

The observation of microplastics as emerging pollutants in UK dairy farms

Olivia Jane Skilbeck

Submitted in accordance with the requirements for the degree of Masters by Research

The University of Leeds: School of Geography

September 2022

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Professor Paul Kay and Dr Laura Carter who have been an amazing support throughout this project. I would also like to thank the farmers who helped give insight into the workings of their farms and allowed me to collect samples over the course of my monitoring period. Finally, to the laboratory staff in the School of Geography who helped me order supplies required for my laboratory analysis and in the Faculty of Biology where I used the EVOS imaging microscope to obtain images for my fluorescence microscopy.

ABSTRACT

Microplastics (MPs) have been widely identified throughout agricultural soils with the application of livestock slurries as organic fertilisers highlighted as a key input of these emerging pollutants into the environment. Despite livestock slurries being identified as key inputs of MPs into agricultural soils, to date little research has investigated the potential routes of exposure for MPs into these slurries. Therefore, this study was designed to assess potential MP inputs into slurries in UK dairy farms through the observation of MP concentrations in a range of agricultural matrices and how these can be transferred into soils. MP concentrations were observed in samples collected at all sites with the largest concentrations measured in sawdust bedding samples at Site B (662 ± 449 MP/kg dw). Dominant polymers identified include polyurethane foam, polyethylene, and polystyrene and abundant morphotypes were film, fibres and fragments. MP concentrations recorded in this study were greater than those previously reported throughout the literature and this study provides valuable insight into potential MP inputs into livestock slurries. Having observed the largest MP concentrations in livestock bedding it was concluded that this was a considerable contributor to MP contamination in slurries with potential transfer pathways through wind blow processes, ingestion, or inhalation. However, there is a need for further work to be conducted on the ingestion and inhalation of MPs present in livestock bedding by cattle to ensure certainty on this MP input into the agricultural system. Due to a lack of evidence for the direct MP transfers between sawdust bedding and slurries it cannot be concluded that the application of these slurries on soils is the sole MP input. Therefore, there is a need for further studies to identify MP inputs into livestock slurries through the use and degradation of plastic materials in the agricultural sector.

CONTENTS

ACKNOWLEDGEMENTS	1
ABSTRACT	2
CONTENTS	3
FIGURE CONTENTS	6
TABLE CONTENTS.....	8
EQUATION CONTENTS	8
ABBREVIATIONS	9
1. INTRODUCTION	10
1.1. PLASTICS.....	10
1.1.1. <i>Global plastic production and usage</i>	10
1.1.1.1. <i>Growth of plastic production globally</i>	10
1.1.1.2. <i>Degradation of plastic in the environment</i>	11
1.2. OVERVIEW OF MICROPLASTIC RESEARCH	12
1.2.1. <i>The presence of microplastics in aquatic environments</i>	12
1.3. IMPACTS OF MICROPLASTICS IN SOILS.....	13
1.3.1. <i>Impacts of microplastics on soil health</i>	13
1.3.1.1. Impacts on soil bulk density	13
1.3.1.2. Impacts on water holding capacity of soil.....	13
1.3.1.3. Impacts on soil stability.....	14
1.3.1.4. Impacts on available nutrients in soils	14
1.3.2. Impact of microplastics on plant growth	14
1.3.3. <i>Soil microbial changes in soils exposed to microplastics</i>	15
1.3.4. <i>Impacts of microplastics on biota</i>	15
1.4. THE PRESENCE OF MICROPLASTICS IN TERRESTRIAL ENVIRONMENTS	16
1.4.1. <i>Microplastic concentration</i>	16
1.4.1.1. Microplastic type in soils.....	17
1.4.2. <i>Inputs of microplastics into agricultural soils</i>	18
1.4.2.1. Microplastic inputs from atmospheric deposition	18
1.4.2.2. Microplastic inputs from agricultural practices	18
1.4.2.3. Microplastic inputs from organic fertilisers	19
1.4.2.4. Microplastic inputs from plastic mulching	20
1.4.3. <i>Microplastic fate in soils</i>	20
1.4.3.1. Microplastic fate influenced by soil biota	21
1.4.3.2. Microplastic fate influenced by soil properties.....	21
1.4.3.3. Microplastic fate influenced by agricultural practices	22
1.4.3.4. Microplastic fate influenced by microplastic characteristics	22
1.5. DEVELOPMENT OF MICROPLASTIC RESEARCH METHODS.....	22
1.5.1. Development of microplastic extraction methods	22
1.5.2. Development of microplastic observation methods.....	23
1.6. CURRENT GAPS IN THE LITERATURE	24
2. AIMS OBJECTIVES AND HYPOTHESES	25
2.1. RESEARCH AIM	25
2.2. RESEARCH OBJECTIVES.....	25
2.3. HYPOTHESES	25
3. MATERIALS AND METHODS.....	26
3.1. FIELD METHODOLOGIES	26
3.1.1. <i>Study sites</i>	26

3.1.1.1.	Study site A	27
3.1.1.2.	Study site B	27
3.1.1.3.	Study site C	28
3.1.2.	<i>Sample collection</i>	29
3.2.	LABORATORY METHODOLOGIES.....	30
3.2.1.	<i>Microplastic extraction from agricultural samples</i>	30
3.2.1.1.	Sample preparation	30
3.2.1.2.	Density separation	30
3.2.1.2.	Filtration.....	30
3.2.2.	<i>Microplastic identification</i>	31
3.2.2.1.	Visual observation.....	31
3.2.2.2.	Fluorescence microscopy.....	33
3.2.2.3.	Polymer identification using chemical spectroscopy	34
3.3.	STATISTICAL ANALYSIS	35
4.	RESULTS	36
4.1.	MICROPLASTIC CONCENTRATIONS IN UK DAIRY FARMS	36
4.1.1.	<i>Microplastic concentrations at Site A</i>	36
4.1.2.	<i>Microplastic concentrations at Site B</i>	36
4.1.3.	<i>Microplastic concentrations at Site C</i>	37
4.1.4.	<i>Differences between microplastic concentrations</i>	38
4.2.	POLYMER TYPES IN AGRICULTURAL SAMPLES	38
4.2.1.	<i>Polyurethane foam microplastic concentrations</i>	39
4.2.2.	<i>Polyethylene microplastic concentrations</i>	39
4.2.3.	<i>Polystyrene microplastic concentrations</i>	39
4.2.4.	<i>Differences of polymer type concentrations between sites</i>	41
4.3.	MATERIALS IDENTIFIED IN CONTROL SOIL SAMPLES.....	41
4.4.	PARTICLE MORPHOTYPES OF EXTRACTED POTENTIAL MICROPLASTICS.....	41
4.4.1.	<i>Concentrations of microplastic films</i>	42
4.4.2.	<i>Concentrations of microplastic fibres</i>	42
4.4.3.	<i>Concentrations of microplastic fragments</i>	42
4.4.4.	<i>Differences of microplastic morphotypes between sites</i>	44
4.5.	MICROPLASTIC IDENTIFICATION TECHNIQUES	44
4.5.1.	<i>Differences between microplastic concentrations obtained from fluorescence microscopy and FTIR spectroscopy</i>	44
5.	DISCUSSION.....	48
5.1.	MICROPLASTIC CONCENTRATIONS IN AGRICULTURAL SAMPLES.....	48
5.1.1.	<i>Livestock feed</i>	48
5.1.2.	<i>Livestock bedding</i>	49
5.1.3.	<i>Livestock slurry</i>	49
5.1.4.	<i>Agricultural soil</i>	50
5.1.5.	<i>Other materials measured</i>	51
5.2.	MICROPLASTIC CONCENTRATIONS IN AGRICULTURAL SAMPLES.....	51
5.2.1.	<i>Evaluation of microplastic extraction methods</i>	51
5.2.1.1.	Density separation	51
5.2.1.1.	Filtration and visual sorting.....	52
5.2.1.2.	Digestion	52
5.2.2.	<i>Evaluation of microplastic identification methods</i>	53
5.2.2.1.	FTIR Spectroscopy	53
5.2.2.2.	Fluorescence microscopy.....	53
5.3.2.2.	Alternative methods	54
6.	CONCLUSIONS	56

REFERENCES.....	57
APPENDICES.....	67
APPENDIX A- PRELIMINARY METHODS AND INTERPRETATIONS.....	67
APPENDIX B- MICROPLASTIC CONCENTRATION RAW DATA TABLES	68
APPENDIX C- CONCENTRATION OF OTHER MATERIALS EXTRACTED FROM AGRICULTURAL SAMPLES IDENTIFIED USING FTIR	
SPECTROSCOPY	69
<i>C1. Observations at Site A.....</i>	<i>69</i>
<i>C2. Observations at Site B.....</i>	<i>69</i>
<i>C3. Observations at Site C.....</i>	<i>70</i>

FIGURE CONTENTS

Figure 1.A. The growth of global plastic production from 1950-2040 (Chen et al., 2021).	10
Figure 1.B. Distribution of most common polymers used in global plastic production (Geyer et al., 2017).	11
Figure 1.C. Distribution of polymer types measured in soil environments (Zhang et al., 2021).	17
Figure 1.D. Distribution of microplastic morphotypes deposited into surface soils due to atmospheric deposition (Allen et al., 2019).	18
Figure 1.E. Microplastic concentrations measured in livestock manures (Beriot et al., 2021; Lwanga et al., 2017; Wu et al., 2021; Yang et al., 2021; Zhang et al., 2022a).....	20
Figure 1.F. Microplastic fates in soils (Guo et al., 2020).	21
Figure 3.A. Site A feed storage for components of total mix ration (a. dry bales b. waste bread feed c. silage).	27
Figure 3.B. Site B outdoor silage clamp showing black plastic sheeting, green netting and tyres.....	28
Figure 3.C. Site C metal slurry store.....	29
Figure 3.D. Site C soil collection field.....	29
Figure 3.E. Potential microplastics in filtered sawdust samples under a high-powered stereo microscope.....	32
Figure 3.F. Common microplastics observed in filtered samples.	33
Figure 3.G. Microplastics identified in sample using Nile Red staining and fluorescence microscopy (a. light source RFP, b. light source Trans, c. light source RFP and Trans).....	34
Figure 4.A. Microplastic concentration collected in agricultural samples across a 6-month monitoring period at Site A.....	36
Figure 4.B. Microplastic concentration collected in agricultural samples across a 6-month monitoring period at Site B.....	37
Figure 4.C. Microplastic concentration collected in agricultural samples across a 6-month monitoring period at Site C.....	38
Figure 4.D. Mean concentration of polymer types (polyurethane foam (PU) polyethylene (PE) and polystyrene (PS)) measured in agricultural samples collected over a 6-month monitoring period.....	39
Figure 4.E. Distribution of polyurethane foam (PUF) microplastics in agricultural samples.	40
Figure 4.F. Distribution of polyethylene (PE) microplastics in agricultural samples.	40

Figure 4.G. Distribution of polystyrene (PS) microplastics in sawdust bedding samples.	41
<i>Figure 4.H. Distribution of particle shapes extracted as potential microplastics from agricultural samples from all sites over a 6-month monitoring period.</i>	<i>42</i>
Figure 4.I. Distribution of film particles in agricultural samples at all sites collected over monitoring period.	43
Figure 4.J. Distribution of fibre particles in agricultural samples at all sites collected over monitoring period.	43
<i>Figure 4.K. Distribution of fragment particles in agricultural samples at all sites collected over monitoring period.</i>	<i>44</i>
<i>Figure 4.L. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from silage samples at Site A over a 6-month monitoring period.</i>	<i>45</i>
<i>Figure 4.M. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from slurry samples at Site A over a 6-month monitoring period.</i>	<i>45</i>
<i>Figure 4.N. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from cattle pellet samples at Site B over a 6-month monitoring period.</i>	<i>46</i>
<i>Figure 4.O. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from sawdust bedding samples at Site B over a 6-month monitoring period.</i>	<i>46</i>
<i>Figure 4.P. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from slurry samples at Site B over a 6-month monitoring period.</i>	<i>47</i>
<i>Figure 4.Q. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from sawdust bedding samples at Site C over a 6-month monitoring period.</i>	<i>47</i>

TABLE CONTENTS

Table 1.1 Global microplastic concentration across differing environments (Büks and Kaupenjohann, 2020; Van Sebille et al., 2015; Uddin et al., 2020).....	12
Table 1.2. Summary of global microplastic concentrations (Büks and Kaupenjohann, 2020; de Souza Machado, 2019; Harms et al., 2021; Piehl et al., 2018; Zhang and Liu, 2018).	17
Table 1.3. The variation in abundance of microplastic morphotype concentration observed in topsoil (Büks and Kaupenjohann, 2020; Harms et al., 2021; Liu et al., 2018; Zhang et al., 2021; Zhang and Liu, 2018).	18
Table 3.1. Site characteristics.	26
Table 3.2. Microplastic characteristics used for identification (Hidalgo-Ruz et al., 2012).	31
Table 3.3. Determining microplastic shape.	32
Table 4.1. Materials extracted from control soils collected from each site was analysed under FTIR spectroscopy.	41

EQUATION CONTENTS

Equation 3.1. Density of sodium dihydrogen phosphate solution.	30
Equation 3.2. Mass of salt for 200 mL sodium dihydrogen phosphate solution, density 1.35 g/mL at 20°C for 40 weight percent with assumptions of 1 g/mL density of water.	30

ABBREVIATIONS

<i>MP</i>	Microplastic
<i>NP</i>	Nanoplastic
<i>PUF</i>	Polyurethane foam
<i>PU</i>	Polyurethane
<i>PE</i>	Polyethylene
<i>HDPE</i>	High density polyethylene
<i>LDPE</i>	Low density polyethylene
<i>PS</i>	Polystyrene
<i>PP</i>	Polypropylene
<i>PVC</i>	Polyvinyl chloride
<i>PET</i>	Polyethylene terephthalate
<i>UV</i>	Ultraviolet
<i>H₂O₂</i>	Hydrogen peroxide
<i>NaOH</i>	Sodium hydroxide
<i>KOH</i>	Potassium hydroxide
<i>NaCl</i>	Sodium chloride
<i>ZnCl₂</i>	Zinc chloride
<i>NaI</i>	Sodium iodide
<i>NaH₂PO₄</i>	Monosodium phosphate
<i>CaCl₂</i>	Calcium chloride
<i>FTIR</i>	Fourier transform infrared
<i>SEM</i>	Scanning electron microscope
<i>EDS</i>	Energy dispersive x-ray spectroscopy
<i>DW</i>	Dry weight
<i>WWTW</i>	Wastewater treatment works
<i>OM</i>	Organic matter
<i>DOM</i>	Dissolved organic matter
<i>DOC</i>	Dissolved organic carbon
<i>DON</i>	Dissolved organic nitrogen
<i>DOP</i>	Dissolved organic phosphorus

1. INTRODUCTION

1.1. PLASTICS

1.1.1. GLOBAL PLASTIC PRODUCTION AND USAGE

1.1.1.1. GROWTH OF PLASTIC PRODUCTION GLOBALLY

Due to desirable characteristics including low production costs, versatility, high resistance to corrosion and durability of plastics, it has been a widely used material for over 60 years with mass production beginning in the 1950s starting at 1.7 million tonnes (Mt) per annum (Chen et al., 2021; Gong and Xie, 2020; Nithin and Goel, 2017; Ryan, 2015). The British Plastics Federation state that plastic usage is imperative for modern society, providing benefits including being light weight, durable, versatile, hygienic, resource efficient and recyclable (Smith, 2022). The production of plastics has risen over the past 6 decades by ~9% per annum with production increasing globally by ~357.5-378 Mt between 1950-2015 (Figure 1.A; Chamas et al., 2020; Chen et al., 2021; Geyer et al., 2017; Okoffo et al., 2021). Suggestions have been made that the growth of global plastic production is driven by an increased demand for single use plastics which contributed 50% of global plastic production in 2018 with future predictions for plastic production to double over the next 20 years (Chen et al., 2021).

