercial polymer blends is a major gap in modern microplastic research.

1.5.4 Pyro-GC/MS

Pyrolysis Gas Chromatography/Mass Spectrometry (Pyro-GC/MS) is a rather more expensive

method for microplastic analysis; however, it may be the least time-consuming and most accurate

method available. Pyro-GC/MS uses a heated chamber to volatilize compounds in an inert

environment (Funck et al., 2020). The volatilized compounds are then sent through a long, thin

coil (or column), where the compounds are separated based on their residence times in the column. The individual compounds can then be identified using a high-energy beam of electrons to excite the bonds in the molecules (Funck et al., 2020). Polymers, however, do not volatilize as they are, but instead break into shorter, simpler molecules, and these "characteristic pyrolysis products" can be detected (Funck et al., 2020). Because each polymer contains unique functional groups and backbones, unique pyrolysis products are given off, and the abundance of these specific products are used as indicators of the original amount of polymer in the sample (Funck et al., 2020). The correlation of these products with the original polymer is typically in the order of $R^2 = 0.83$ up to $R^2 = 0.99$. Perhaps the greatest advantage, however, is the ability of this method to analyze raw samples, with no need for a complicated extraction procedure, which can introduce error and losses into the measurement (Funck et al., 2020). Instead, field samples may be directly analyzed in the pyro-GC/MS, with little to no extraction or pretreatment necessary. Another benefit to this method is that pyro-GC/MS analysis has the ability to measure particle size distribution, something not feasible using FTIR or Raman spectroscopy (Funck et al., 2020).

1.6 Extraction methodologies for microplastics in soils

A variety of methods are available for the extraction of microplastics from soils; these methods work by exploiting the physical and chemical differences between plastics and the major constituents of soils. Specifically, extraction methods must be able to isolate microplastics from a heterogeneous mixture of sand, silt, clay, and organic matter, without altering the properties of the microplastics.

1.6.1 Digestion methodologies

Due to the resistant nature of most polymers, organic matter may be chemically destroyed without significant alteration to the plastics; this process is typically referred to as digestion and is commonly employed in the extraction of microplastics from soils. Many chemicals are available

for digestion of organic matter in soil samples and broadly fall into the categories of acids, bases, and oxidizers. A good chemical for digestion should be able to thoroughly remove organic material in a timely manner, while causing as little alteration to the plastics as possible.

Acidic digestion involves organic matter removal using strong acids. Common acids such as HCl, H_2SO_4, and HNO_3 have been investigated as possible chemicals for the digestion of organic matter; however, the corrosive nature of these acids often leads to the oxidation and destruction of microplastics, particularly for polyamide (PA or nylon) (Pfeiffer & Fischer, 2020). For example, the digestion protocol for organic matrices in marine samples recommended by the International Council for the Exploration of the Sea (ICES) calls for a 4:1 (v:v) mixture of 69% HNO_3 and 70% $HClO_4$ followed by 5 h incubation at room temperature and subsequent heating to 80˚C for 20 min (Enders et al., 2017). This protocol was demonstrated to completely dissolve common polymers such as nylon-6, polyurethane, and black tire rubber elastomer (Enders et al., 2017). In the case of nylon-6, complete dissolution was observed within a few seconds (Enders et al., 2017). Structural damage was also observed in other tested polymers such as acrylonitrile butadiene styrene (ABS), polyvinyl chloride (PVC), and polymethyl methacrylate (PMMA) (Enders et al., 2017). Color leaching was observed in polyethylene terephthalate (PET) and polystyrene (PS), while softening and expansion of polycarbonate (PC) was also observed (Enders et al., 2017). Less potent acids such as 10% HCl and 20% HNO_3 still partially dissolve PA, and do a poor job of eliminating all forms of plant organic matter (Pfeiffer & Fischer, 2020). The same study found plant matter could be nearly completely destroyed using 65% HNO_3 above 40˚C, though this resulted in significant reduction of PA mass (Pfeiffer & Fischer, 2020). 35% HNO_3 at 60˚C for 1 h were also found to destroy PA and fuse PET and HDPE (Catarino et al., 2017).

Bases have been investigated as well, with NaOH and KOH being the most commonly studied. Though alkaline digestion induces less damage to microplastics when compared with acidic

digestion, significant alterations and losses have been observed using NaOH, primarily in PET samples (Pfeiffer & Fischer, 2020). 10 M NaOH at 60˚C for 24 h has also been reported to degrade cellulose acetate (CA) (Dehaut et al., 2016). KOH is a much more gentle alternative, however, and some studies have found this base to induce only negligible changes to most polymers, with the exception of CA (Munno et al., 2018; Pfeiffer & Fischer, 2020). Others, however, have found 10% KOH to induce losses to PVC and PET, while causing yellowing of PA (Dehaut et al., 2016; Karami et al., 2017). KOH may also be poor for digestion of vegetal matter, and at least one study has found little advantage of KOH over DI water alone (Monteiro et al., 2022).

Perhaps one of the most effective methods for digestion used in microplastic extraction is Fenton oxidation, due to its ability to destroy organic matter with little to no alteration to plastics. The reagent is prepared by adding an iron-based free radical initiator (typically $FeSO_4$) to a solution of 30% H_2O_2 and adjusting the solution to a pH of 3 (Lavoy & Crossman, 2021). The reaction of Fenton reagent with organic matter is vigorous and exothermic, and thus may induce alterations and losses of microplastics; however, these effects are highly temperature-sensitive and losses during Fenton oxidation are identical to those losses caused by heating control samples in water (Munno et al., 2018; Pfeiffer & Fischer, 2020), indicating that these losses are due solely to the heat produced by the reaction, and thus may be mitigated by controlling the temperature of the reaction. Indeed, Fenton oxidation, when maintained under 60˚C, has been shown to not significantly alter microplastics, as verified using FTIR and Raman spectroscopy (Hurley et al., 2018; Lavoy & Crossman, 2021; Munno et al., 2018; Pfeiffer & Fischer, 2020). Fenton's reagent also produces a large amount of gas during reaction, which under certain conditions, may cause foaming (Karami et al., 2017). If foaming becomes excessive, it may overtop the container, potentially losing sample and spilling caustic H_2O_2 outside of the sample flask.

Another promising alternative for digestion is NaOCl, or common bleach. 6 hours of digestion in 7% NaOCl at 50˚C was found to be even more effective than Fenton, and no significant changes were noted in any of the tested polymers with the sole exception of PA (Bottone et al., 2021; Monteiro et al., 2022). Another study found no changes to PA or any tested polymers, even at concentrations and temperatures of 10% and 70˚C (Pfeiffer & Fischer, 2020). NaOCl is of particular interest due to its ability to break the strong bonds between functional groups in lignin and cellulose, both of which are often present in field samples and are highly resistant to many chemicals (Monteiro et al., 2022). NaOCl has been shown to outperform even Fenton's reagent in terms of organic matter destruction (Bottone et al., 2021; Pfeiffer & Fischer, 2020)

The most common digestion protocol in published microplastic research appears to be H_2O_2, which was used in 35% of microplastic extractions in a critical review of 20 articles; however, 60% of those studies used no digestion protocol whatsoever, so of the articles that implemented a digestion step, 87.5% used 30% H_2O_2 (Joana Correia Prata et al., 2019). Digestion is an important step in microplastic extraction from soils; however, this method fails to remove soil grains from the solution, therefore another process is necessary for a complete extraction protocol.