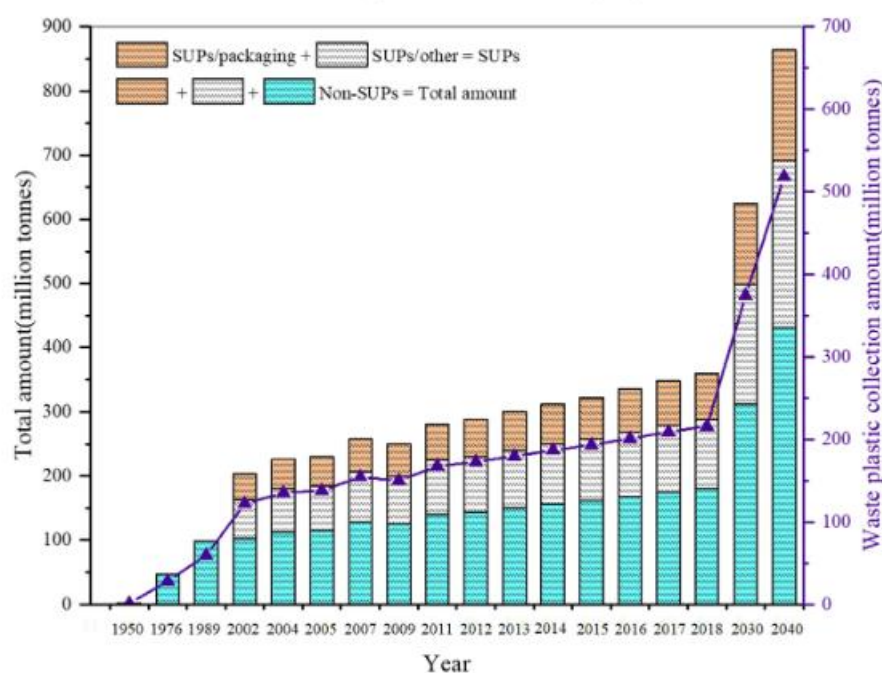


FIGURE 1.A. THE GROWTH OF GLOBAL PLASTIC PRODUCTION FROM 1950-2040 (CHEN ET AL., 2021).

Polymers that account for 92% of plastics include polyethylene (PE) (36%), polypropylene (PP) (21%), polyvinyl chloride (PVC) (12%), polystyrene (PS), polyethylene terephthalate (PET) and polyurethane (PU) (<10% each) with PP and PE used most widely in today's 'throw away' culture as single use plastics (Figure 1.B; Chen et al., 2021; Geyer et al., 2017). With over 90 million tons produced each year, PE is the most widely used polymer globally growing in production and consumption during the 1990s through products such as packaging, plastic bags and other consumer plastics (Demirors, 2011; Okoffo et al., 2021).

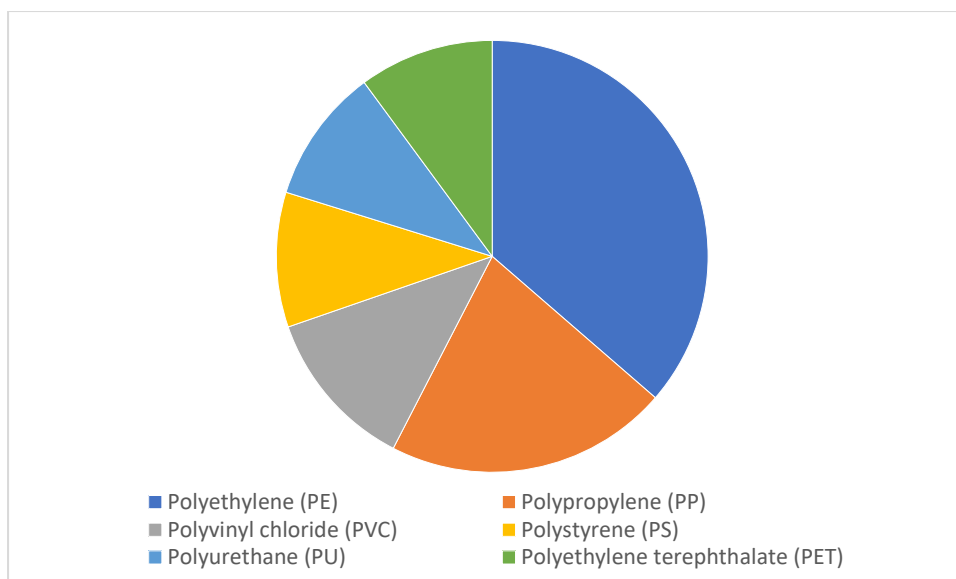


FIGURE 1.B. DISTRIBUTION OF MOST COMMON POLYMERS USED IN GLOBAL PLASTIC PRODUCTION (GEYER ET AL., 2017).

1.1.2. DEGRADATION OF PLASTIC IN THE ENVIRONMENT

Plastic degradation occurs in the environment through processes including photodegradation, biodegradation, chemical, thermal and mechanical degradation (Beriot et al., 2021; Liu et al., 2022). Plastic properties can be altered physically and chemically due to these processes, promoting the fragmentation of plastics and the production of microplastics into the environment (Prata et al., 2021; Zhang et al., 2021). The high durability of plastics increases resistance against degradation, elevating plastic longevity with rate of degradation influenced by plastic composition and environmental conditions (Ángeles-López et al., 2017; Laing et al., 2022; Roy et al., 2011; Zhang et al., 2021).

Photodegradation is the breakdown of plastics due to the exposure to ultraviolet (UV) radiation and has been considered the most important degradation process for the initial breakdown of plastics (Zhang et al., 2021). This process is suggested to reduce the flexibility of plastics making them more brittle and susceptible to break, resulting in the release of MPs and volatile pollutants into the environment (Liu et al. 2022; Ng et al., 2018). The rate of plastic decomposition by photodegradation is suggested to increase in topsoils due to elevated exposure to UV radiation (He et al., 2018).

The exposure of plastics to light, temperature and microbial activity can impact chemical degradation including hydrolysis (requires H₂O) and oxidation (requires O₂) (Chamas et al., 2020). Thermal degradation occurs due to the occurrence of thermo-oxidative reactions at high temperatures promoting the breakdown of polymer chains due to increased energy inputs (Zhang et al., 2021). HDPE and LDPE may contain unsaturated (C=C) bonds which are susceptible to oxidation by radicals such as O₃ or NO_x resulting in unstable hydroperoxides (Chamas et al., 2020). This promotes photo-oxidation rates in LDPE and HDPE, however, this only occurs in the presence of sunlight (Chamas et al., 2020). Degradation of PET most commonly occurs via thermal oxidation in the presence of O₂ in the environment (Chamas et al., 2020).

In addition to the biochemical degradation of plastics, further plastic degradation can occur during physical digestive processes following exposure to biota (Zhang et al., 2021). The presence of bacteria, fungi soil mites and digging mammals in soils can promote biological degradation of plastics in soils (Zhang et al., 2021; Zhou et al., 2020). In well oxygenated soils plastic degradation is

suggested to increase due to concentrations of microorganisms on the plastic surface in addition to the inability of soil microbes to mineralise polymers (Ng et al., 2018; Roy et al., 2011). Despite LDPE having a high molecular weight making it difficult to degrade in the environment, microorganisms and bacteria in soils have been shown to be highly effective in degrading LDPE (Abraham et al., 2016).

Mechanical degradation processes that can result in the production of MPs can include collision and abrasion with sediments and rocks (Zhang et al., 2021). Mechanical degradation is responsible for the production of tyre MPs and synthetic MP fibres (Zhang et al., 2021). This may promote the entrance of MPs into agricultural environments through the use of common practices including tillage and ploughing.

1.2. OVERVIEW OF MICROPLASTIC RESEARCH

Microplastics (MPs) have widely been accepted as persistent and emerging pollutants in the environment and are defined as plastic particles <5 mm which was first introduced by Thompson et al. (2004). These pollutants have been measured in environments including oceans and freshwaters, terrestrial environments and in the atmosphere, with inputs and concentrations in each environment varying and driven by anthropogenic activity (Table 1.1; Büks and Kaupenjohann, 2020; Gong and Xie, 2020; Zhang et al., 2020c). MPs originate from two sources: primary MPs directly inputted into the environment and secondary MPs released from the degradation of larger plastic materials (Gong and Xie, 2020). MP research has largely focused in Western Europe, specifically the UK, Netherlands and Belgium although China has also contributed considerable to the research field with only 20% of the top 20 countries where MP studies have been located being developing countries (Zhang et al., 2020c). Early MP research focused on the presence of this pollutant in aquatic environments with research only recently having a stronger focus towards the presence of MPs in terrestrial environments, as opposed to oceans and rivers, and their potential impacts (Ryan, 2015).

TABLE 1.1 GLOBAL MICROPLASTIC CONCENTRATION ACROSS DIFFERING ENVIRONMENTS (BÜKS AND KAUPENJOHANN, 2020; VAN SEBILLE ET AL., 2015; UDDIN ET AL., 2020).

Environment	MP Concentration
Oceans	15-51 trillion MP particles in global oceans
Rivers	1.94-106 MP particles m ⁻³
Soils	<1-12,760 MP/kg ⁻¹ dw soil

1.2.1. THE PRESENCE OF MICROPLASTICS IN AQUATIC ENVIRONMENTS

Plastics have been recognised as a significant pollutant in marine environments since the 1970s but were initially believed to undergo complete degradation due to photodegradation and oxidation (Ryan, 2015). However, it was not until 2004 when the term ‘microplastic’ was introduced following the identification of large concentrations of this pollutant in the environment with published work in the field significantly increasing from 2011 (Ryan, 2015; Zhang et al., 2020c). The occurrence of a series of international conferences surrounding impacts of marine litter stimulated following research leading to a shift in focus for research with new studies concentrating on finding effective solutions for dealing with the issue (Ryan, 2015).

The current wave of research into marine MPs was driven by the discovery of the ‘garbage patch’ in the North Pacific with Thompson et al. (2004) extracting MP particles from UK beach and estuarine sediments originating from the breakdown of larger plastic materials (Ryan, 2015). This paper paved

the way for research focusing on plastic longevity and the potential of plastics to act as vectors for harmful pollutants, transferring these through the food chain (Thompson et al., 2004).

Having been considered critical pathways for the transfer of MP pollution into marine environments, an increasing number of studies began to identify and quantify MPs in freshwater environments (Blair et al., 2019; Zhang et al., 2020c). MP concentrations have since been identified in river and lake water and sediment in North America, Asia and Europe (Blair et al., 2019; Eerkes-Medrano et al., 2015). Key MP sources are often anthropogenic such as WWTW effluent with early reviews highlighting the closer proximity to MP sources seen in riverine environments in comparison to marine environments influences MP characteristics (Eerkes-Medrano et al., 2015; Mason et al., 2016).

1.3. IMPACTS OF MICROPLASTICS IN SOILS

1.3.1. IMPACTS OF MICROPLASTICS ON SOIL HEALTH

Soil health is greatly impaired due to the presence of MPs due to decreases in bulk density, alterations to water dynamics, changes to soil structure and increased evapotranspiration (de Souza Machado, 2018; Guo et al., 2020). MP shape plays a critical role in impacts on soil health with polyester fibres altering bulk density, water holding capacity and soil structure due to the high flexibility of these particles causing them to be more readily entangled in soil particles increasing compaction of soil aggregates (de Souza Machado, 2018; Zhou et al., 2020).

However, controversies surrounding the impacts of MPs on soil health exist with some studies suggesting the presence of MPs improve soil health due to the addition of humic-like materials promoting stability of soils, water holding capacity and concentrations of nutrients highlighting the need for further research (Guo et al., 2020).

1.3.1.1. IMPACTS ON SOIL BULK DENSITY

Soil bulk density is affected by MPs in soils with the addition of polyester fibres to soils leading to significant decreases in soil bulk density due to the fragmentation of macropores by these fibres that often create clods when entangled with other fibres and soil particles, elevating the number of micropores (Hu et al., 2022; Li et al., 2020). MP fragments in soils can lead to significant decreases in soil bulk density due to the lower densities of MPs in comparison to soil organic matter which can increase aeration in soils (de Souza Machado, 2018; Hu et al., 2022; Khalid et al., 2020). Soil bulk densities do not show similar changes due to the addition of MP beads and films (Hu et al., 2022; Li et al., 2020).

However, controversies on this research topic have been highlighted in the literature with conflicting results showing no alterations in soil bulk density when exposed to $\leq 0.3\%$ polyester microfibrils and fragments however this may be due to the small quantity of MPs added to soil samples (Hu et al., 2022; Li et al., 2020).

1.3.1.2. IMPACTS ON WATER HOLDING CAPACITY OF SOIL

Due to poor permeability of MPs, high MP concentrations in soils can impact water permeability and soil water holding capacity which have subsequent impacts on alterations to evaporation and water availability to plants (Guo et al., 2020; Zhao et al., 2022; Zhou et al., 2021). Impacts of MPs on the hydrological system of soils is dependent on MP shape leading to varying results throughout this field (Guo et al., 2020; Hu et al., 2022; Rillig, 2019; Zhou et al., 2021)

MP concentrations impacts soil water holding capacity with increases in the quantity of water stable aggregates due to the presence of MP fibres due to their ability to connect to soil minerals and OM

(Zhou et al., 2021). Results vary between studies with a decrease in water holding capacity observed with the presence of MP fibres by Guo et al. (2020). Water loss from soils has also been shown to increase in the presence of PE films due to increases in evaporation with further alterations in evaporation observed by the presence of MPs due to changes in permeability, water holding capacity and water transfers through soils which could promote the drying of soils (Guo et al., 2020; Rillig, 2019; Zhou et al., 2021).

Alterations to soil water holding capacity could have subsequent impacts on soil moisture and evapotranspiration rates which both play an important role in ecosystems and climate systems (de Souza Machado et al., 2018). Further changes to soil water systems could aggravate the negative impacts of drought resulting from impacts of changes in Earth's climate system (Zhou et al., 2021).

1.3.1.3. IMPACTS ON SOIL STABILITY

Soil stability is impacted due to the presence of MPs in soils with micro-aggregates having stronger bonds between particles compared to macro-aggregates (Boots et al., 2019). Close to 75% of MPs present in soils are attached to soil aggregates leading to impacts on the ability for the formation of soil aggregates (Zhao et al., 2022). This has detrimental effects on soil stability and has been observed in most MP types (Zhao et al., 2022). Soil erosion can be further impacted by MPs as they influence the clumping of soil particles which in turn could impact soil erosion (de Souza Machado, 2018).

1.3.1.4. IMPACTS ON AVAILABLE NUTRIENTS IN SOILS

The presence of MPs in soils is suggested to elevate DOM concentrations with an experiment lasting 30 days showing increased MP concentrations in soils increasing DOM and subsequently promoting the release of nutrients (dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP)) (Guo et al., 2020; Xu et al 2020b; Zhao et al., 2022).

The cycling of nitrogen through soil ecosystems is impacted by the presence of MPs in soils as these interfere with bacteria key to the nitrogen cycle in soils, however, soils have the ability to counteract these impacts on the cycling of nitrogen due to nitrification and denitrification (Zhao et al., 2022).

Further theories suggested in the literature include alterations in nutrient flows with studies suggesting the presence of biodegradable plastics leading to fixation of N to starch released from the biodegradable plastics impacting plant growth (Zhou et al., 2020). Carbon transfers between plants and soils have also been suggested to be negatively impacted by the presence of MPs suggesting that the MPs in soils can impact terrestrial C stores with elevations in CO₂ emissions from soils observed due to higher PVC concentrations in soils (Zang et al., 2020). This may be a resulting factor from reduced soil bulk density and elevated soil aeration (Zang et al., 2020).

1.3.2. IMPACT OF MICROPLASTICS ON PLANT GROWTH

MP's impacts on soil properties has observed subsequent influences on biodiversity, soil functions and productivity ultimately impacting food production and plant productivity in a time when food security is already stretched by a growing global population, changes in land use and climatic changes (Khalid et al., 2020; Molotoks et al., 2020; Zhang et al., 2020a; Zhou et al., 2020).

Negative impacts on plant development resulting from MP exposure include reduced growth of above ground matter, root development and seedling growth; elevate oxidative stresses and alter nutrient content in plants (Enyoh et al., 2020; Mateos-Cárdenas et al., 2021; Wang et al., 2021; Wang et al., 2020). However, impacts on plant development are largely dependent on MP concentration and exposure time (Enyoh et al., 2020; Liu et al., 2018; Mateos-Cárdenas et al., 2021; Wang et al., 2021). Exposure to concentrations >4713 MP/kg soil resulted in negative impacts on

seed development observed following an exposure time of 72 hours (Mateos-Cárdenas et al., 2021; Wang et al., 2021). Furthermore, a 23% decrease of aboveground plant development was observed alongside a reduction of below ground productivity of 8% due to the presence of MPs in soils (Boots et al., 2019; Wang et al., 2020; Zang et al., 2020; Zhu et al., 2019).

Comparatively, elevated plant development has been observed in studies exposing plants to low MP concentrations (<2782 MP/kg soil) where elevated leaf growth and root development were promoted following exposure to PS fragments and PET fibres respectively (Wang et al., 2021). Increased root biomass has been measured with longer and finer roots in soils exposed to MPs due to increased plant stress caused by MP exposure alongside elevated nutrient concentrations due to heightened enzyme activity and soluble nutrient accumulation and reduced soil bulk density allowing for improved root penetration (Boots et al., 2019; de Souza Machado, 2019; Hu et al., 2022; Rillig, 2019; Zhou et al., 2020). Differences in results between studies may be due to variation between crop and MP type and concentration in studies making comparison between studies difficult (Zhou et al., 2020).

MPs and nanoplastics (NPs) have been observed to be toxic to plants as they contain toxic elements such as heavy metals, antibiotic resistance genes, pathogens and other organic contaminants which can often be released upon MP degradation (Wang et al., 2021). These chemicals can impact plant growth and cause the uptake of heavy metals by plants which can be transferred through the food chain (Mohajerani and Karabatak, 2020; Zang et al., 2020).

The uptake of plastic particles into plants has also been demonstrated, with particle uptake being dependent on size, molecular weight and charge of the particle (He et al., 2018; Mateos- Cárdenas et al., 2021). The presence of MPs in soils leads to blockages of 78% of seed pores and plants exposed to MP concentrations in soils reducing water and nutrient uptake (Boots et al., 2009; Khalid et al., 2020). Due to the small size of particle needed for plant uptake, research has focused on the uptake of nanoplastics (NPs) with particle uptake observed through root systems of particles ranging in size from 20 nm - 2 µm, however, it is important to note that this is species dependent (Mateos- Cárdenas et al., 2021; Wang et al., 2021). Despite plant cells having pore sizes of 3.5-5.2 nm, plants are able to take up larger particles through endocytosis which has been used to explain the presence of larger plastic particles observed in plant systems (Wang et al., 2021).

1.3.3. SOIL MICROBIAL CHANGES IN SOILS EXPOSED TO MICROPLASTICS

The presence of MPs in soils has been suggested to alter soil microorganisms due to changes in porosity and soil moisture, ultimately impacting oxygen transfers through soils (Guo et al., 2020; Zhou et al., 2020). These impacts may be both positive and negative. The presence of polystyrene NPs has been found to reduce bacteria in soils critical to nitrogen fixation and the breakdown of OM (Mohajerani and Karabatak, 2020; Rillig, 2019). Conversely, inputs of plastics into soils are suggested to favour soil microbial communities due to greater incorporation of ¹⁴C which facilitates microbial growth due to MPs acting as habitats for microbial activity (Zang et al., 2020). Impacts of MPs on microbial communities can have subsequent impacts on plant growth due to alterations in mineral absorption and soil nitrogen availability (Khalid et al., 2020).

1.3.4. IMPACTS OF MICROPLASTICS ON BIOTA

MP exposure to biota lead to alterations in mortality rate, growth, food intake, organ damage, immune response and observations of early signs of disease (Enyoh et al., 2020; He et al., 2018; Lwanga et al., 2016; Rodrigues-Seijo et al., 2016). The degree of toxicity is dependent on MP concentrations, exposure duration and polymer type with severe impacts to biota resulting from exposure to concentrations >100 MP/kg for a period of 60 days which causes organ damage,

increased mortality, reduced growth and decreased reproductive outputs with PS MPs showing strongest impacts on *Caenorhabditis elegans* (Enyoh et al., 2020; Lei et al., 2018; Lwanga et al., 2017). Even when exposed to the lowest MP concentrations (62.5 mg/kg soil dw) histopathological damages were identified such as damages to the gut (prevalent at concentrations of >125 mg/kg soil dw) (Rodrigues-Seijo et al., 2016). Similar results were observed due to MP exposure of PE MPs <150µm at concentrations between 28-60% dry weight compared to growth rate measured at MP concentrations of 7% dry weight and control levels due to elevated uptake of MPs which reduces nutrient uptake (Lwanga et al., 2016).

In addition to direct impacts caused by the ingestion of MPs by biota, MP toxicity to biota can also be driven by the release of harmful chemicals such as heavy metals, colourants, plasticisers, antibiotics and pathogens (He et al., 2018; Song et al., 2019; Zhang et al., 2020a; Zhou et al., 2020). Desorption rates of zinc from MPs was observed to be elevated in earthworm guts compared to levels in soils (40-60% and 2-15% respectively) suggesting MP can make elements such as zinc more bioavailable (Hodson et al., 2017). The ingestion and excretion of MPs has been observed by biota throughout the literature with MPs observed in organism gut and tissue leading to the leaching of toxic chemicals in biota and into the food chain (Beriot et al., 2021; Heinze et al., 2021; Song et al., 2019).

1.4. THE PRESENCE OF MICROPLASTICS IN TERRESTRIAL ENVIRONMENTS

Despite MP pollution in marine environments receiving higher coverage throughout the scientific literature, soil environments are estimated to contain MP concentrations 4-23 times greater than concentrations in the oceans (de Souza Machado et al., 2017; Xu et al., 2020b; Zang et al., 2020). Few studies have focused on the presence of MPs in soils with the first paper only published in 2012. This lack of research may be due to issues with MP extraction methods from solid environmental matrices, the separation between research in marine and terrestrial ecology and greater concerns surrounding the uptake of MPs into food chains through the presence of filter feeders in marine environments (Rillig, 2012).

MPs have been observed in a range of soil environments including agricultural soils, domestic gardens, floodplain soils, urban soils and coastal areas suggesting that MP pollution in terrestrial environments is widespread (Rillig, 2012; Xu et al., 2020b). Sources of MPs into these environments include plastic mulching, application of sewage sludges and organic fertilisers and atmospheric deposition (Beriot et al., 2021; Nizzetto et al., 2016; Rillig, 2012; Xu et al., 2020b; Wu et al., 2021; Zhou et al., 2022).

1.4.1. MICROPLASTIC CONCENTRATION

MPs have been observed as emerging pollutants in soil environments with concentrations believed to be greater than those observed in oceans due to agricultural practices utilising plastics as well as the incorrect disposal of >30% of waste plastics globally (Büks and Kaupenjohann, 2020; de Souza Machado et al., 2017; Zang et al., 2020). Following degradation of this waste plastic, MPs are released and can move throughout the environment by water and wind and the ingestion from biota (Zhang et al., 2020a). MP concentrations in soils have been measured globally, however, studies have not been evenly dispersed worldwide with studies heavily focused in Europe and North America compared to countries in Asia and Africa (Table 1.2; Büks and Kaupenjohann, 2020).

TABLE 1.2. SUMMARY OF GLOBAL MICROPLASTIC CONCENTRATIONS (BÜKS AND KAUPENJOHANN, 2020; DE SOUZA MACHADO, 2019; HARMS ET AL., 2021; PIEHL ET AL., 2018; ZHANG AND LIU, 2018).

Area	MP concentration
<i>Global estimations</i>	<i>1167-13,000 particles kg⁻¹ dry soil</i>
China	1076 particles kg ⁻¹
Southwestern China	7100 – 42,960 particles kg ⁻¹
Europe	2914 particles kg ⁻¹
Switzerland	~0.002% of soil weight
Northern Germany	3.7 ± 11.9 MP per kg dry weight (dw)

Variation in MP concentration measures in soils is impacted by location with elevated concentrations recorded in soils surrounding heavy industry and areas of urbanisation as well as in floodplain soils, with observed impacts of soil depth and method of collection and extraction on MP concentration (Büks and Kaupenjohann, 2020; Koutnik et al., 2021; Zhu et al., 2019).

To date, there is no legislation requiring microplastic monitoring in soil environments, leading to uncertainties surrounding large-scale concentrations (Büks and Kaupenjohann, 2020). The development of models to predict large-scale MP concentration due to varying MP inputs offers a means of filling this knowledge gap (Büks and Kaupenjohann, 2020; Nizzetto et al., 2016a).

1.4.1.1. MICROPLASTIC TYPE IN SOILS

In excess of 20 polymer types have been measured in soil environments including PE, PP, PS, PVC and PET (Figure 1.C; Harms et al., 2021; Zhang et al., 2021). The most abundant MP morphotype observed in topsoil environments has been shown to vary include fragments, fibres, films and pellets (Table 1.3; Büks and Kaupenjohann, 2020; Harms et al., 2021; Liu et al., 2018; Zhang et al., 2021; Zhang and Liu, 2018). Higher concentrations of microfibrils may be due to elevated retention rates compared to MP fragments caused by entanglement of fibres with soil particles (Crossman et al., 2020; Horton and Dixon, 2017). In deeper soil horizons fibres were still observed to be the highest MP morphotype at 38% (Liu et al., 2018).

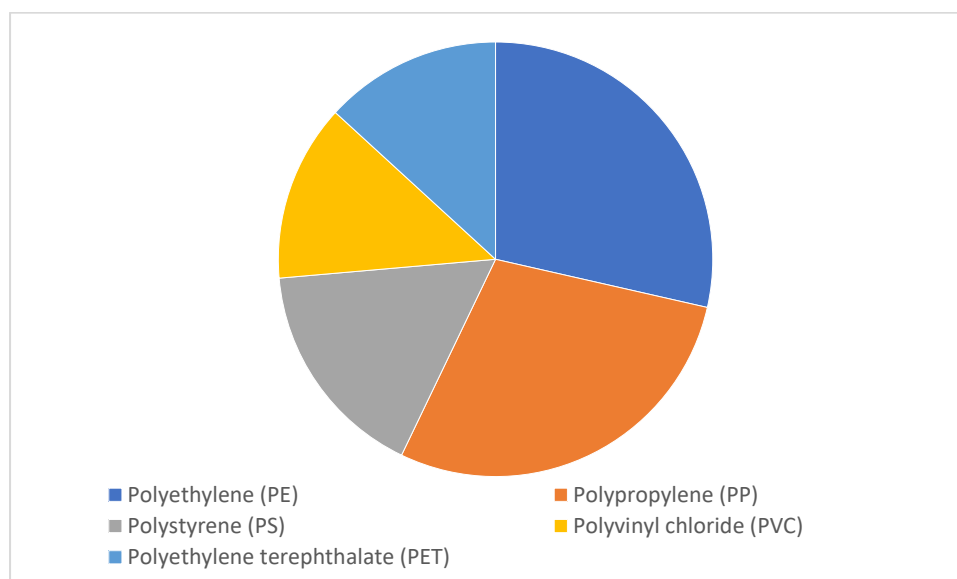


FIGURE 1.C. DISTRIBUTION OF POLYMER TYPES MEASURED IN SOIL ENVIRONMENTS (ZHANG ET AL., 2021).

TABLE 1.3. THE VARIATION IN ABUNDANCE OF MICROPLASTIC MORPHOTYPE CONCENTRATION OBSERVED IN TOPSOIL (BÜKS AND KAUPENJOHANN, 2020; HARMS ET AL., 2021; LIU ET AL., 2018; ZHANG ET AL., 2021; ZHANG AND LIU, 2018).

Morphotype	Concentration (%)
Fragments	28-87
Fibres	83-92
Films	61-63
Pellets	2-10

1.4.2. INPUTS OF MICROPLASTICS INTO AGRICULTURAL SOILS

The improper disposal of plastic is the largest contributor to plastic inputs into soils with other inputs including atmospheric deposition and agricultural practices such as the application of plastic mulches, sewage sludges and wastewater irrigation (He et al., 2018; Piehl et al., 2018; Rillig, 2012; Zhou et al., 2020).

1.4.2.1. MICROPLASTIC INPUTS FROM ATMOSPHERIC DEPOSITION

The introduction of MPs into surface soils can be influenced by atmospheric deposition acting as a transfer pathway of MPs from urban environments and landfill sites to rural environments (Zhang et al., 2020a; Zhu et al., 2019). Sources of microplastic pollution transported through areal deposition include microfibrils originating from textiles, the re-emission of MP fragments from rubber tyres released from their wear and tear as well as dust from urban environments, marine environments and agricultural soils (Brahney et al., 2021; Kumar et al., 2020). Common MP types deposited through atmospheric deposition include fragments, films and fibres with variations in concentrations varying (Figure 1.D; Allen et al., 2019; Bläsing and Amelung, 2018; Xu et al., 2020a).

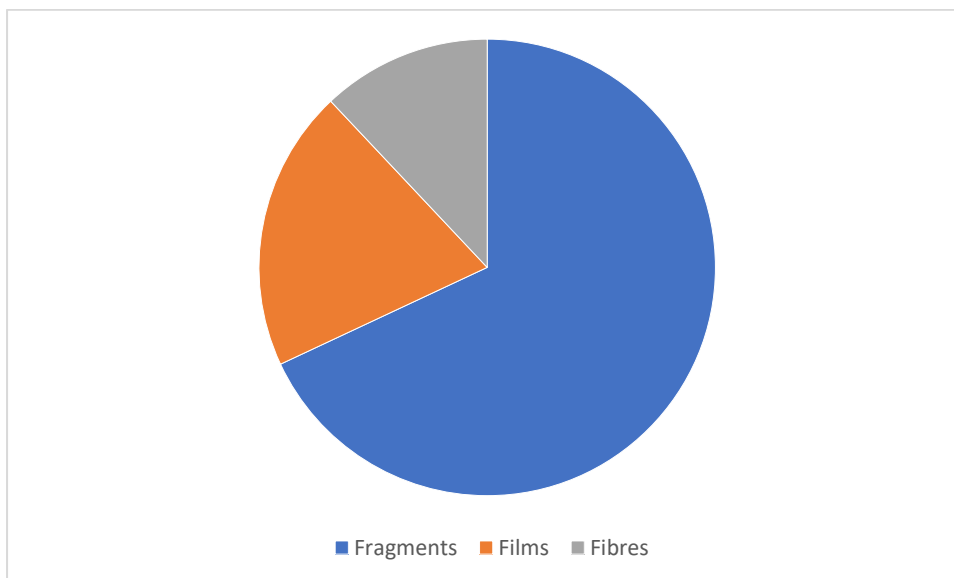


FIGURE 1.D. DISTRIBUTION OF MICROPLASTIC MORPHOTYPES DEPOSITED INTO SURFACE SOILS DUE TO ATMOSPHERIC DEPOSITION (ALLEN ET AL., 2019).