1.6.2 Flotation methodologies

One simple and highly effective method for separation of microplastics from sand and silt grains is density separation using a dense brine solution. This method works by suspending contaminated sediment in a solution with a greater density than the plastic particles and lower density than the sand grains. This causes the microplastics to float to the surface while the sediments sink to the bottom, allowing the plastics to be decanted, pipetted, or overflowed off the surface. Several solutes are available for this purpose, each with unique benefits and drawbacks. In general, flotation using a brine solution tends to be quite effective, especially when the brine solution is denser than the plastics of interest. For example, using a saturated NaCl solution (SG =

1.17), PET (SG = 1.38) recovery was roughly 50%; however, using a NaI solution (SG = 1.57), the recovery increases to >95% (Quinn et al., 2017). Despite this, a critical review of 20 articles found NaCl to be the most common solute for separation of microplastics from soils, being used in roughly 65% of articles (Joana Correia Prata et al., 2019).

Separation in brine solution is not instantaneous, and some time is required for separation of plastics and sediment. Many studies use a time interval of 10 min (Konechnaya et al., 2020; Quinn et al., 2017), but this duration is not standardized, with different studies using anywhere between 2 min and 6 h (Hidalgo-Ruz et al., 2012). Regardless, settling time can be reduced and efficiency increased by centrifugation of the samples. Using this method, 94% of plastics can be extracted from sediments with less than 10 min of centrifugation at 3700 rpm, though lower accelerations did not result in much difference in recoveries (Grause et al., 2022). 5 min of centrifugation at 3500 rpm also resulted in 94% recovery of microplastics (Monteiro et al., 2022). Though this process is efficient, dense brine solutions can be quite costly, and heavy solutes such as $ZnCl_2$ and NaI tend to be hazardous to both humans and the environment (Frost, 1959; Stock et al., 2019).

Because flotation of soils in brine solutions does not chemically alter the solutions, it is possible to reuse these solutions multiple times by filtering out suspended particles, though solutions may become discolored after multiple uses (Rodrigues et al., 2020). Still, due to the costs and hazards associated with density separations, it is preferable to minimize the use of these solutions, necessitating the implementation of a safer, more economical form of density separation.

1.7 Overview of elutriation

Elutriation is a method of density separation that works by exploiting the differences in settling velocities between particles. Under discrete settling conditions, particle settling velocity is governed by Stokes' Law, which states that a particle settling in a fluid will accelerate due to

gravity until such a velocity that its drag force balances the gravitational and buoyant forces acting on the particle (Steele & Sterner, 1983). At this point, the particle reaches its terminal settling velocity. For a smooth, spherical particle settling in a uniform fluid medium, the terminal settling velocity, v_s, given by the equation:

$$v_s = \sqrt{\frac{4gd}{3C_D}\left(\frac{\rho_p - \rho_f}{\rho_f}\right)} \quad where \begin{cases} g = gravitational\ acceleration \\ d = particle\ diameter \\ C_D = particle\ drag\ coefficient \\ \rho_p = particle\ density \\ \rho_f = fluid\ density \end{cases}$$

The drag coefficient, C_D, can then be estimated for all flow regimes by inputting the Reynolds number, Re, into the Brown-Lawler Correlation:

$$C_D = \frac{24}{Re}(1 + 0.15Re^{0.681}) + \frac{0.407}{1 + \frac{8710}{Re}}$$

Taking sphericity into account, the Reynolds number is given by:

$$Re = \frac{\psi d v_s \rho_p}{\mu} \quad where \begin{cases} \psi = particle\ sphericity \\ \mu = fluid\ dynamic\ viscosity \end{cases}$$

In a steady, upward fluid stream, the net velocity of a particle from an Eulerian reference frame is equal to the difference between the upward fluid velocity, v_u, and the particle's settling velocity. Assuming positive velocity in the upward direction and negative velocity in the downward direction, the net velocity of the particle, v_{net}, is then given by the equation:

$$v_{net} = v_u - v_s$$

Therefore, if the particle's settling velocity exceeds the upward fluid velocity, the particle will move downward. Likewise, if the particle's settling velocity is less than the upward fluid velocity, the particle will move upward. By exploiting this principle, it is possible to safely,

efficiently, and inexpensively separate heterogeneous mixtures of particles. Due to the low cost

and high extraction efficiency, elutriation has a wide range of uses in various applications.

1.8 Uses of elutriation

1.8.1 Mining and Recycling

Perhaps one of the oldest uses of elutriation is in mineral extraction operations. During the

isotopic study of lead, it is necessary to isolate galena (PbS_2), to relatively high purity,

necessitating the use of time-consuming methods such as superpanning, electromagnetic

separation, and/or hand-picking (Frost, 1959). Alternatively, flotation in a heavy solution could

also be used, though as previously discussed, adequate solutes are typically costly, hazardous, and

corrosive (Frost, 1959). This makes elutriation a promising alternative for extraction of minerals

from the surrounding lighter rock, as the process can be carried out relatively quickly without the

need for hazardous or expensive chemicals (Frost, 1959). Elutriation has also been used in the

recycling industry as a method for recovering non-ferrous metals from automotive scrap (Steele

& Sterner, 1983). After shredding, iron and steel may be easily recovered using a large

electromagnet; however, other useful and recyclable materials such as copper, aluminum, nickel,

zinc, and stainless steel remain in the rejects. In order to recover these metals, the Bureau of

Mines designed an 18-inch-diameter elutriation column in which nonmagnetic shredder rejects

were added to a rising column of water (Steele & Sterner, 1983). This system was able to achieve

99% recovery of the metals, with a purity of around 93%, significantly reducing the labor

required to recover the nonferrous metals (Steele & Sterner, 1983).

1.8.2 Soil fractionation

Elutriation is also a commonly-employed method for fractionating soils. One study, which

investigated the composition of suspended river sediments, used a series of elutriation columns to

extract and fractionate soils based on their settling velocities (Walling & Woodward, 1993).

15

Though this system was capable of fractionating soils into distinct size categories, further analysis of these particles revealed that most of the particles in each size category were composites of smaller particles bound together with clay and organic matter (Walling & Woodward, 1993). Because elutriation exploits the differences in settling velocities between particles, this method is unable to discern between individual particles and aggregated particles. Therefore, without pretreatment to disrupt and thoroughly disperse aggregates, the results of elutriation are not necessarily indicative of the actual composition of the soil, especially for clayey and organic-rich soils. Another study, which attempted to accurately fractionate silt, addressed this problem. Traditionally, little emphasis has been placed on the study of the silt fraction of soils as this fraction is often difficult to fractionate by traditional sieving or pipette methods (Follmer & Beavers, 1973). The fine nature of these particles reduces the efficiency of traditional dry sieving techniques, leading to poor fractionation of silt grains (Follmer & Beavers, 1973). In an attempt to quickly and efficiently fractionate silt particles, this study developed a sequential elutriation system (Follmer & Beavers, 1973). Three conical glass containers were set up in series with increasing diameters as to decrease the upflow velocity in each sequential column. This system allowed for the fractionation of silt grains, and the size ranges of each fraction could be estimated using Stokes' Law. As previously discussed, however, the legitimacy of these results are based on the assumption that particles in the sample settle independently and discretely, and as such, are contingent on the proper dispersion of the soil. In order to achieve sufficient dispersion of the soils, samples were pretreated with 15% H_2O_2 and heated to remove organic matter; then Calgon dispersing agent (a mixture of Na_2CO_3 and $Na_6[(PO_4)_6]$) was added and the sample agitated overnight (Follmer & Beavers, 1973). Sand was removed using a sieve and processed separately, so only the silt and clay fractions were present in the elutriator during operation (Follmer & Beavers, 1973). Using the elutriation method, between 94-99% of silt was recovered, and the percent difference between the elutriation method and the standard pipette method was calculated to be only 3.3% (Follmer & Beavers, 1973).

1.8.3 Nematodes

Elutriation has also been used with great success in the extraction of organisms from soils. In these studies, nematodes must be carefully separated from the surrounding soil in extremely high purity so as to make quantification steps easier (Seinhorst, 1962). In addition, these organisms must be separated from many types of soils, including clayey and loamy textures, which complicate the extraction procedure due to the tendency of these soils to aggregate. These soils therefore required pretreatment to disrupt aggregates, allowing for the discrete settling of particles in the system. Soils were dispersed using a vibromixer, which uses an oscillating plate with conical holes to vigorously agitate water and break apart composite particles (Seinhorst, 1962). Using this method, 500g of clay soil could be dispersed in 750g of water in 7 min, and the extraction efficiency of all soils was also increased by pretreatment with vibromixing. Furthermore, vibromixing was able to disrupt organic matter aggregates formed by organic matter and fungal hyphae without damaging the delicate nematodes (Seinhorst, 1962). The efficacy of the elutriation method was corroborated by another study, which not only found it to be equally as efficient as other methods, but also quicker and easier to perform (Whitehead & Hemming, 1965).

1.8.4 Microplastics

Elutriation has been implemented as a common method for microplastic extraction due to its low cost, high efficiency, and safety. This method was pioneered in 2013 using a simple 15cm-diameter vertical column (Claessens et al., 2013). Water was pumped upward through the column at a constant rate (determined through trial and error), and the lighter microplastics flowing from the top of the column were collected on a 38μm sieve. Elutriation was followed by centrifugation in NaI solution three times, then digestion in various solutions (Claessens et al., 2013). This process resulted in variable recovery efficiencies depending on polymer type and shape. A major

issue with using this method, however, is the use of only a single upflow velocity; due to the variability of natural soil and microplastic particle sizes, there may be some overlap between the settling velocities of the heaviest plastic particles and the lightest sand particles, resulting in the infiltration of sand in the final product. This was addressed in the study by subsequent flotation in NaI solution, allowing the lighter microplastics to be separated from the remaining sand (Claessens et al., 2013). Essentially, elutriation in this protocol works as a rough mass-reduction step, decreasing the scale and complexity of subsequent steps in the extraction. One group attempted to improve on this method by taking a granulometric approach to elutriation (Kedzierski et al., 2016). The idea behind this approach was to reduce the range of possible particle sizes in the column during elutriation, and then performing elutriation in multiple batch processes. To achieve this, the soil was sieved through a stack of progressively finer sieves, and then each fraction was elutriated at a specific flowrate (Kedzierski et al., 2016). With all particles in a narrow size range, the light plastic particles could be easily separated from the heavier sand particles by tuning the upflow velocity through the column for each size fraction. Indeed, this method achieved excellent recovery efficiencies, with >90% of denser polymers being recovered with minimal sand infiltration (Kedzierski et al., 2016). This method was replicated using a smaller glass column which achieved 80% and 72% recovery of PET and PVC respectively (Hengstmann et al., 2018). One major advantage of this method is that sand infiltration may be reduced to such a degree that flotation becomes unnecessary, eliminating the need to use large amounts of NaI or $ZnCl_2$, both of which are comparatively costly and hazardous.

1.9 Research gaps

Currently, few studies have focused on the extraction of microplastics from freshwater sediments. Studies on microplastics in terrestrial systems are so sparse that the research gap has been described as a critical gap in modern research, as most microplastic pollution originates from land-based sources (Birch et al., 2020). These soils, which are often loamy and organic-rich,

present unique challenges compared with coarse sandy soils at every step of extraction. During

elutriation, clay aggregates must be thoroughly dispersed in order to free microplastic particles

from the surrounding matrix. Often this aggregation presents a challenge when attempting to

fractionate clayey soils accurately, and attempts to accurately fractionate clayey soils using

successive elutriation columns have reported inaccurate results due to the aggregation of clays

(Walling & Woodward, 1993). In addition, silty soils contain a significant fraction of soil grains

smaller than 0.05mm in diameter. These particles are small enough that settling velocities of such

particles may overlap significantly with microplastic particles of larger diameters, leading to

infiltration of silt and smaller sand particles in the final product.

1.10 Conclusion

Microplastics are an emerging contaminant which necessitate a great amount of further research.

The majority of these contaminants wash into ecosystems from urban centers, though the sources

are numerous and diffuse, complicating the process of modeling fate and transport processes. The

relative lack of understanding of fate and transport of microplastics is largely due to the lack of

standardized extraction and quantification methodologies, which makes direct comparisons

between studies difficult. Notably, the methodologies required for the extraction of microplastics

from a broad range of soils remains a critical gap in modern research, and as such, no

standardized methodology for extracting microplastics from soils currently exists. Currently,

many individual methodologies are available for separating microplastics from specific

constituents of soil (e.g. digestion, flotation, elutriation); however, the exact combination and

specific details of each step are not standardized, nor is it certain which protocol or protocols

would be most effect over a broad range of soils.

Because microplastics and soils are both heterogeneous mixtures of components with different

properties, it is likely that no single methodology will be optimal for all soils. As such, in order to

achieve the greatest extraction efficiency, a combination of methods will need to be tailored for each individual soil. In order to save crucial time, money, and resources, it is preferable that this extraction methodology can be determined without performing multiple extractions using various methods. Therefore, the effect of soil constituents (e.g. clay, silt, sand, organic matter) on the extraction of microplastics requires further study. If any correlations between soil constituents and extraction methods exist, it would allow for the rapid and precise selection of an optimal extraction protocol based on simple, readily-obtainable soil parameters.

CHAPTER II

SUITABILITY OF ELUTRIATION FOR THE EXTRACTION

OF MICROPLASTICS FROM NATURAL SOILS

Abstract

This study evaluates if elutriation, a process commonly employed in the extraction of

microplastics from marine soils, improves the extraction of microplastics from soils with varied

textures. Microplastic pollution in marine environments has been extensively studied in modern

literature; however, information about the prevalence of microplastics in freshwater and terrestrial

systems is lacking. Up to 80% of marine microplastic pollution comes from land-based sources

and washes into oceans through freshwater systems; thus, understanding of microplastic

contamination of freshwater systems is of great importance to the larger field of microplastic

research. The lack of published literature regarding the extent of microplastic contamination of

terrestrial ecosystems is in part due to the lack of any standardized extraction methodology for

microplastics from high-clay and organic-rich soils. In this study, we obtained five samples with

textures varying from, 0.1-8.6% organic matter, 16.7-96.3% sand, 2.5-45% silt, and 1.3-50.0%

clay and compared the extraction efficiency of an extraction process that included elutriation with

a typical direct digestion process. All soils were subjected to microplastic extraction with and without elutriation. This was followed by digestion in 7.5% NaOCl, then flotation in 5.1M $ZnCl_2$ solution. The number of particles was quantified through staining and fluorescence microscopy. The amount extracted varied from 3,300-188,900 particles/kg in elutriated soils and from 3,600-153,700 in direct digested soils. Linear regression was used to determine the relationship between relative number of particles extracted (with elutriation vs without elutriation) and combinations of soil parameters. Multiple correlations were observed, suggesting that elutriation may be effective as an extraction step for high-sand and silt soils, but should be avoided for high-clay and organic matter soils. Further work requires validation of plastic composition of the particles using Attenuated Total Reflectance – Fourier Transformed Infrared spectroscopy and quantifying the amount of remaining metals and salts using Inductive Coupled Plasma Mass Spectroscopy and Emission Dispersive X-ray spectroscopy. The information gathered in this study should help other groups in determining the extent of microplastic contamination in terrestrial ecosystems by providing viable extraction methodologies for a range of soil types. A rigorous understanding of microplastic prevalence in the environment will aid in determining the impacts associated with microplastic contamination.