1.4.2.2. MICROPLASTIC INPUTS FROM AGRICULTURAL PRACTICES

MPs in agricultural soils often derive from agricultural practices with the majority being polypropylene (50.51%) and polyethylene (43.43%) (He et al., 2018). Agricultural techniques commonly introducing MPs into soil environments include the application of biosolids and other

organic fertilisers, the use of wastewaters from wastewater treatment works (WWTWs) in crop irrigation and plastic mulches on arable soils (Zang et al., 2020; Zhang et al., 2020a; Zhu et al., 2019).

1.4.2.3. MICROPLASTIC INPUTS FROM ORGANIC FERTILISERS

Biosolids including sewage sludge and composts are key sources of MPs into soils transferring ~90% of MPs into agricultural soils due to the application of sludge and organic fertilisers (Beriot et al., 2020; Zhu et al., 2019). A bioproduct from the WWTW process, the application of biosolids onto agricultural land has been widely adopted with 75% of biosolids used in this cost-effective method of recycling nutrients and organic matter back into soil systems, increasing soil organic matter (SOM), water holding capacity and soil stability (Biosolids Assurance Scheme, 2019; Crossman et al., 2020; Lu et al., 2012; Nicholson et al., 2018). The retention of 90% of MPs post wastewater treatment has led to MP concentrations in biosolids ranging from 0.018-8700 MP kg⁻¹ with common polymer types and morphotypes including PE, PP, PVC, PET and PS and fibres (Bläsing and Amelung, 2018; Corradini et al., 2019; Crossman et al., 2020; Nizzetto et al., 2016a; Zhang et al., 2020a). Biosolids applied to agricultural soils in the EU have previously been treated to meet pollution standards with ~150,000 ha of agricultural land receiving biosolids in the UK, increasing the potential for MP inputs into soils (Biosolids Assurance Scheme, 2019; Brandes et al., 2021; Mohajerani and Karabatak, 2020). European soils exposed to sewage sludge and biosolids have been observed to contain MP concentrations of 26,000-430,000 tonnes with the development of models suggesting the application of sewage sludge onto agricultural soils could result in concentration estimations of 5.8 kg ha⁻¹ a⁻¹ (Büks and Kaupenjohann, 2020; Crossman et al., 2020; Mohajerani and Karabatak, 2020; Nizzetto et al., 2016a).

Livestock manures are also used to fertilise agricultural land as a method of recycling nutrients into soil and can contain MPs with concentrations measured in poultry, cow, sheep and pig manures (Figure 1.E; Beriot et al., 2021; Lwanga et al., 2017; Wu et al., 2021; Yang et al., 2021; Zhang et al., 2022a). MP concentrations of ~2 x 10³ MP/kg⁻¹ – 3.5 ± 1.71 million MP ha⁻¹ a⁻¹ have been measured in soils treated with these manures with dominant morphotype being fragments and polymer composition including polypropylene (47.8%), polyester (39.1%), rayon (7.07%) and polyethylene (5.98%) (Beriot et al., 2021; Wu et al., 2021; Yang et al., 2021). Despite the little research on this source of MPs into agricultural soils, the long term application of contaminated livestock manures as organic fertilisers could lead to the transfer of these pollutants into soil environments causing subsequent impacts on soil and plant health (Zhou et al., 2022).

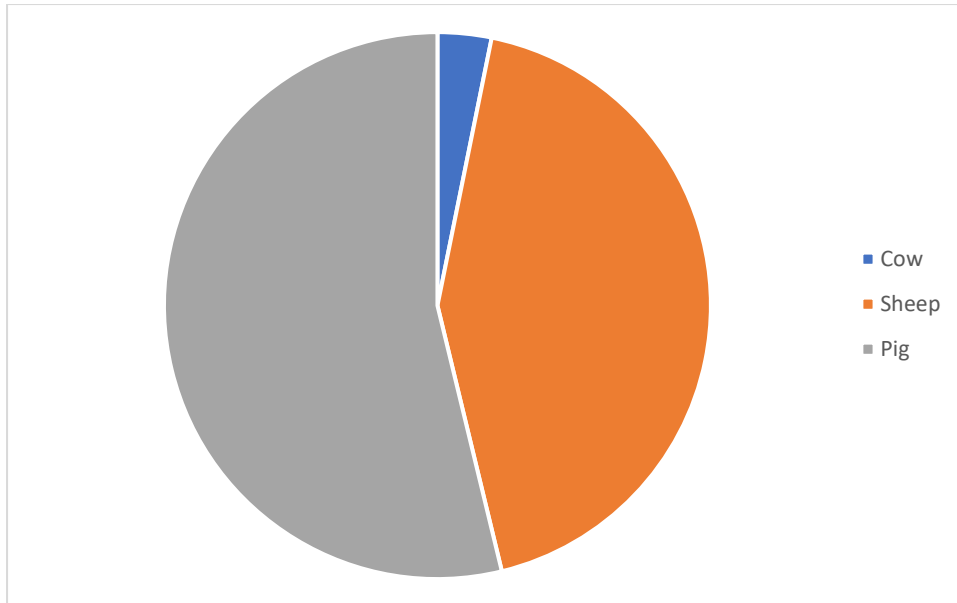


FIGURE 1.E. MICROPLASTIC CONCENTRATIONS MEASURED IN LIVESTOCK MANURES (BERIOT ET AL., 2021; LWANGA ET AL., 2017; WU ET AL., 2021; YANG ET AL., 2021; ZHANG ET AL., 2022A).

1.4.2.4. MICROPLASTIC INPUTS FROM PLASTIC MULCHING

Plastic mulching is used to improve crop yield through increasing soil temperatures and moisture content but can subsequently be incorporated into soils or left on the surface to degrade (Beriot et al., 2021; Steinmetz et al., 2016; Zang et al., 2020). This has been found to result in soil MP concentrations of $0.07 \text{ MP kg ha}^{-1}$ which were observed to be led to the lower MP concentrations in soils compared to the application of sewage sludge and composts (Brandes et al., 2021).

1.4.3. MICROPLASTIC FATE IN SOILS

MP fate through soils can be influenced by bioturbation, agricultural practices, characteristics of MPs and soil properties (Figure 1.F; Bläsing and Amelung, 2018; Guo et al., 2020; Khalid et al., 2020; Xu et al., 2020b). The downward movement of MPs into deeper soil horizons through pathways such as macropores allows the vertical movement of MPs in soils leading to variations in MP concentrations in soil columns (Horton and Dixon, 2017; Möller et al., 2020; Xu et al., 2020b). Concentrations of MPs have been observed to vary through the soil column with topsoils (0-3cm) having the highest concentrations at $78 \pm 12.91 \text{ MP/kg}$ which decline to $62.5 \pm 12.97 \text{ MP/kg}$ at depths of 3-6 cm (Liu et al., 2018; Horton and Dixon, 2017; Möller et al., 2020; Zhou et al., 2020; Zhu et al., 2019). Larger MPs are located in the upper soil horizons (0-6 cm) whilst smaller MPs have been observed to migrate into deeper soil horizons at a depth of 8-10.5 cm due to movement by earthworms (Liu et al., 2018; Zhang et al., 2021; Zhou et al., 2020).

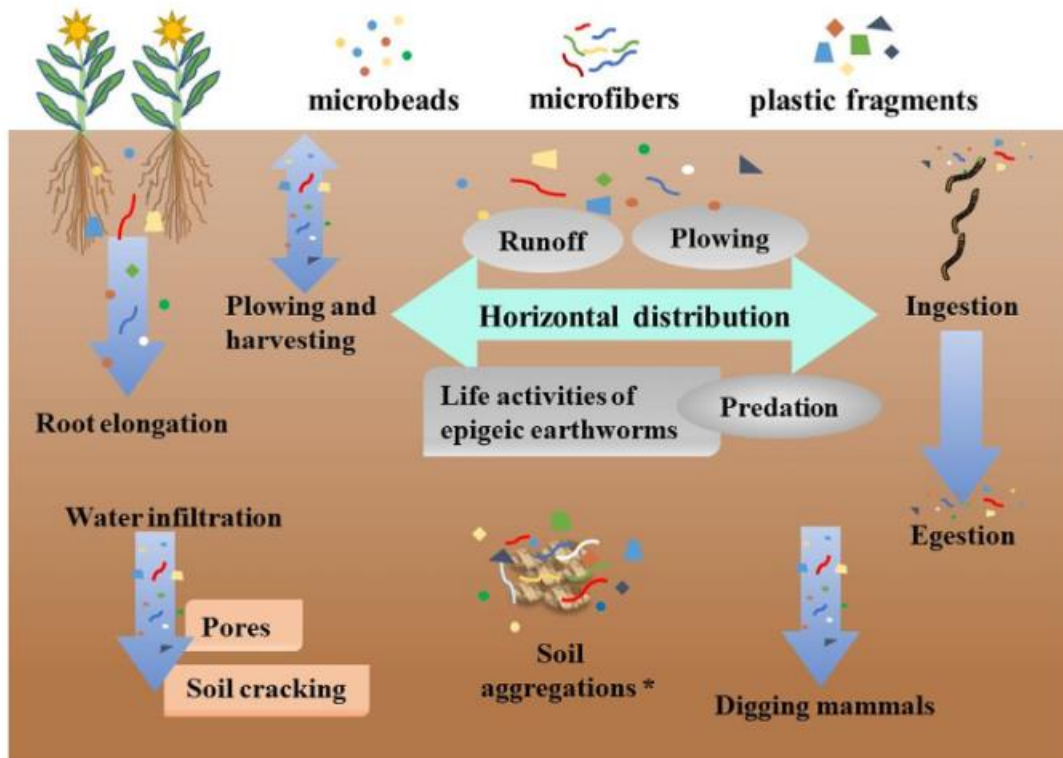


FIGURE 1.F. MICROPLASTIC FATES IN SOILS (GUO ET AL., 2020).

1.4.3.1. MICROPLASTIC FATE INFLUENCED BY SOIL BIOTA

The fate of MPs in soils can be influenced by bioturbation by fauna such as earthworms which occurs due to the ingestion and excrement of MPs or through the external attachment, promoting vertical and horizontal MP movement into deeper soil horizons (Figure 1.F; Guo et al., 2020; Heinze et al., 2021; Li et al., 2020; Xu et al., 2020b; Zhou et al., 2020). Biota such as collembola species (*Folsomia candida* and *Proisotoma minuta*), mites (*Hypoaspis aculeifer*) and earthworms have been suggested to lead to movement of MPs (Li et al., 2020). Earthworms and fungal mycelia can facilitate the degradation of MPs in soils due to bacteria aiding the breakdown of MPs and the creation of nanoplastics (NPs) which can be more readily moved through the environment (Guo et al., 2020; Kumar et al., 2020).

Bioturbation can also occur due to the presence of plant roots in soils which can lead to the creation of preferential flow pathways (Figure 1.F; Crossman et al., 2020; Guo et al., 2020; Li et al., 2020). The transport of MPs can be enhanced due to the presence of macropores left behind after the decomposition of a plant, however, little research has focused on the impact of vegetation on MP transfers in soils (Li et al., 2020).

1.4.3.2. MICROPLASTIC FATE INFLUENCED BY SOIL PROPERTIES

Soil texture can impact MP transfers due to pore size, soil organic carbon, clay content, pH, soil mineral composition, OM content and ionic strength (Xu et al., 2020b; Zhou et al., 2020). The opening of large cracks in soils due to repetitive wetting and drying of soils could facilitate the movement of MPs into deeper soil horizons at a faster rate through macropores (Figure 1.F; Crossman et al., 2020; Guo et al., 2020; Li et al., 2020; Rillig et al., 2017; Zhou et al., 2020). The presence of macropores (pores >0.08 mm) elevated MP movement through soils due to the reduced impact of sedimentation and elevated transfer of particles by water movement through soils, however for leaching to occur pores must be greater than MP particle size (Bläsing and Amelung,

2018; Rillig et al., 2017). Leaching has been suggested to be a large contributor to the movement of MPs through soils with MP movement elevated due to high water velocities with the potential for MP of entering groundwater sources due to the leaching of MPs $\sim 3.7 \mu\text{m}$ up to depths of 70 cm (Li et al., 2020; Xu et al., 2020b). The presence of soil pores further influences MP retention rates in soils with greater retention rates due to elevated quantities of micropores as well as increases in MP retention in agricultural and forest soils in comparison to urban soils due to reduced surface runoff and elevations in soil permeability (Bläsing and Amelung, 2018; Horton and Dixon, 2017; Nizzetto et al., 2016b). This can heighten the possibility for interaction between MPs and soil biota, elevating impacts in these soil types (de Souza Machado et al., 2017).

1.4.3.3. MICROPLASTIC FATE INFLUENCED BY AGRICULTURAL PRACTICES

Agricultural practices can promote MP movement through soils with practices such as tillage, irrigation, drainage, ploughing and harvesting promoting the transfer of surface MPs into deeper soil horizons (Figure 1.F; Guo et al., 2020; He et al., 2018; Zhang and Liu, 2018; Zhou et al., 2020). The depth of MP redistribution is determined by the depth of the agricultural practice with the first 20-30 cm of soil typically impacted as a result of ploughing, however the impact of this practice on MP movement is questioned due to more prevalent processes of MP fate such as leaching (He et al., 2018; Rillig et al., 2017). The harvesting of crops can also influence MP redistribution in soils due to the removal and decomposition of roots, enhancing the presence of macropores, however, this generally occurs at shallower depths (Guo et al., 2020; Rillig et al., 2017).

1.4.3.4. MICROPLASTIC FATE INFLUENCED BY MICROPLASTIC CHARACTERISTICS

Polymer type, MP size, shape, charge, density and hydrophobicity impact MP movement through soils (Rillig et al., 2017; Xu et al., 2020b; Zhou et al., 2020). MPs ranging in size from $0.1-1 \times 10^3 \mu\text{m}$ have been suggested to migrate into deeper soil horizons with larger MPs being retained in soils (Heinze et al., 2021; Li et al., 2020; Zhou et al., 2020). Spherical and granular (beads and fragments) MPs migrate more readily into deeper soil horizons with fibres and films having greater difficulties moving through soils due to different interactions between soil particles (Crossman et al., 2020; Rillig et al., 2017; Zhou et al., 2020). Comparatively, MPs with hydrophobic surfaces are more readily retained in soils compared to MPs with hydrophilic surfaces with degradation of the surface of a MP further altering MP fate in soils due to changes with interactions between other particles (Rillig et al., 2017; Xu et al., 2020b).

1.5. DEVELOPMENT OF MICROPLASTIC RESEARCH METHODS

The study of MPs has faced challenges due to uncertainties surrounding the correct definition (Blair et al., 2019). With no standardised definition identifying the lower size limit of a MP and inconsistencies between extraction methods and MP quantification the comparison of studies in this field is difficult (Blair et al., 2019; Gong and Xie, 2020; Hurley et al., 2018; Prata et al., 2019; Rillig, 2012). This has impacted MP monitoring and understanding of pollution risk, leading to uncertainties surrounding MP sources, fates and impacts in the environment (Blair et al., 2019).