2.1 Introduction

2.1.1 Background

Microplastics are broadly defined as plastic polymer particles with a diameter in the range of 1-5000μm and fall into two distinct categories based on their origins (Koelmans et al., 2019). Primary microplastics are polymer particles manufactured to a size less than 5mm in diameter and include items like exfoliating products and plastic pellets used in the manufacture of larger plastic items (Koelmans et al., 2019). Secondary microplastics are polymer fragments and fibers generated from the breakdown of larger plastic debris in the environment (Koelmans et al., 2019).

Secondary microplastics comprise the overwhelming majority of plastics and are estimated to make up approximately 91% of land-based plastic output (Birch et al., 2020). These secondary microplastics enter the environment through various mechanisms and processes. There are many processes which generate microplastics; however, the most dominant mechanisms are mechanical abrasion and UV radiation. Mechanical abrasion is the simplest and most direct method of microplastic generation. Common examples include wear of tires and brake pads, as well as fibers generated from machine washing of clothing (Zhang et al., 2021). Mechanical abrasion is possibly the most common route for introduction of microplastics into the environment, as tire wear particles and synthetic fibers comprise 29% and 34% of marine microplastics respectively (Boucher & Friot, 2017). Another important vector for degradation of plastics in the environment is by exposure to UV radiation. UV radiation from natural sunlight is powerful enough to cleave bonds between atoms in polymers, initiating free radical interactions between the polymer and gaseous oxygen (O'Connor et al., 2016). Photoinitiation of free radical reactions in polymers causes surface oxidation, as well as a decrease in the mechanical properties of the polymers, such as embrittlement, cracking, pitting, and discoloration (G. Liu et al., 2019; O'Connor et al., 2016; Wu et al., 2020). The deterioration of mechanical properties can lead to production of microplastics directly or may exacerbate microplastic generation by other mechanisms, such as mechanical abrasion.

Once in the environment, the distribution and transport of microplastics is not well understood. Though microplastics are present in nearly every location on Earth, the spatial and temporal distribution of microplastics, which would typically be used to determine exposure risks, are not well known (Birch et al., 2020). The gap in modern knowledge of microplastic distributions is mostly attributable to a total lack of any standardized protocol for extraction, identification, and quantification of these contaminants (Birch et al., 2020).

Many methodologies have been developed for the identification and quantification of microplastics; however, no standardized methodology currently exists. Comprehensive reporting on the prevalence of microplastics generally requires two separate measures: quantification and characterization.

2.1.2 Extraction and analytical techniques

Quantification is simply a measure of the quantity of plastics in the sample, and is often reported in terms of pieces/kg or mg/kg. Both of these metrics have drawbacks, for example, knowing the total mass of microplastics in a sample gives no information on the particle size distribution, which may affect toxicity to organisms (Wang et al., 2020); however, knowing the number of particles in a sample gives no information regarding the total amount of microplastics present. One very precise method to obtain plastic mass is pyrolysis-gas chromatography/mass spectrometry (pyro-GC/MS). This method allows for the quantification of plastic mass, as well as the particle size distribution, with little to no prior treatment of samples (Funck et al., 2020). Pyro-GC/MS comes with the drawback of extreme equipment cost, which many labs would struggle to afford. Another common method for quantification of particles is by Nile Red staining coupled with fluorescence microscopy (Joana C Prata et al., 2019). The particles in these collected images can then be counted using an algorithm such as MP-VAT (Joana C Prata et al., 2019). Of course, neither of these methods gives any information regarding the specific polymers found in a sample.

Characterization is an important step in microplastic analysis as different polymers each have a variety of different physical and chemical properties, all of which may affect their impact on the environment (Hidalgo-Ruz et al., 2012). In addition, properties such as surface morphology and chemistry are important in determining the transport of these polymers through the environment, as well as their toxic effects on organisms (J. Liu et al., 2019; Wang et al., 2020). The most

commonly applied methods for identification of microplastics are Fourier transform infrared

(FTIR) spectroscopy and Raman spectroscopy (Cabernard et al., 2018; Xu et al., 2019).

2.1.3 Extraction methodologies

The most common methods for eliminating organic matter from samples involve incubation in an

oxidizing solution at elevated temperatures. Thus far, the most commonly applied method for

digestion is by using 30% H_2O_2 (Joana Correia Prata et al., 2019). This method is more effective

for destroying organic matter than many acidic and basic treatments, while also leaving plastics

mostly unaltered chemically (Hurley et al., 2018; Pfeiffer & Fischer, 2020). H_2O_2 can be further

activated by addition of $FeSO_4$ and dilute H_2SO_4 to create what is commonly known as Fenton's

reagent (Hurley et al., 2018). Though effective, Fenton's reagent is exothermic and evolves a

large amount of CO_2 and O_2 gas. The heat from this reaction can damage plastics, and the rapid

gas production can lead to foaming if not carefully controlled. A promising alternative, however,

is NaOCl, which displays superior qualities for organic matter destruction (Bottone et al., 2021;

Pfeiffer & Fischer, 2020). At the current moment, less information regarding the efficacy of

NaOCl for digestion is available compared with Fenton's reagent, though comparisons of the two

show superior organic matter destruction for NaOCl, with little alteration to plastics (Bottone et

al., 2021; Pfeiffer & Fischer, 2020).

Flotation in a brine solution allows for the easy separation of microplastics from sand and silt

grains. Flotation is commonly used as a final step to clean up extracted microplastics and involves

suspension of soil in an aqueous salt solution, whereby lighter microplastics can be decanted from

the surface after a period of settling (Koelmans et al., 2019). The most commonly used solute for

this method is NaCl which can obtain a specific gravity of 1.2, lower than common polymers

such as PVC and PET (Joana Correia Prata et al., 2019). Other common solutes such as $ZnCl_2$

allow for specific gravity well in excess of 1.4; however, such solutes are often expensive and

corrosive (Frost, 1959; Joana Correia Prata et al., 2019). Fortunately, solutions of $ZnCl_2$ can be filtered and reused multiple times if proper care is taken to maintain the specific gravity of the solution (Rodrigues et al., 2020). The nature of these solutes necessitates a safer density separation alternative

2.1.4 Elutriation

Elutriation is the process of separating mixtures of particles using an upward fluid stream. The principle works by exploiting differences in particles' settling velocities. For discretely settling particles, this process is governed by Stokes' Law, which states that a particle settling in a fluid medium will accelerate to such a velocity that the drag force balances the gravitational and buoyant forces. At this point, the particle reaches its terminal settling velocity which is determined by the properties of the particle and the fluid medium (Frost, 1959). By suspending a heterogeneous mixture in an upward fluid stream, the upward velocity can be tuned to such a value that particles can be effectively separated from one another based on density, shape, and size (Frost, 1959). This method has been applied in many unique areas of research and industry.

Elutriation has been implemented as a method for extraction of materials from soils across many different fields. The use of elutriation has been implemented to separate heavier minerals from lighter rock in mining operations, as well as to separate metals from plastic scrap in automotive recycling (Frost, 1959; Steele & Sterner, 1983). Elutriation has also been employed to great success in the extraction of nematodes from soils, though the properties of certain soils lead to some difficulties with the extraction process. Loamy and clayey soils tend to aggregate, entrapping nematodes in the soil matrix and thereby decreasing the efficiency of extraction (Seinhorst, 1962). This problem is easily solved however by pretreatment through sonication or vibromixing to thoroughly disperse clay aggregates, allowing for the free, discrete settling of particles (Seinhorst, 1962).

More recently, elutriation has been implemented to separate microplastics from soils. This method was pioneered in 2013 using a simple, 15-cm diameter vertical column elutriator (Claessens et al., 2013). The upflow velocity was optimized through experiment, and elutriation was followed by centrifugation in NaI solution and digestion in different chemicals (Claessens et al., 2013). This method was improved on by fractionating soils prior to elutriation then running each mass fraction at a unique flowrate, determined through experiment (Kedzierski et al., 2016). This method was able to achieve 90% recovery of heavier polymers, without using heavy brine solutions (Kedzierski et al., 2016). The method was replicated using a small glass column, and was able to achieve 72% recovery for PET (Hengstmann et al., 2018). Elutriation is clearly a promising method for the rapid, effective, and safe extraction of microplastics from soils; however, no research thus far has investigated the extraction of microplastics from clayey soils using elutriation.

2.1.5 Objectives

Given the prior success in implementing elutriation in marine sediments, the objective of this study is to investigate whether or not elutriation is a viable alternative for the extraction of microplastics from soils of varying composition, and to correlate the effectiveness of elutriation with readily-measurable soil constituents. In doing so, microplastic contamination can also be evaluated in Oklahoma's terrestrial ecosystems.

2.2 Materials and Methods

2.2.1 Reduction of contamination

Prior to usage, all materials were sonicated for 30 minutes in DI water. Plastic usage was kept to a minimum, and whenever possible, procedures were performed in a laminar flow cabinet. Samples were handled in a clean lab while wearing 100% cotton lab coats.

2.2.2 Location and Procedures

Four soils of varying texture and organic matter content were selected from local sources

including creeks, rivers, and applied biosolids. The first sample labeled "Neighborhood" was

taken from the Arkansas River in Tulsa, OK. The next sample labeled "Couch Park" was

obtained from Boomer Creek in Couch Park located in Stillwater, Oklahoma. Another sample,

labeled "Biosolids" was obtained from a field containing applied biosolids sourced from the

Stillwater, Oklahoma Wastewater Treatment Plant. The last sample, labeled "Yard Grill" was

obtained from the Arkansas River near the Yard Grill restaurant in Tulsa, Oklahoma. Each

sample was obtained using a shovel to sample the top 10cm of soil just above the water line. An

arbitrarily large sample mass was collected and placed in a clean bucket, then labeled and sealed

until further processing. Coordinates for all samples are given in Table 1.

2.2.3 Soil preparation

An arbitrarily large mass of each soil was spread out onto a sheet of aluminum foil, and then

placed into an oven at 50˚C. This temperature was selected in order to avoid affecting the

composition of potential microplastics in the samples. Oven vents were fully opened, and allowed

to dry for 24 h. Samples were then removed from the oven and manually worked through a 12-

inch-diameter, 4.75 mm sieve to break up aggregates and remove large rocks and plant debris.

Non-clayey samples were typically dried at this point; however, clayey samples were placed back

into the oven at 50˚C for another 24 h. Once dried, samples were weighed out into 10g samples.

Samples were stored in glass beakers and capped with aluminum foil. Samples were then labeled

with name, date, and description and stored in a refrigerator prior to elutriation.

Roughly 300g of prepared soil from each sample was also sent to Oklahoma State University's

Soil, Water, and Forestry Analytical Laboratory (SWFAL) for a soil texture analysis. This

provides the percentage of clay, silt, and sand as a percentage of the mineral fraction of the soil.

Due to the lack of nearby available clay soil, 200g of the Biosolids sample was separated from the rest of the sample, and 70g of lab-grade bentonite clay (Spectrum) was added to the sample to increase the clay percentage. DI water was added to the mixture, and the damp soil was kneaded by hand until thoroughly homogenized. The soil was then dried and sieved as previously discussed.

Separately, 10g samples of each soil were weighed out in triplicate in small aluminum dishes. The soil was then placed into an oven at 105˚C for 24h. The masses of each soil were recorded, and then placed into an oven at 550˚C for 30min. The masses of each soil were again recorded, and the percentage of organic matter calculated.

2.2.4 Soil disaggregation

Each 10g sample was added to a 100mL glass beaker. 50mL of DI water was then added to achieve a mass-to-volume ratio of 1g dry soil to 5mL DI water. The mixture was allowed to sit overnight in order to thoroughly wet and soften soil aggregates. A probe-type sonicator was lowered into the water to roughly half the depth of the water and centered in the beaker. At 20% power, 400J were applied for every 1g of dry soil. Once sonicated, the probe was lifted out of the sample and rinsed into the beaker with a wash bottle of DI water.

2.2.5 Elutriation

Each sonicated soil sample was subjected in triplicate to one of the following methods:

Direct Digestion (Control): Sonicated samples were each thoroughly rinsed in a 45μm sieve.

Glass Column Elutriatior: Sonicated samples were added to a glass elutriation column (Diameter = 5cm, Height = 30cm) and elutriated into a 45μm sieve at an upflow velocity of 1.3cm/s for 15min. The sonicated samples were added while the column was in the process of filling, and the time was recorded from the point at which all sample had been added to the column.

Large Column Elutriator: Sonicated samples were added to the mixing container (Volume = 2L, Hydraulic Retention Time = 2.4min) and elutriated into a 45μm sieve at an upflow velocity of 1.3cm/s for 15min. The flow to and from the mixing container was set at steady-state prior to operation, and the elutriator (3 identical columns in parallel: Diameter = 10cm, Height = 1m) was allowed to flow continuously for several minutes prior to addition of the sample. Time was recorded from the point at which all sample had been added to the mixer.

All samples were rinsed three times from the 45μm mesh into a 250mL beaker and capped with aluminum foil until further processing. Every sample was subjected to the following steps:

2.2.6 Digestion

A 47mm vacuum filtration apparatus was assembled with a 20μm stainless steel mesh filter. The elutriated sample was then poured into the filtration apparatus, and the beaker was thoroughly rinsed into the filtration apparatus 3 times with DI water to minimize losses. Once fully filtered, the funnel and mesh were rinsed into a 250mL Erlenmeyer flask using a rinse bottle containing 7.5% NaOCl. The Erlenmeyer flask was then filled to 50mL with 7.5% NaOCl, and the flask was capped with aluminum foil and labeled with name, date, and description. The sample was then placed into an orbital incubator at 50˚C and 300rpm for 24h.