1.5.1. DEVELOPMENT OF MICROPLASTIC EXTRACTION METHODS

Methods for the extraction of MPs from contaminated solid environmental matrices such as soils, sediments and slurries is yet to be standardised, however, pre-processing methods commonly include digestion, density separation and filtration to allow for MP separation from impurities (Gong and Xiu, 2020; Hurley et al., 2018; Rillig, 2012).

The presence of organic matter (OM) in environmental samples is suggested to hinder MP detection with OM mistaken for MPs, resulting in the avoidance of solid environmental matrices for the extraction of MPs (Prata et al., 2019). To allow for the removal of organic substances from samples

acid, alkaline or enzyme digestion can be performed with Fenton's reagent, 35% hydrogen peroxide (H₂O₂), sodium hydroxide (NaOH) and potassium hydroxide (KOH) widely being employed. These methods have been applied at temperatures up to 70°C (<40°C preferred to minimise MP damage) for a period of 12-72 hours with this allowing for MP extraction rates of 66-80% (CSEAS Indonesia, 2020; Ding et al., 2020; Gong and Xie, 2020; Hurley et al., 2018; Prata et al., 2019; Yang et al., 2021). However, this process can lead to polymer damage and can be highly time consuming with large variation in study conclusions over effectiveness of this method (Hurley et al., 2018). Current studies on the digestion of OM to aid the recovery of MPs have been limited with studies having strong focuses on general OM digestion. Further research is needed to develop methods for the extraction of MPs from samples with high OM contents (Prata et al., 2019).

Density separation is a widely adopted method for the extraction of MPs from samples with MP density ranging between 0.8-1.4 g cm⁻³ therefore allowing for separation from denser sediment matrices through the addition of a high-density saline solution (CSEAS Indonesia, 2020; Gong and Xie, 2020; Yang et al., 2021; Zhang et al., 2020b). Sodium chloride (NaCl) is widely used with a density of 1.2 g cm⁻³, however, due to its inability to remove higher density polymers other saline solutions such as zinc chloride (ZnCl₂), sodium iodide (NaI) and monosodium phosphate (NaH₂PO₄) with densities between 1.4-1.6 g cm⁻³ have been used in replace of this (Gong and Xie, 2020; Zhang et al., 2020b). Despite allowing for the extraction of denser MPs, environmental concerns and cost have limited the use of these saline solutions (Zhang et al., 2020b).

1.5.2. DEVELOPMENT OF MICROPLASTIC OBSERVATION METHODS

The development of the visual identification of MPs in samples has allowed for advancements in this research field with frequently adopted techniques including optical microscopy, spectral analytical methods (Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy and pyrolysis gas-chromatography mass-spectroscopy), scanning electron microscopy (SEM), fluorescence microscopy and thermal analysis (Cowger et al., 2020; Picó and Barceló, 2020; Shim et al., 2017; Song et al., 2015; Veerasingam et al., 2021).

Visual analysis of MPs using the naked eye was a commonly adopted technique prior to the universal use of the term 'microplastic' allowing for the physical identification and sorting of larger MP particles commonly with a size range of 1-5 mm (Shim et al., 2017). A stereo microscope can be used for the identification of MPs with a size of 100 µm allowing for more accurate identification of particle characteristics (Hidalgo-Ruz et al., 2012; Shim et al., 2017). Despite this method providing a simple and fast method of MP identification, smaller MP particles and MPs with similar visual characteristics to sediments are often overlooked (Shim et al., 2017; Song et al., 2015; Thompson et al., 2004).

Spectral analytical methods including FTIR, and Raman spectroscopy have been universally used for polymer identification as they have the ability to accurately detect chemical makeup and bonds of a material with the application of a non-invasive method (Shim et al., 2017). Studies assessing the accuracy of FTIR and Raman spectroscopy in comparison to visual observation methods have suggested that spectral analysis of MPs allows for the identification of a higher concentration of MPs as well as the distinction between synthetic and natural fibres with Raman spectroscopy and µ-FTIR a highly effective method of the identification of polymers <20µm (Araujo et al., 2018; Ivleva et al., 2016; Song et al., 2015). Pyrolysis gas-chromatography mass-spectroscopy has been favoured as a method for the accurate identification of MPs due to its ability to detect MP with small dimensions and can be beneficial for identifying polymer mass (Ivleva et al., 2016; Picó and Barceló, 2020). However, due to low run times and higher accuracy in results obtained from other methods, this method has not always been favoured (Ivleva et al., 2016; Picó and Barceló, 2020).

The adoption of SEM for the visual analysis of MPs allows for the high-magnification of particle topography which can be paired with energy-dispersive X-ray spectroscopy (EDS) to identify particle makeup (Shim et al., 2017). However, SEM-EDS is costly and time-consuming limiting sample numbers that can be analysed (Shim et al., 2017).

Fluorescence microscopy is suggested to be a reliable and feasible MP identification technique due to the wide availability of materials needed for the completion of this method (Hengstmann and Fischer, 2019). The use of Nile red staining and visual analysis of samples under exposure to UV light allows for the time efficient and accurate microscopy of large MPs enabling large sample populations to be analysed using this technique (Hengstmann and Fischer, 2019). These techniques have enabled further progression in the visual identification of MPs although further analysis through spectral analysis is beneficial (Gong and Xie, 2020).

Polymers can be further identified through the use of thermal analysis which identifies polymers based on thermal stability through measuring physical and chemical alterations (Shim et al., 2017). This method can be applied through the use of differential scanning calorimetry, however, this method requires the use of a reference material in order for polymer identification which may limit MP identification in environmental samples when the material is not known prior to analysis (Shim et al., 2017).

Due to the complexity of identifying MPs in samples it has been suggested that the adoption of a multi analytical method including chemical classification allows for elevation in method robustness (Cowger et al., 2020). Ubiquitously implemented methods include the combination of microscopy to physically characterise MPs alongside spectroscopy to allow the identification of the chemical profile of the plastic (Shim et al., 2017).

1.6. CURRENT GAPS IN THE LITERATURE

With the development of research measuring MP concentrations in terrestrial environments since 2012 due to the expansion of extraction and observation methods, studies have been able to improve understanding of MP sources into these environments (Rillig, 2012). MP research still remains in its early stages of development with gaps in the literature existing in the presence of MP concentrations in the agricultural sector. The presence of MPs has been identified in livestock feeds and manures with concentrations in manures playing a key role into the transfer of MPs into agricultural soils through the application of livestock slurries (Beriot et al., 2020; Lwanga et al., 2017; Wu et al., 2021; Yang et al., 2021; Zhou et al., 2022). However, further research is needed on other potential sources of MPs into the agricultural sector which may have the potential to influence the input of MPs into slurries. With the cattle and dairy industry producing the largest quantity of manure in the UK it is vital that this product can be recycled and reused as organic fertiliser on agricultural land. Few studies have previously focused on the presence of MPs in UK dairy farms, therefore, through considering a range of agricultural matrices including livestock feeds and soils this study will assess the entrance and potential movement of MPs in three UK dairy farms.

2. AIMS OBJECTIVES AND HYPOTHESES

2.1. RESEARCH AIM

To investigate potential pathways for the entrance of MPs into slurries in UK dairy farms through the observation of MP concentrations in a range of agricultural matrices and how these can be transferred into agricultural soils through the application of livestock slurries as organic fertilisers.

2.2. RESEARCH OBJECTIVES

1. Identify sources of MP contamination in agricultural samples collected from UK dairy farms (livestock feeds and bedding) through the identification of MP concentrations, polymer type and shape.
2. Assess the quantity of MPs in agricultural soils exposed to the application of livestock slurries through the use of FTIR spectroscopy, identifying MP polymer type and shape in livestock slurries applied to soils as well as in soil samples.

2.3 HYPOTHESES

H₁: MP concentrations measured in cattle slurries will contain similar polymer type and shape as MP concentrations measured in livestock feeds and bedding.

H₂: MP concentrations measured in agricultural soils exposed to the application of cattle slurry will contain similar polymer types and shapes as MP concentrations measured in livestock slurry.

3. MATERIALS AND METHODS

3.1. FIELD METHODOLOGIES

3.1.1. STUDY SITES

Three study sites were chosen in the North East of England in County Durham. All sites were predominantly dairy farms using agricultural techniques ubiquitous within UK dairy farming (Table 3.1). These sites have heard sizes of 150-600 which is greater than average UK heard size (148-174 cattle per herd) (March et al., 2014; Uberoi, 2021). Therefore, sites analysed in this study are largely representative of large-scale UK dairy farms.

TABLE 3.1. SITE CHARACTERISTICS.

	SITE A	SITE B	SITE C
Livestock Quantity	600	200	150
Livestock Breed	Holstein Friesian	Holstein Friesian	Holstein Friesian Friesian Norwegian reds
Housing	Housed in cubicles, bedded on sawdust on rubber matting	Housed in cubicles, bedded on sawdust on rubber matting	Housed in cubicles, bedded on sawdust on rubber matting (sawdust from waste of cuttings of kitchens)
Pasture Allowance	Three months in summer	No pasture allowance	Two hours in summer
Feed Composition	Total mix ration comprising of silage, whole crop wheat, home grown brewers draft, rolled barley, soya, SelcoPlus (brewery biproduct), enpro syrup (biproduct), concentrate, waste bread and rape meal	Silage, rolled barley, protein mix (soya and wheat distillers)	Silage, hay and meal (meal includes soybean meal, molasses, barley and minerals)
Silage Storage	Stored in silage clamp, double sheeted, clingfilm on top of grass then black sheet, green net on top and topped with tyres	Stored uncovered in shed and in silage clamp covered with plastic sheeting	Stored in silage clamp covered with green plastic sheet topped with tyres. Some silage stored in wrap
Manure Spreading	Umbilical slurry spreading using a dribble bar spread up to four times a year	Slurry spread twice a year in spring and autumn	Slurry spread six times a year
Other Fertilisers	Digestate spread on grassland	Fertiliser spread on fields	

3.1.1.1. STUDY SITE A

Site A was located in County Durham, England. It is 900 acres and is predominantly a dairy farm with 600 dairy cows of Holstein Friesian breed. Cattle were housed in cubicles bedded on rubber mattresses topped with sawdust produced from by-product of timber from a kitchen manufacturer. Feed consisted of a total mix ration of silage, whole crop wheat, home grown brewers draft, rolled barley, soya, SelcoPlus (brewery by-product), enpro syrup (by-product), concentrate, waste bread and rape meal (Figure 3.A). On observation, waste bread contained large plastic fragments from packaging not fully removed at the de-packaging plant. Silage is stored in a silage clamp which is double sheeted with clingfilm directly on top of the grass followed by a black polyethylene sheet and green netting with tyres placed on top to keep sheeting in place. This also represents an obvious potential source of plastic in feed. Sheeting is replaced annually. Dry bales used are not wrapped as the use of a bale clamp is more efficient due to the small quantity of bales created at this site. Cattle pellets consisting of cereals, crop seeds, legume seeds, forage and dried beet pulp were given to cattle during milking and were stored in a metal feeding system. Pasture allowance was limited to three months during summer with indoor feeding of cattle using the total mix ration continuing during this time allowing feed inputs to be largely controlled year-round. The UK dairy industry has observed a decline in cattle grazing with 55% of UK dairy farms feeding cattle indoors during summer months due to the ability to control sources of nutrients the livestock is receiving (March et al., 2014). Slurry was collected from the cattle cubicles by a stainless-steel automatic scraper, transferring slurry into a pit below the cattle shed which is then transferred into an underground lined lagoon. Small quantities of slurry and digestate were spread onto grassland 4 times a year by umbilical slurry spreading using a dribble bar.



FIGURE 3.A. SITE A FEED STORAGE FOR COMPONENTS OF TOTAL MIX RATION (A. DRY BALES B. WASTE BREAD FEED C. SILAGE).

3.1.1.2. STUDY SITE B

Site B was located in County Durham, England. It is 500 acres and is predominantly a dairy farm with a cattle population of 200 Holstein Friesian dairy cows. Cows are housed year-round where no pasture allowance occurred, allowing complete control over feed intake of livestock which is an increasingly popular technique adopted by larger UK dairy farms (March et al., 2014). Cattle were

housed in cubicles with rubber mattresses topped with sawdust with a ventilation system allowing for the circulation of air in the cattle barn. Sawdust was supplied by a wood shavings contractor who predominantly create sawdust using waste kitchens. Feed was comprised of silage, rolled barley and a protein mix of soya and wheat distillers. Silage bales were wrapped in Visqueen, a widely used low density polyethylene plastic sheeting and stored in sheds with some stored in silage clamps which was covered with plastic sheeting replaced annually and green netting to reduce the quantity of tyres needed to stabilise the sheeting (Figure 3.B). Cattle pellets were fed to cattle during the milking process and stored in metal feeding systems. Slurry was transferred from cubicles to an underground slurry store via a stainless-steel automatic scraper and was then pumped to a concrete underground store and then to a lagoon lined with plastic. Slurry was spread twice a year (spring and autumn) where grasses are grown and cut for silage in addition to the application of a chemical fertiliser.



FIGURE 3.B. SITE B OUTDOOR SILAGE CLAMP SHOWING BLACK PLASTIC SHEETING, GREEN NETTING AND TYRES.

3.1.1.3. STUDY SITE C

Site C was located in County Durham, England. It is 250 acres with 150 Holstein Friesian, Friesian and Norwegian red cows and is predominantly a dairy farm. Cows were housed in cubicles with rubber mat flooring topped with sawdust obtained from waste cuttings of kitchens. A silage blend was the main feed used at this site consisting of silage, hay and meal including soybean meal, molasses, barley and minerals. Barley used in feed was mostly grown on site with soya imported from America. Silage was cut three times annually and covered in a clamp with black low-density polyethylene sheeting topped with green plastic sheeting topped and tyres, with the sheeting replaced annually. Bales are also used to feed livestock at this site which were wrapped in plastic wrap. Pasture allowance at this site was two hours per day in the summer, with livestock housed in cubicles in the winter to control feed intake. Cattle pellets were supplied to cows during the milking process and were stored in metal feeding systems. Slurry was transferred to an underground slurry pit using a stainless-steel automatic scraper and stored in a metal slurry store (Figure 3.C). Slurry was spread six times a year on fields where silage is cultivated with the addition of solid muck spread one to two times annually to the same fields.



FIGURE 3.C. SITE C METAL SLURRY STORE.

3.1.2. SAMPLE COLLECTION

Samples of silage, cattle pellets, slurry, sawdust bedding and soils were collected from each farm with three replicate grab samples taken of each matrix. This was replicated six times with four-week increments starting in January. A stainless-steel trowel was used to collect silage, cattle pellets, slurry and sawdust bedding which was washed and air dried between each sample collection to reduce contamination (Gong and Xie, 2020). Soil samples were collected from a pre-determined field used for the growth of silage which remained constant throughout the study and with coordinates for sampling within the field created randomly prior to each visit (Figure 3.D). Soil was collected using a soil corer which sampled the top 10 cm of soil and vegetation was removed from the sample. Control soils were collected using the same technique with samples taken from each site along the verge of the field where no fertiliser is applied. Samples were placed in sustainable glass jars to reduce plastic contamination and stored in an environmental cold room at 4°C for 48 hours prior to freezing.



FIGURE 3.D. SITE C SOIL COLLECTION FIELD.