2.2.7 Flotation and Filtration

After digestion procedures, a 47mm vacuum filtration apparatus was assembled with a 20μm stainless steel mesh filter. The digested sample was then slowly poured into the filtration funnel and the flask rinsed into the filtration apparatus three times with DI water. Occasionally, the mesh would clog, however, when this happened, the liquid was allowed to filter, then the film overlaying the mesh was disrupted using a wash bottle of DI water. When the mesh was clean, more sample was added, and this process repeated until all sample was filtered. Once completely filtered, the sample was rinsed one more time with DI water to remove the remaining NaOCl. The

30

funnel and mesh were then rinsed into a small beaker using a wash bottle of 5.1M (SG = 1.5)

$ZnCl_2$ solution. Care was taken to minimize the volume of $ZnCl_2$ solution used. The contents of

the beaker were then rinsed into 15mL polypropylene vials (Falcon). Each vial was then vortexed

until the soil was thoroughly dispersed. The vials were then placed in a centrifuge at 4900rpm for

10 min. A 47mm vacuum filtration apparatus was assembled with a 20μm stainless steel mesh

filter. The vials were removed from the centrifuge, taking care to not mix or invert the tubes. The

supernatant of each Vial was then decanted onto the filtration apparatus. The tubes were then

refilled to 10mL using 5.1M $ZnCl_2$ solution, vortexed until thoroughly dispersed, then centrifuged

at 4900rpm for 10 min. The supernatant was again decanted onto the 47mm vacuum filter. The

Vials were again filled to 10mL, centrifuged, and decanted once more for a total of 3 times. The

filtered sample was then thoroughly rinsed with DI water. A 13mm vacuum filtration apparatus

was then assembled with a 25mm, 0.2μm aluminum oxide membrane. The 20μm mesh and 47mm

funnel were then rinsed into the 13mm apparatus with DI water, and the sample allowed to filter

completely. The walls were then rinsed with DI water to ensure the sample is retained on the

aluminum oxide membrane and not the filter funnel. The apparatus was then carefully

disassembled, and the aluminum oxide membrane placed in a glass petri dish and labeled with

name, date, and description. The filtered $ZnCl_2$ solution was then emptied into a waste container

and sealed for filtration and reuse.

2.2.8 Quantification and Characterization

Three to four drops of 2μg/L Nile Red-methanol solution were placed onto the extracted

microplastics such that the microplastics were thoroughly wetted. 10 min were allowed for

staining of the microplastics then the filter was carefully transferred to a 47mm glass frit filter

unit with no funnel. The vacuum was turned on and 300μL of hexanes were used to rinse the

excess nile red dye from the filter. This was achieved by dropping the hexanes around the

perimeter of the filter in such a way as to not disrupt the microplastics. Once dry, the filter was

then transferred to a 6-well Greiner plate, and capped with aluminum foil to avoid the disturbance of microplastics due to static charge on the acrylic plate lid. Each filter was then imaged using fluorescence microscopy (Cytation 5 Imager), and the images of the filters exported as PNG files at 1:1 pixel resolution. The particles in each image were then counted using ImageJ-MPVAT (Joana C Prata et al., 2019). Based on the quantity of particles observed, denoted by n, the following equation gives the number of particles to be analyzed using FTIR-ATR:

$$Particles\ analyzed\ with\ FTIR\ ATR = \begin{cases} n\ for\ n \leq 5 \\ 5\ for\ 5 < n \leq 50 \\ 0.1n\ for\ 50 < n \leq 300 \\ 30\ for\ n > 300 \end{cases}$$

The average number of particles and standard deviation for each flow regime and soil was calculated, and then t-test was then used to check for statistically-significant differences between flow regimes.

2.3 Results and Discussion

2.3.1 Soil characteristics

Table 1: Sampling locations with soil texture and organic matter percentage

| Sample	Coordinates	Texture			Organic Matter
	(Latitude, Longitude)	% Clay	% Silt	% Sand	% Organic Matter
Neighborhood	35.9969810532°N, -95.9432463466°W	1.3	2.5	96.3	0.1
Yard Grill	36.1042630780°N, -97.0421747433°W	8.8	6.3	85.0	2.4
Couch Park	36.0911311334°N, -97.0197627590°W	16.3	28.8	55.0	5.1
Biosolids	36.1340376518°N, -95.9925524560°W	32.5	45.0	22.5	8.6
BS-50	36.1340376518°N, -95.9925524560°W	50.0	33.3	16.7	6.0
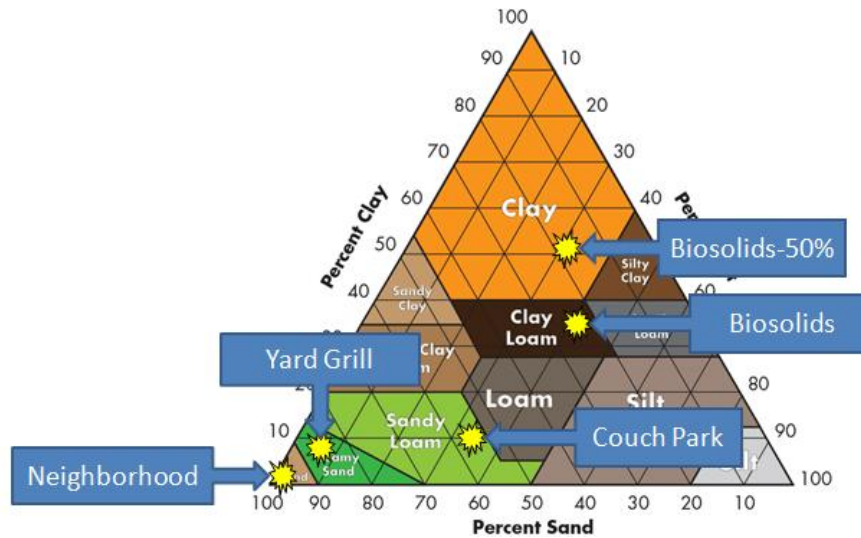
Figure 1: Soil texture of each sample overlaid on the soil texture triangle

The five soils differed greatly in organic matter content. Neighborhood had the lowest organic matter at only 0.1%, while Biosolids had 8.6%. Yard Grill and Couch Park fell in between these two samples, at 2.4% and 5.1% organic matter, respectively. The impact of different organic matter compositions (e.g. lignin, cellulose, hemicellose, etc) and size fractions (e.g. plant organic matter, mineral-associated organic matter, etc) was not investigated, though different types and degrees of decomposition likely will impact the way in which organic matter interacts with other soil constituents.

The five soils also greatly differed in terms of soil texture (Figure 1). The Neighborhood sample had the lowest clay and silt content, and was classified as "sand". Yard Grill was classified as "loamy sand" and had higher clay and organic matter content compared with the Neighborhood sample. Couch Park had nearly double the clay content of Yard Grill and much higher silt content. Couch Park was classified as a "sandy loam" though this soil was very near the threshold for "loam" soil. Biosolids had higher clay and silt fractions, and was classified as a "clay loam" and very nearly "silty clay loam". The Biosolids-50% sample had the highest clay content, with lower sand, silt, and organic matter than the regular Biosolids sample. The Biosolids-50% sample

was classified as a "clay" soil. It should be noted that though a texture analysis was performed, the particular clay minerals present were not determined, which may have an impact on the interactions between clay and other soil constituents as well as microplastics. The soil grain size distribution was also not determined, which could impact the settling characteristics and passing fraction during sieving. Both of these characteristics should be investigated in future research.
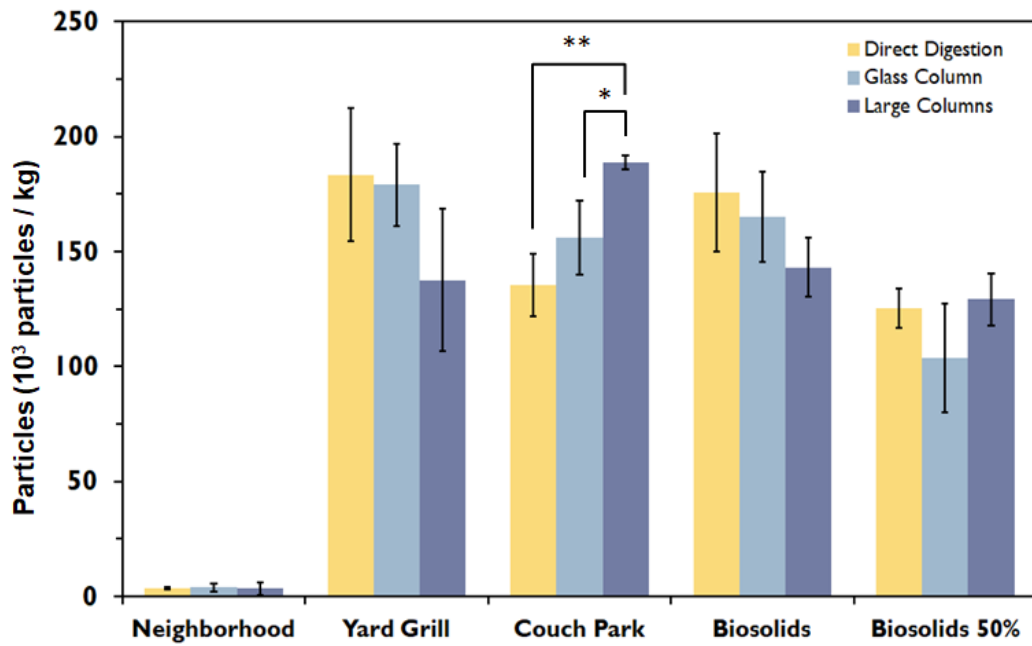
2.3.2 Abundance of microplastics in samples

Figure 2: Average particles/kg for three extraction methods performed on five different soil samples. Error bars show ± 1 standard deviation. (* denotes $p < 0.05$; ** denotes $p < 0.01$).
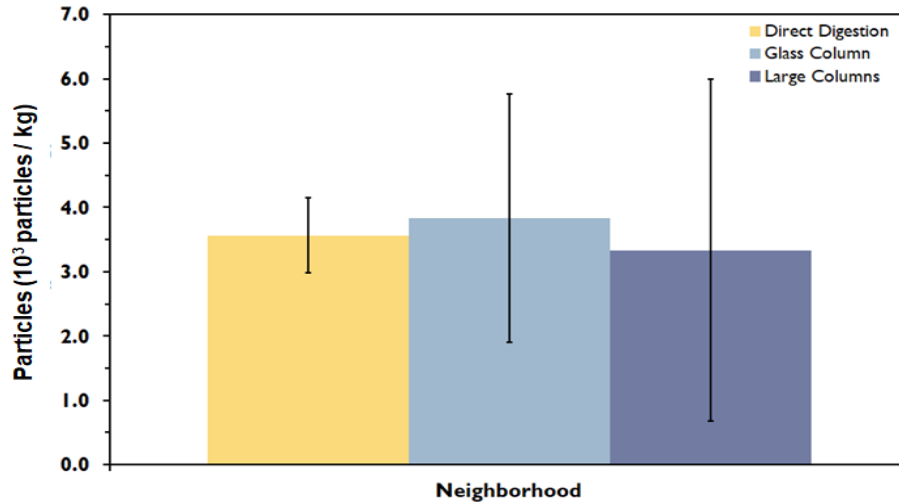
Figure 3: Average particles/kg for three extraction methods performed on Neighborhood soil sample. Error bars show ±1 standard deviation.

2.3.3 Comparison of extraction methods

No statistically significant differences were observed between the direct digestion method and glass column methods (Figure 2). In most samples, the direct digestion method resulted in greater particle recovery compared with the glass column elutriator, with the exception of the Neighborhood and Couch Park samples. The Couch Park sample is a notable outlier as it showed a modest improvement over the direct digestion method ($p = 0.085$).

2.3.4 Correlation of soil parameters with relative particle extraction

Comparing the amount of particles observed in the glass column and direct digestion methods, a trend was observed with clay content (Figure 4a). In general, elutriation resulted in a greater number of extracted particles from samples with lower clay contents, whereas direct digestion was more effective for samples with higher clay contents. The point at which direct digestion and elutriation are equally effective is at 19.4% clay, meaning that soils with higher clay content will yield more particles using direct digestion, and soils with lower clay content will yield more

particles using pretreatment with elutriation. This relationship can possibly be explained by two phenomena. Elutriation is more effective at removing large sand particles, so for coarse, sandy soils (and therefore lower-clay soils), elutriation will result in greater sample mass reduction, decreasing the amount of bulk soil in subsequent steps, and thereby reducing interference in the extraction procedure. In clayey soils, however, not only is there less sand to be removed, but clay may form flocs which could entrain microplastics, causing them to sink in the column (and therefore will not be recovered). If flocculation is decreasing the efficiency of elutriation, then organic matter should also have an effect on efficiency as both clay and organic matter interactions play a major role in particle agglomeration.

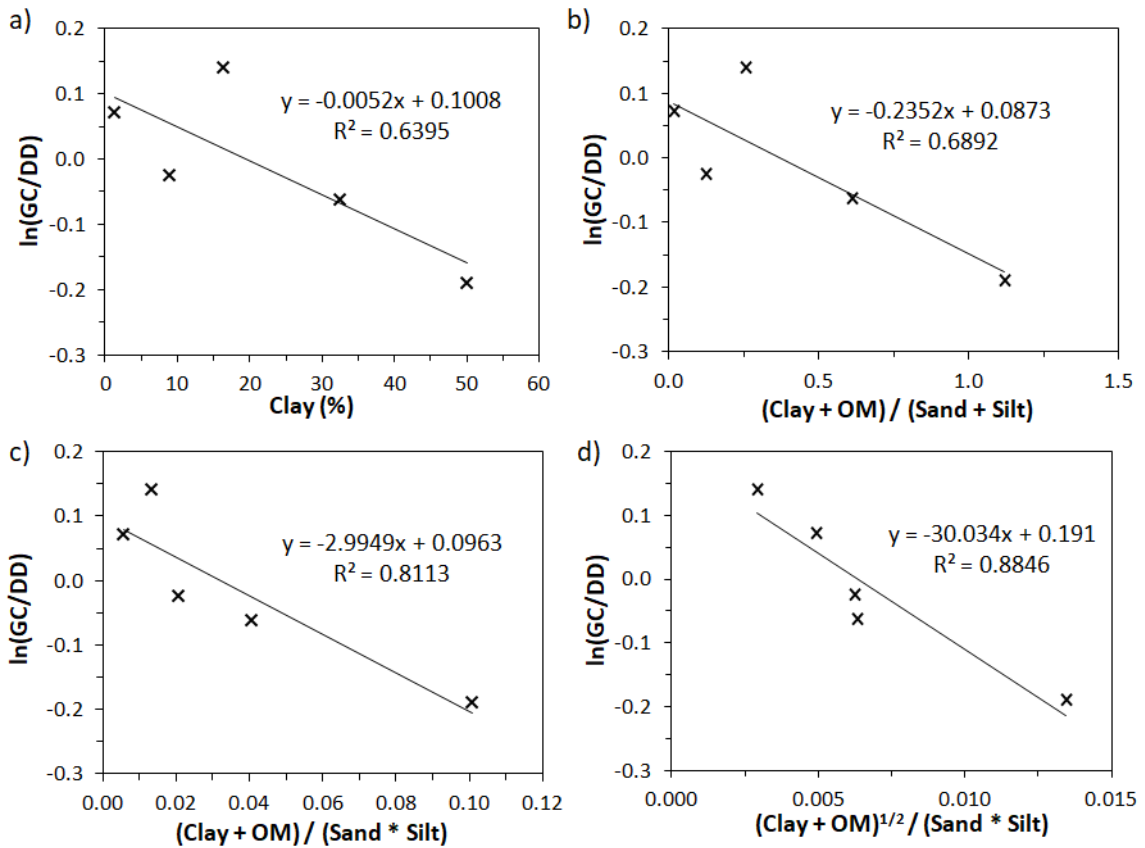Figure 4: Relative particles extracted (expressed as ln(glass column (GC) / direct digestion (DD))) as a function of: a) clay content, b) (clay + organic matter (OM))/(sand + silt), c) (clay + OM)/(sand * silt), and d) (clay + OM)$^{1/2}$/(sand * silt).