3.2. LABORATORY METHODOLOGIES

3.2.1. MICROPLASTIC EXTRACTION FROM AGRICULTURAL SAMPLES

3.2.1.1. SAMPLE PREPARATION

All samples collected in the field were weighed into aluminium containers (to reduce plastic contamination) and stored in a chest freezer at a temperature of -20°C for one week prior to being transferred into a freeze dryer at a temperature of -52°C for 48 hours to remove moisture. Freeze drying allowed for the removal of moisture without the application of heat, reducing potential microplastic damage (CSEAS Indonesia, 2020). The removal of moisture from all samples allowed for the homogenisation of freeze-dried samples using a pestle and mortar to breakdown OM, releasing potential MPs.

Three subsamples with weights depending on sample density (larger weights were taken from cattle pellet and soil samples while smaller weights were taken from silage, sawdust and slurry samples as these were less dense) were taken from each sample using balance scales measuring to 4 decimal points and placed into glass beakers that were previously rinsed with deionised water to remove any potential MP contamination.

3.2.1.2. DENSITY SEPARATION

Due to higher MP recovery rates than NaCl, NaH₂PO₄ was used for density separation at 1.35 g/mL as well as to allow for the extraction of denser MPs such as polycarbonate which were identified in large concentrations during the pilot (Appendix A; Equation 3.1-3.2) (Engineering ToolBox, 2017; Zhang et al., 2020b). Deionised water was used to create NaH₂PO₄ solution to minimise potential MP contamination from the use of tap water. Saline solution was added to subsamples in quantities between 100 and 200 mL ensuring all the sample was covered by solution. Samples were manually stirred for 5 minutes to ensure sample and saline solution were fully incorporated. Samples were covered with aluminium foil to avoid airborne MP contamination and left at ambient temperature for 24 hours; improved MP retention occurring with increased settling times (Zhang et al., 2020b).

EQUATION 3.1. DENSITY OF SODIUM DIHYDROGEN PHOSPHATE SOLUTION.

$$[\text{mass of salt}] / ([\text{mass of salt}] + \text{density of water}) = \text{concentration}$$

EQUATION 3.2. MASS OF SALT FOR 200 ML SODIUM DIHYDROGEN PHOSPHATE SOLUTION, DENSITY 1.35 G/ML AT 20°C FOR 40 WEIGHT PERCENT WITH ASSUMPTIONS OF 1 G/ML DENSITY OF WATER.

$$[\text{mass of salt}] / ([\text{mass of salt}] + 200 \text{ g}) = 0.4$$

$$[\text{mass of salt}] = 0.4[\text{mass of salt}] + 0.4 \times 200 \text{ g}$$

$$0.6[\text{mass of salt}] = 40 \text{ g}$$

$$[\text{mass of salt}] = 80 / 0.6$$

$$[\text{mass of salt}] = \mathbf{133 \text{ g}}$$

3.2.1.2. FILTRATION

The supernatant for each sample was separated from the solid material by decanting it into a clean glass beaker, which was covered using aluminium foil and left to settle for a further six hours to remove the remaining solid material from sample. The supernatant underwent vacuum filtration using glass microfiber filters GF/A with a diameter of 47 mm to filter solid material remaining in the supernatant after density separation. Filtered samples were removed from the vacuum filter and placed into pre-labelled glass petri dishes for MP identification.

Waste-waters created as a by-product of laboratory methods in this study were autoclaved to kill bacteria prior to disposal.

3.2.2. MICROPLASTIC IDENTIFICATION

3.2.2.1. VISUAL OBSERVATION

Prior to visual observation, samples on filter papers were left covered in glass petri dishes at room temperature to dry for a period of 24 hours. Filtered samples underwent preliminary visual observation using a high-powered stereo microscope (LEICA S6D). Potential microplastics were identified based on common characteristics, comprising texture, structure, thickness and colour (Table 3.2; Figure 3.E). Potential microplastics were removed from the sample using forceps under the microscope and placed in a pre-labelled glass petri dish. Microplastic shape and colour was noted upon extraction of potential microplastics. Shape was identified using characteristics of the particles (Table 3.3; Figure 3.F) and the number of potential microplastics extracted from each filtered sample was recorded.

TABLE 3.2. MICROPLASTIC CHARACTERISTICS USED FOR IDENTIFICATION (HIDALGO-RUZ ET AL., 2012).

Characteristic	Description
<i>Size</i>	Microplastics will be <5mm in size.
<i>Texture</i>	When prodded with metal forceps microplastics will bounce. If particle breaks when pressure is applied using metal forceps this is not counted as plastic.
<i>Structure</i>	Microplastic particles will have no organic structures. Take care to note if organic material is visible on sections the plastic surface or throughout the particle.
<i>Thickness</i>	Microplastic particles should be a consistent thickness throughout, however, splitting of a microplastic may be observed in fibres.
<i>Colour</i>	Homogenous colour should be shown throughout microplastic. Aging or biofouling of plastic may disguise colour or cause bleaching.



FIGURE 3.E. POTENTIAL MICROPLASTICS IN FILTERED SAWDUST SAMPLES UNDER A HIGH-POWERED STEREO MICROSCOPE.

TABLE 3.3. DETERMINING MICROPLASTIC SHAPE.

Microplastic Shape	Description
<i>Fragment</i>	Angular and irregular
<i>Film</i>	Irregular and thin
<i>Fibre</i>	Thin and often straight
<i>Pellet</i>	Circular and bead like
<i>Foam</i>	Irregular and porous

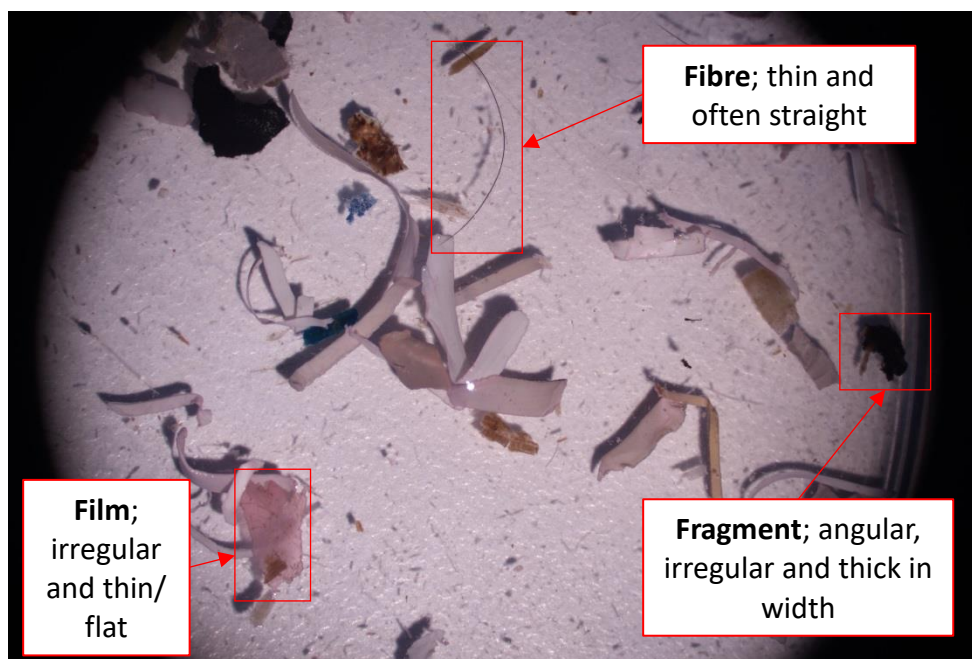


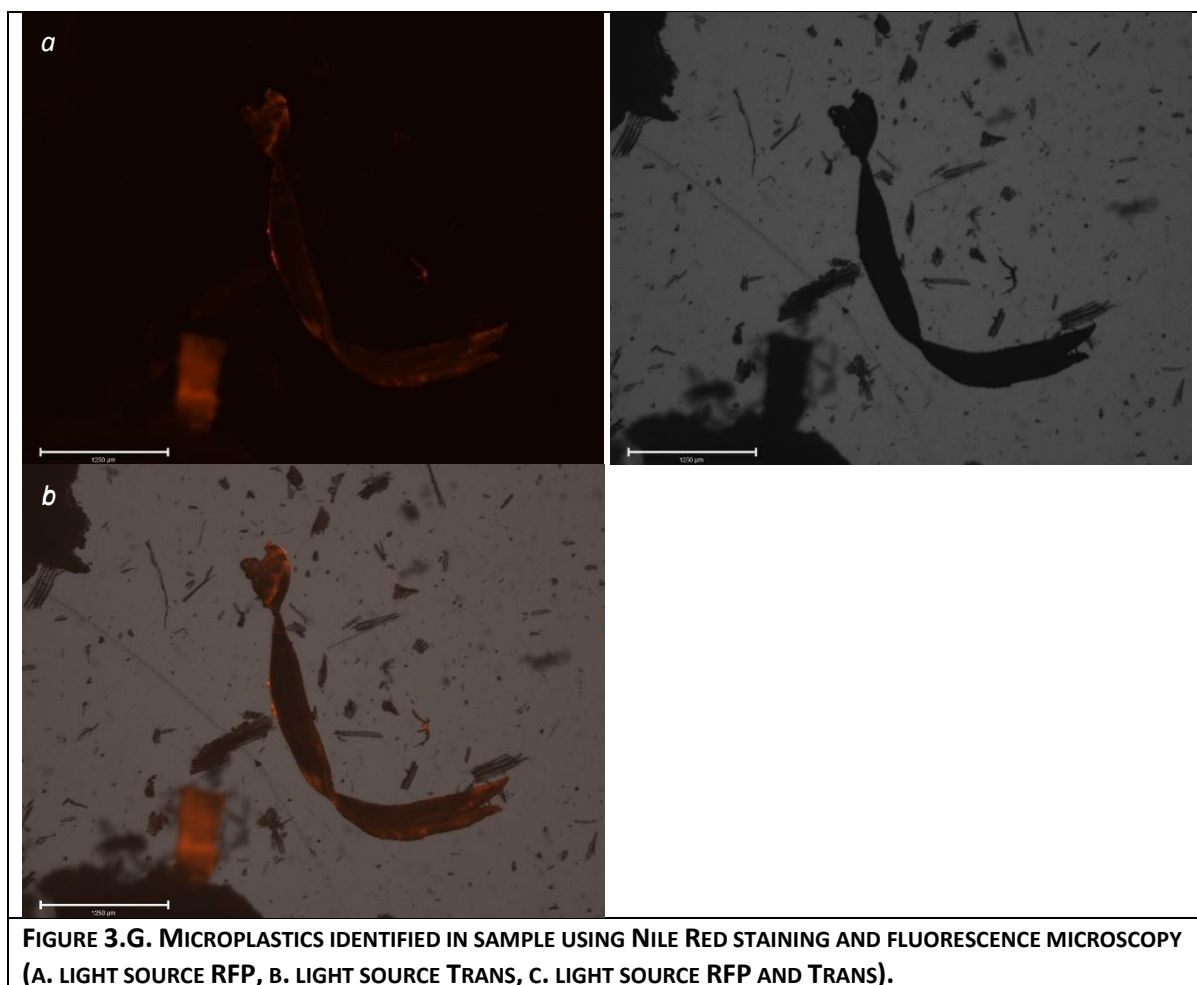
FIGURE 3.F. COMMON MICROPLASTICS OBSERVED IN FILTERED SAMPLES.

3.2.2.2. FLUORESCENCE MICROSCOPY

Where the number of extracted potential microplastic particles exceeded ten, fluorescence microscopy was used to distinguish between plastic particles and natural materials. Samples containing ten potential MPs were exposed to fluorescence microscopy to distinguish between synthetic and natural materials.

Nile Red staining was applied to potential MP samples as this binds to the surface of plastics causing them to fluoresce under an EVOS microscope when light cube red fluorescent protein (RFP) was applied. A concentrated solution of the Nile Red dye was created by dissolving 1 mg in 1mL of acetone (Maes et al., 2017). To each petri dish containing extracted potential microplastics 6mL of deionised water was added, followed by 0.083 mL of concentrated Nile Red dye solution to each sample allowing for dilution of the dye concentrate.

Samples were covered and left for two hours to allow the dye to bind to the particles before imaging was conducted. Samples were imaged using light cubes RFP and Trans which allowed images of fluorescence (RFP light cube) and no fluorescence (Trans light cube) to be captured (Figure 3.G). The use of Nile Red dye allowed for rapid MP identification with rates reported at ~98% in the literature and common polymers correctly identified including polyethylene, polypropylene, polystyrene, polycarbonate, polyurethane and poly(ethylene-vinyl acetate) (Shim et al., 2016). As a lipophilic dye, Nile Red allows for the staining of lipid materials such as polymers due to their hydrophobicity (Maes et al., 2017). In addition to this, Nile Red has the ability to stain polymers in contaminated samples which was beneficial as the samples of extracted potential microplastics sometimes contained contaminating particles such as sawdust (Shim et al., 2016).



3.2.2.3. POLYMER IDENTIFICATION USING CHEMICAL SPECTROSCOPY

When potential MP population exceeds 10 a 10% sample of potential microplastics 0.3-5 mm of size were randomly selected (based on size and shape to ensure a true representation of particles) for analysis using Fourier-transform infrared spectroscopy (FTIR spectrometry) to identify material composition (Agilent 4300 handheld FTIR spectrometer). FTIR was adopted as a technique for polymer identification due to the reliable and fast polymer identification of MPs >300 µm with an identification rate of 96% of particles (Käppler et al., 2016; Löder and Gerdts, 2015; Veerasingam et al., 2021). A diamond attenuated total reflectance (ATR) sample interface was used with the sample placed in contact with the diamond window to identify polymer type. ATR is beneficial for larger microplastic particles allowing for accurate readings in <60 seconds (Löder and Gerdts, 2015). The crystal was cleaned between samples to ensure no contamination. Material composition of the particles were recorded with a match rate ≥ 0.7 to ensure certainty on the spectral reading.

MicroLab PC was utilised with the qualitative search method to compare collected infrared spectrum from a sample to a predefined spectral library using the Library Search Demo ATR Library (Agilent Technologies, 2020). This method relied on the use of infrared radiation creating a spectrum for each sample which consisted of absorption peaks, corresponding to the frequency of bonds between atoms in the material (Löder and Gerdts, 2015; Veerasingam et al., 2021). Therefore, no two compounds produce the same infrared spectrum due to the unique atom combination of each material (Veerasingam et al., 2021). In addition to polymer identification, FTIR was used for the

identification of other molecular groups present in the material which allowed for the identification of non-synthetic materials in this study (Veerasingam et al., 2021).

3.3. STATISTICAL ANALYSIS

Statistical software MiniTab18 and RStudio was used for statistical analyses in this study. Anderson-Darling normality testing was used to test for normality of each data set prior to further statistical analysis due to the high level of power of this test with a smaller sample size (p value of >0.05 used to identify normal data) (Razali and Wah, 2011; Yazici and Yolacan, 2007). Descriptive statistics were calculated for each data set allowing for the identification of measures of central tendency and measures of dispersion within data.

Statistical differences were identified between MP concentrations in agricultural samples (silage, cattle pellets, sawdust bedding, slurry and soil) between sample sites through the application of a one-way ANOVA (parametric) or Kruskal-Wallis (non-parametric) test which was determined by the normality of the data set. A statistical significance between data sets was identified by a p value <0.05 . This allowed potential sources of MP contamination to be identified.

As an annex of the Students T-Test and Mann-Whitney U test, one-way ANOVA and Kruskal-Wallis tests allow for differences to be identified between continuous data collected from ≥ 3 categorical variables. Post-hoc tests were completed following a significant result to reduce type 1 error using a Dunn's post hoc test as this method accounts for tied ranks and is widely accepted in the literature to provide accurate results (Elliott and Hynan, 2011). This allows for clarity of where the differences lie between samples.

4. RESULTS

4.1. MICROPLASTIC CONCENTRATIONS IN UK DAIRY FARMS

Microplastics were observed in 28% of samples collected and analysed from sites in this study. MPs were observed in silage, cattle pellet, slurry and soil samples at Site A (Figure 4.A). MP concentrations were observed in silage, cattle pellets, sawdust bedding and slurry samples collected at Site B (Figure 4.B); and MPs were only measured in cattle pellet, sawdust bedding and slurry samples at Site C (Figure 4.C).

4.1.1. MICROPLASTIC CONCENTRATIONS AT SITE A

At Site A MPs were detected in high concentrations in slurry samples (mean MP concentration over monitoring period; 265 ± 185 MP/kg dry weight (dw)) with lowest concentrations measured in soil samples (mean MP concentration over monitoring period; 8 ± 20 MP/kg dw) (Figure 4.A). Highest concentrations of MPs were observed in slurry sample collected during sample collection 3 (529.59 MP/kg dw) leading to variability being observed within MP concentrations measured at Site A throughout the monitoring period.

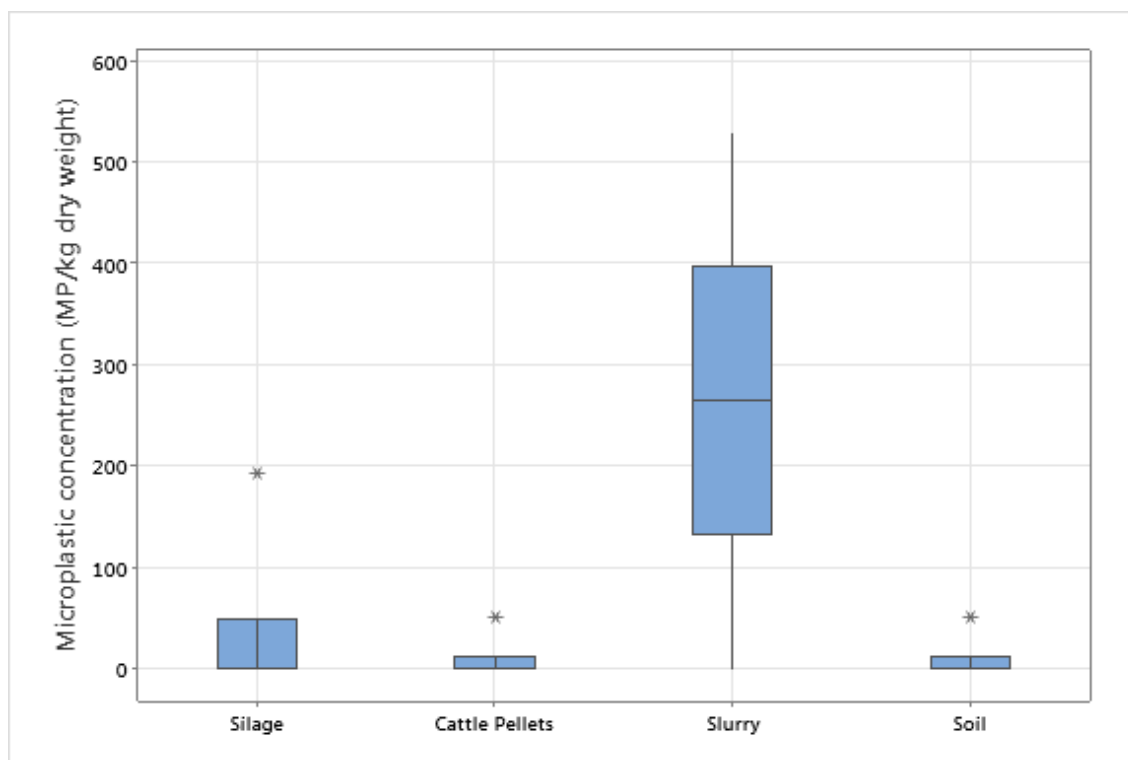


FIGURE 4.A. MICROPLASTIC CONCENTRATION COLLECTED IN AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE A.

4.1.2. MICROPLASTIC CONCENTRATIONS AT SITE B

At Site B MPs were most dominant in sawdust bedding samples across the monitoring period (average MP concentration over monitoring period; 662 ± 449 MP/kg dw) with lowest concentrations measured in cattle pellet samples (mean MP concentrations over monitoring period; 27 ± 45 MP/kg dw) (Figure 4.B). Highest MP concentrations were measured in sawdust bedding samples during collection 3 and 4 (1083 MP/kg dw) with sawdust bedding samples also showing the greatest range of MP concentration throughout the 6-month monitoring period (1083 MP/kg dw).

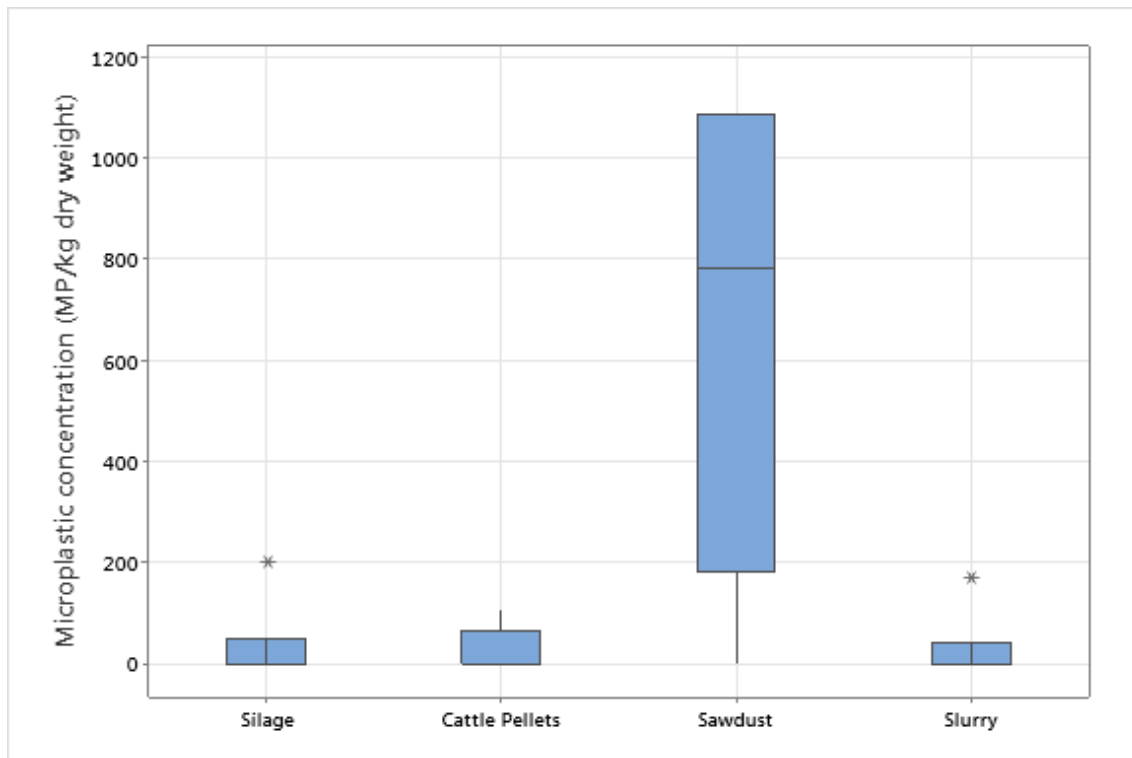


FIGURE 4.B. MICROPLASTIC CONCENTRATION COLLECTED IN AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE B.

4.1.3. MICROPLASTIC CONCENTRATIONS AT SITE C

MP concentrations were most dominant at Site C in sawdust bedding samples (mean MP concentration over monitoring period; 275 ± 207 MP/kg dw) (Figure 4.C). In samples where MP concentrations were recorded, lowest MP concentrations were measured in cattle pellet samples (mean MP concentration over monitoring period; 9 ± 23 MP/kg dw). Greatest MP concentration measured at Site C was collected during sample collection 2 (590 MP/kg dw). Largest variation in MP concentrations measured over the 6-month monitoring period at Site C were observed in sawdust bedding samples (472 MP/kg dw).

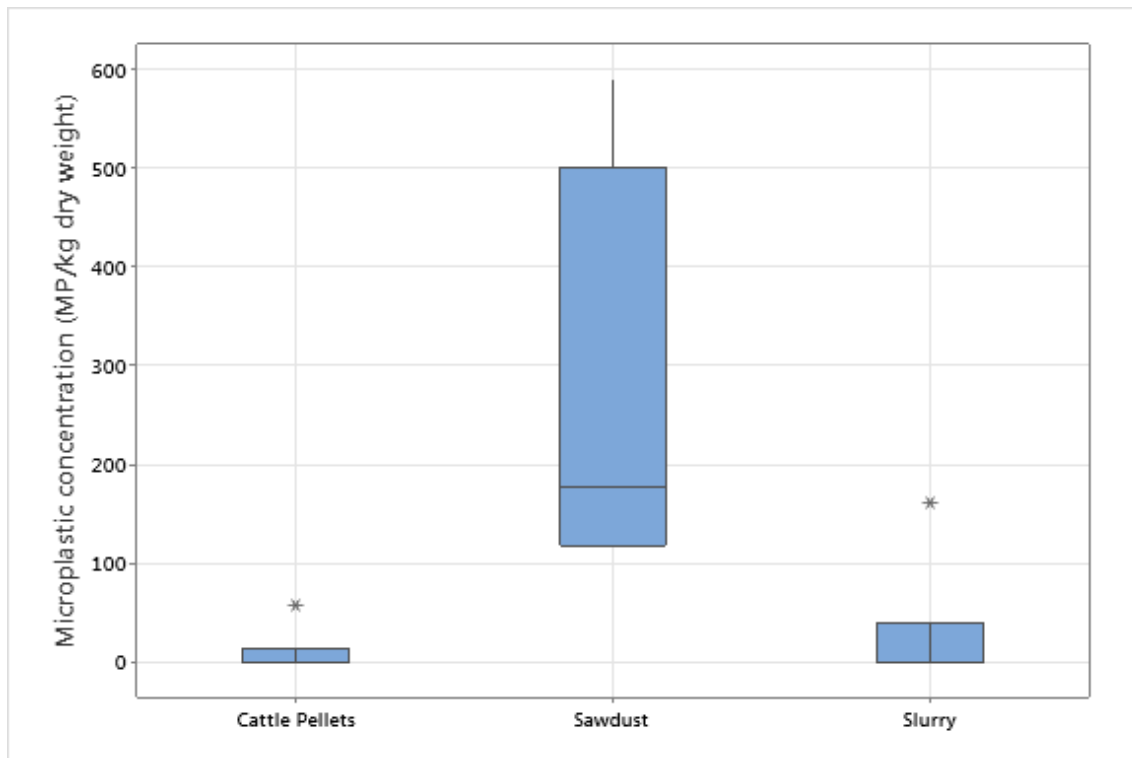


FIGURE 4.C. MICROPLASTIC CONCENTRATION COLLECTED IN AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE C.

4.1.4. DIFFERENCES BETWEEN MICROPLASTIC CONCENTRATIONS

Differences in MP concentrations between sites were observed in sawdust bedding samples collected at Site A and Site B (Dunn's test: $Z=-3.24$, $p=0.004$) as well as in MP concentrations measured in slurry samples between Sites A and B and Sites A and C (Dunn's test: $Z=2.65$, $p=0.02$; $Z=2.71$, $p=0.02$ respectively). No significant differences in MP concentrations were observed in silage, cattle pellet or soil samples between sites (Dunn's test: (a-b) $Z=-0.1$, $p=1$; (a-c) $Z=0.84$, $p=1$; (b-c) $Z=0.94$, $p=1$; (a-b) $Z=-0.85$, $p=1$; (a-c) $Z=-0.15$, $p=1$; (b-c) $Z=0.71$, $p=1$; (a-b) $Z=1.22$, $p=0.66$; (a-c) $Z=1.22$, $p=0.66$; (b-c) $Z=0.001$, $p=1$ respectively).

4.2. POLYMER TYPES IN AGRICULTURAL SAMPLES

Three dominant polymer types were measured; polystyrene (PS), polyurethane foam (PUF) and polyethylene (PE) with PS making up 46% of MPs measured in all samples. PUF were the most dominant MP measured and were primarily identified in slurry samples (56%) whereas PE and PS were most dominant in sawdust bedding samples (75% and 100% respectively) (Figure 4.D, 4.E, 4.F and 4.G).

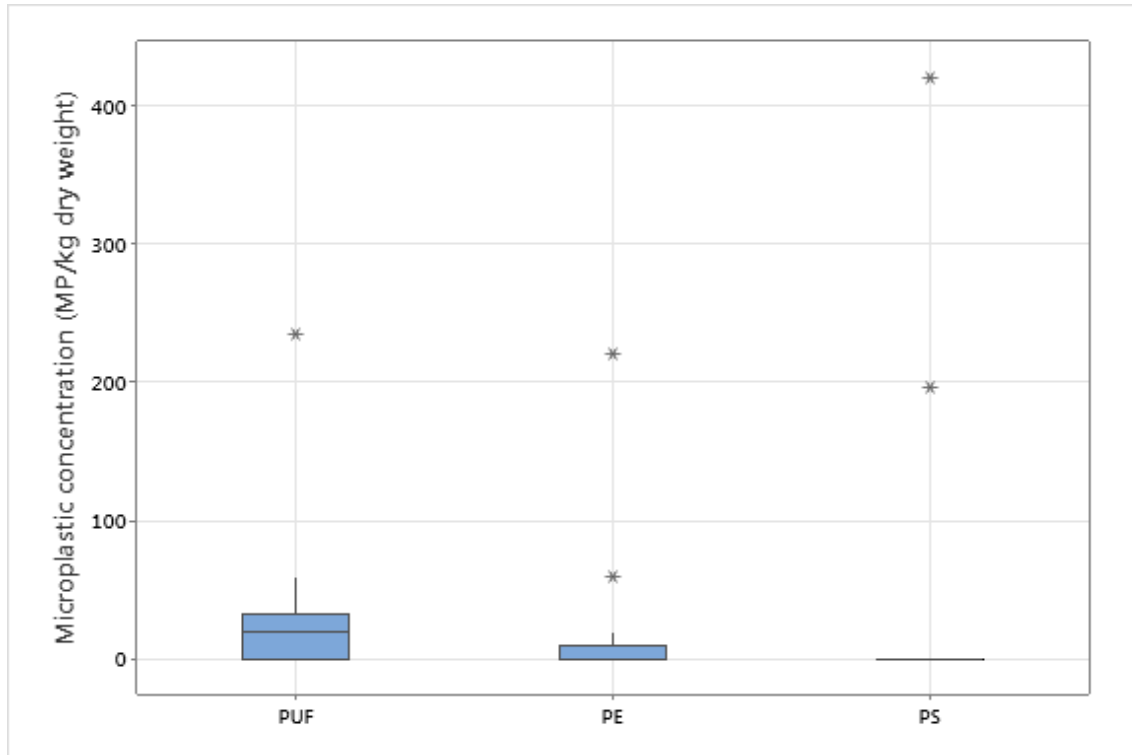


FIGURE 4.D. MEAN CONCENTRATION OF POLYMER TYPES (POLYURETHANE FOAM (PU) POLYETHYLENE (PE) AND POLYSTYRENE (PS)) MEASURED IN AGRICULTURAL SAMPLES COLLECTED OVER A 6-MONTH MONITORING PERIOD.