Considering multiple major constituents of soil (clay, silt, sand, and organic matter), a better correlation can be drawn (Figure 4b). As can be deduced from Figure 4b, clay- and organic-rich soils yield more particles using the direct digestion method, whereas sand- and silt-rich soils yield more particles using pretreatment with elutriation. Using this more precise correlation, the point at which the two methods result in equal recovery lies at the point where the equation on the x-axis equals 0.371, with higher values favoring the direct digestion method and lower values favoring pretreatment with elutriation. In this correlation, all terms are given equal weight, and the equation represents a simple ratio of active components (clay and organic matter) to more inert components (silt and sand).

The relationship in Figure 4c demonstrates a similar concept to Figure 4b, however, the multiplication of sand and silt suggests two separate mechanisms which each work to alter extraction efficiency. For example, a high abundance of silt and sand may disrupt particle flocculation, increasing elutriation efficiency, while sand also contributes to sample mass reduction as previously discussed. Silt may also help to chauffeur microplastics from the column as the rapid efflux of silt from the column produces a reverse-settling effect. Alternatively, high silt and sand content may contribute to blanket settling of particles during flotation. In addition to the interference caused by excess sand and silt, a more even mix of sand and silt may indicate a more well-graded soil, which due to the lower pore space and greater shear strength (Li et al., 2021), could be more likely to capture and entrain particles compared with a poorly-graded soil. Elutriation prior to this step would selectively remove the heavier sand grains, resulting in a more poorly-graded soil. If soil gradation plays a role in extraction efficiency, a composite parameter such as coefficient of uniformity may be more predictive of elutriation efficiency than sand and silt contents. In this case, grain size distribution would be an important tool for determining the optimum extraction procedure for soils.

Figure 4d shows a similar relationship to Figure 4c, however with diminishing effects on elutriation performance as clay and organic matter contents increase. This could be due simply to non-linear competing effects of the four soil constituents, or could be more complicated. One potential explanation is that higher-clay and -organic soils produce larger flocs which are better at capturing microplastics and sinking out of the column; however, producing larger flocs requires proportionally more clay and organic matter as floc sizes increase, therefore diminishing the effects on elutriation efficiency as clay and organic matter contents increase.

2.3.5 Large column elutriator

Pretreatment with the large column elutriator resulted in significantly larger particle recovery for the Couch Park sample ($p = 0.008$) (Figure 2). This result outperformed the glass as well ($p = 0.03$) In general however, the large column elutriator performed rather inconsistently compared with the other two methods. The variability in performance between samples prevented any observable trends in extraction from being identified.

2.3.6 Microplastic prevalence

The amount of microplastics observed in field samples varied from 3,300 to 188,900 particles/kg of dry soil. The Neighborhood had by far the lowest particle content with 3,300-3,900 particles/kg. This number agrees well with current literature (Frederick Büks & Martin Kaupenjohann, 2020; Uddin et al., 2021). The other samples were far higher, with anywhere from 136,500-188,900 particles/kg. This falls in the upper range of microplastic concentration in published literature and is similar to the concentration of microplastic found in dust collected from roads (F. Büks & M. Kaupenjohann, 2020; Qiu et al., 2020). The high abundance of microplastics in these soils is likely due to the locations from which they were collected: Yard Grill and Couch Park were sourced from waterways in urban areas, and the Biosolids sample contained applied biosolids from the wastewater treatment plant, which could introduce high

amounts of microplastic. The Neighborhood sample was also sourced from a waterway in an urban area; however, unlike the Yard Grill sample, which was sourced from the same river in the same urban area, this soil was sandy and very low in organic matter content. It is possible that organic matter plays an important role in the retention of microplastics in soils. For a better understanding of fate and transport of microplastics, this process should be investigated further.

2.4 Conclusion

2.4.1 Comparison of extraction methodologies

The information gathered in this study should help to determine the optimal extraction methodology for microplastic extraction from soils. While all methods performed similarly, the evidence gathered suggests that elutriation can be used to increase particle extraction for sandy and silty samples; however, elutriation should be avoided for clayey and organic-rich soils. The evaluation to determine optimal extraction method can be performed using simple, readily-available methods prior to selection of extraction methodologies.

2.4.2 Microplastic prevalence

The evidence gathered here suggests that high concentrations of microplastics are found in terrestrial ecosystems of Oklahoma, namely the Arkansas River, Boomer Creek, and fields amended with biosolids. Further work is required to identify the specific polymers present in these locations though FTIR spectroscopy.

2.4.3 Future research

The absolute efficiency of each extraction methodology needs to be determined. Though the information gathered shows which method results in the greatest particle extraction, it is not possible to determine the efficiency of any of these methods, so it is not possible to report on the actual quantity of microplastics in any sample.

Particle quantity is not the only available metric for determining the best methodology however, and filter cleanliness should also be investigated. In future work, the composition of the finished product should be analyzed using Energy Dispersive x-ray Spectroscopy (EDS) in order to quantify the abundance of microplastic-related elements (e.g. carbon, nitrogen) with soil-related elements (e.g. silicon, iron, potassium,, etc). The inclusion of EDS will allow for the quantification of leftover soil constituents, such as sand, silt, and clay, and will provide a metric to express sample purity.

2.4.4 Impact

The information gathered here will provide a valuable tool in the future research of microplastics. Currently, no published research has quantified the effect of soil constituents on microplastic extraction in an environmentally-relevant scenario. Because no standardized extraction methodology currently exists for microplastics in natural soils, and extended period of trial-and-error is often required to select an effective procedure, using up precious time and resources. Using the information gathered in this study, future research groups will be able to circumvent this wasteful time period by quickly selecting the appropriate method based solely on readily-measureable characteristics of soils. The ability to quickly and inexpensively select an extraction methodology should help to facilitate the development of a standardized methodology for the extraction of microplastics from soils, which will allow for the precise measurement and monitoring of microplastics in the environment. With a standardized extraction procedure, it will be possible to conduct environmental risk assessment, set and monitor allowable standards, and model fate and transport mechanisms of these emerging contaminants.

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APPENDICES

Table 2: Averages and standard deviations for samples

Sample	Large Columns		Direct Digestion		Glass Column	
	Avg	StDev	Avg	StDev	Avg	StDev
Neighborhood	3.3	2.7	3.6	0.6	3.8	1.9
Yard Grill	137.8	21.9	183.5	28.8	179.1	17.9
Couch Park	188.9	3.0	135.6	13.6	156.1	16.2
Biosolids	143.1	12.9	175.8	25.6	165.3	19.5
Biosolids 50%	129.2	11.2	125.4	8.5	103.7	23.4

VITA

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