4.2.1. POLYURETHANE FOAM MICROPLASTIC CONCENTRATIONS

PUF was observed to be the most dominant polymer type accounting for 57% of polymers measured with this polymer type measured in the majority of samples collected throughout monitoring period (Figure 4.D and 4.E). Largest PUF concentrations were measured in slurry samples (mean MP concentration over monitoring period: 97 ± 120 MP/kg dw) with lowest concentrations observed in Site A cattle pellets (mean MP concentration over monitoring period: 12 ± 14 MP/kg dw) (Figure 4.E).

4.2.2. POLYETHYLENE MICROPLASTIC CONCENTRATIONS

PE MPs were identified in the lowest concentrations in all agricultural samples collected from sample sites (11% of identified MPs) and was identified in all agricultural samples excluding silage samples (Figure 4.D and 4.F). The largest proportion of PE MPs were recorded in sawdust bedding samples (mean concentration collected over monitoring period: 80 ± 122 MP/kg dry weight) (Figure 4.D and 4.F).

4.2.3. POLYSTYRENE MICROPLASTIC CONCENTRATIONS

PS was measured in high concentrations in sawdust samples collected at Site B and C leading to highly skewed data (Figure 4.D and 4.G). This polymer represents 32% of MPs measured in this study (mean concentration measured in sawdust bedding across all sites: 206 ± 211 MP/kg dry weight).

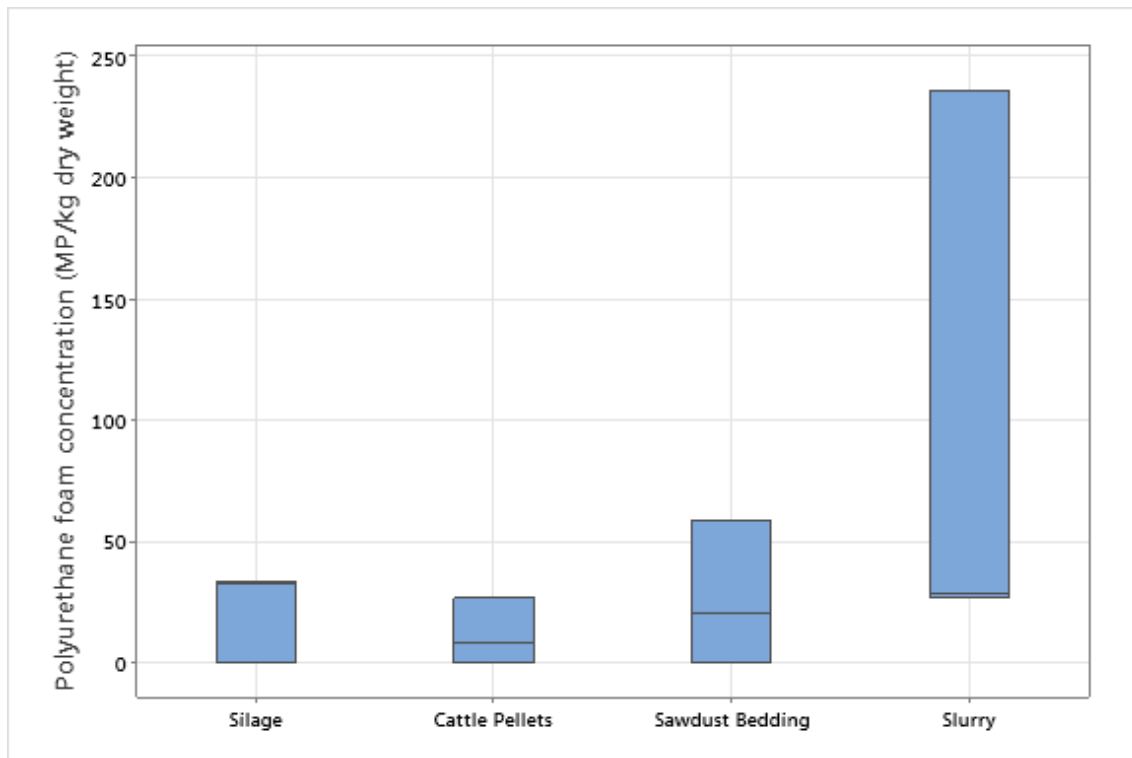


FIGURE 4.E. DISTRIBUTION OF POLYURETHANE FOAM (PUF) MICROPLASTICS IN AGRICULTURAL SAMPLES.

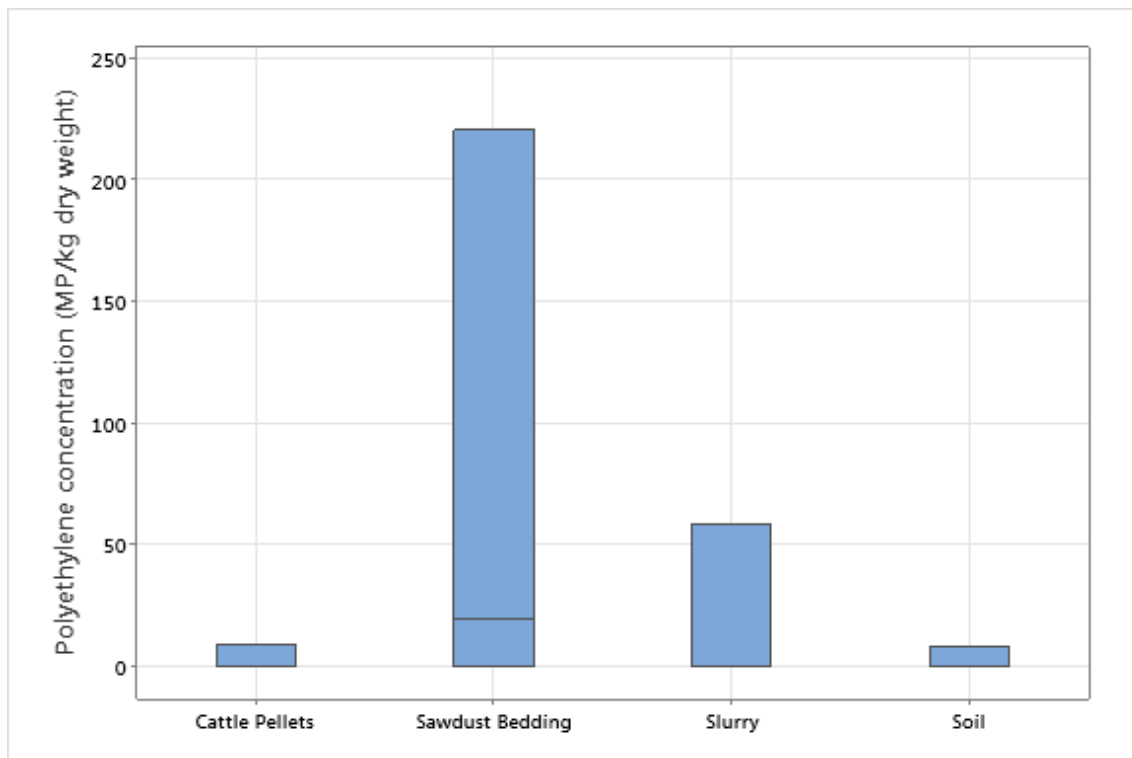


FIGURE 4.F. DISTRIBUTION OF POLYETHYLENE (PE) MICROPLASTICS IN AGRICULTURAL SAMPLES.

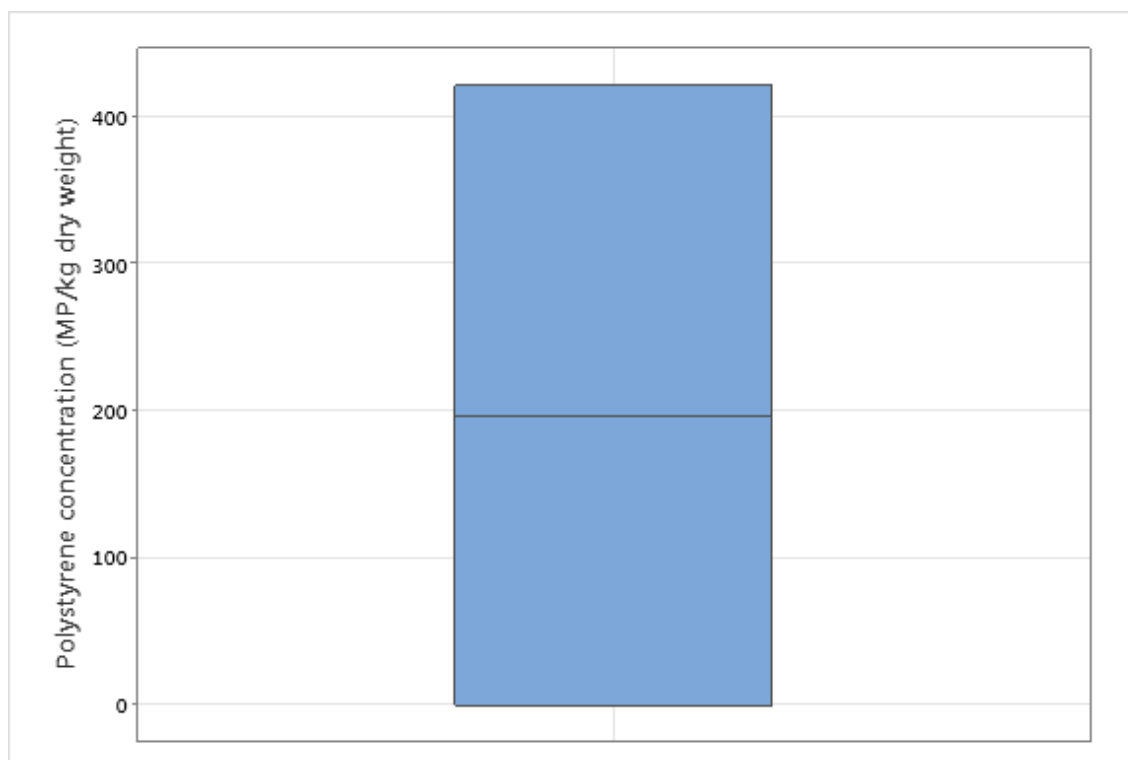


FIGURE 4.G. DISTRIBUTION OF POLYSTYRENE (PS) MICROPLASTICS IN SAWDUST BEDDING SAMPLES.

4.2.4. DIFFERENCES OF POLYMER TYPE CONCENTRATIONS BETWEEN SITES

Significant differences were only observed in PS concentrations measured in sawdust samples between Site A and B (Dunn's test: $Z=-3.30$, $p=0.003$). No differences were observed between PE and PUF concentrations measured in agricultural samples between sites.

4.3. MATERIALS IDENTIFIED IN CONTROL SOIL SAMPLES

Material extracted from control soil collected from each site was analysed under FTIR spectroscopy, measuring a limited number of materials with only two potential MPs extracted which were not identified to be polymer materials (Table 4.1).

TABLE 4.1. MATERIALS EXTRACTED FROM CONTROL SOILS COLLECTED FROM EACH SITE WAS ANALYSED UNDER FTIR SPECTROSCOPY.

Site	Description	Material
A	Film	Cardboard
B	Film	Wood

4.4. PARTICLE MORPHOTYPES OF EXTRACTED POTENTIAL MICROPLASTICS

Three particle morphotypes were identified in this study; film, fibre and fragment with film particles being the most dominant morphotype, making 50% of observed particle shapes in all samples collected over monitoring (Figure 4.H). Film, fibres and fragment particles were all dominant in sawdust bedding samples with measured concentrations recorded at 786 ± 681 particles/kg dw, 134 ± 96 particles/kg dw and 385 ± 381 particles/kg dw respectively whereas fibre particles were most dominant in slurry samples (Figure 4.I, 4.J and 4.K).

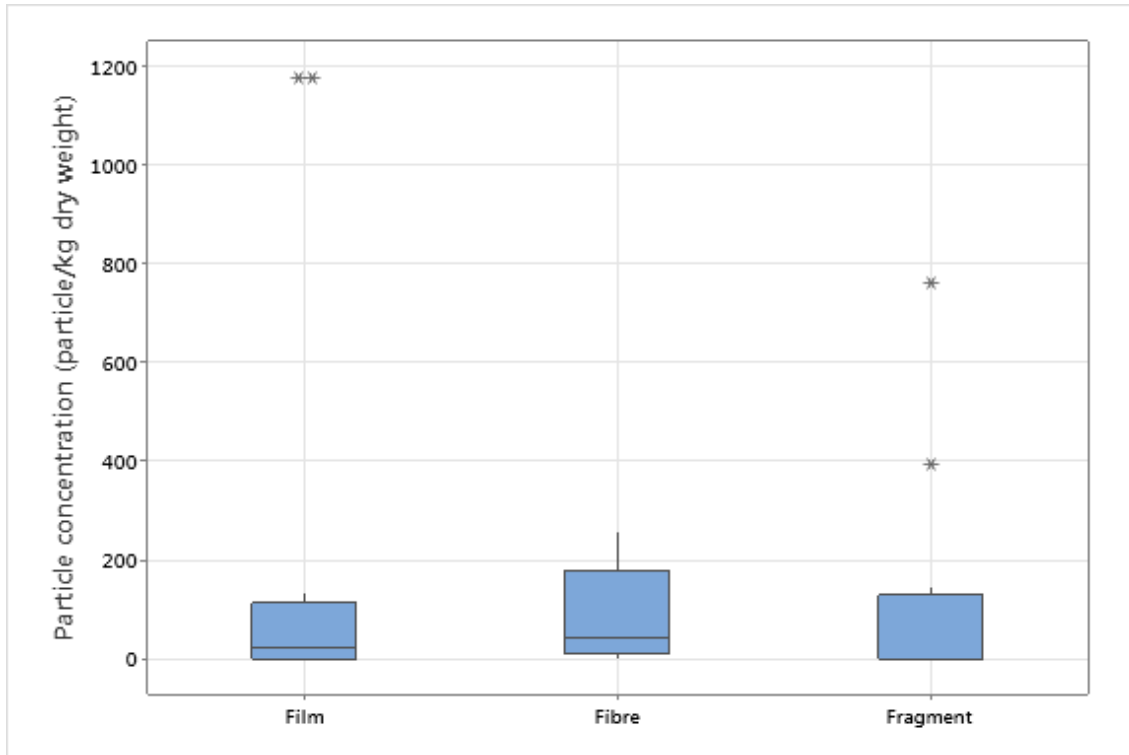


Figure 4.H. Distribution of particle shapes extracted as potential microplastics from agricultural samples from all sites over a 6-month monitoring period.

4.4.1. CONCENTRATIONS OF MICROPLASTIC FILMS

Films were measured as the most dominant particle shape across sample collection (mean particle concentration across all samples and sample sites: 187 ± 405 particles/kg dw) with 84% of these particles observed in sawdust bedding samples collected from Site B and C. Films were measured in cattle pellet samples in very small concentrations (3 ± 5 particles/kg dw).

4.4.2. CONCENTRATIONS OF MICROPLASTIC FIBRES

Fibres were measured in the smallest concentration across all samples and sample sites (mean concentration: 82 ± 90 particles/kg dw) (Figure 4.H). This morphotype was most abundant in sawdust bedding samples (mean particle concentration across all sample sites: 134 ± 96 particle/kg dw) (Figure 4.J). In sawdust bedding samples, fibres were most prevalent in samples collected from Site C (49% of fibre particles measured across sites).

4.4.3. CONCENTRATIONS OF MICROPLASTIC FRAGMENTS

Fragments were measured in large concentrations in sawdust bedding samples collected across sites over monitoring with overall mean concentration measured across all samples and sites 104 ± 210 particles/kg dw (Figure 4.H). Site A was recorded to have the largest concentration of fragment particles in samples with 100% of fragment particles collected from soil samples from Site A.

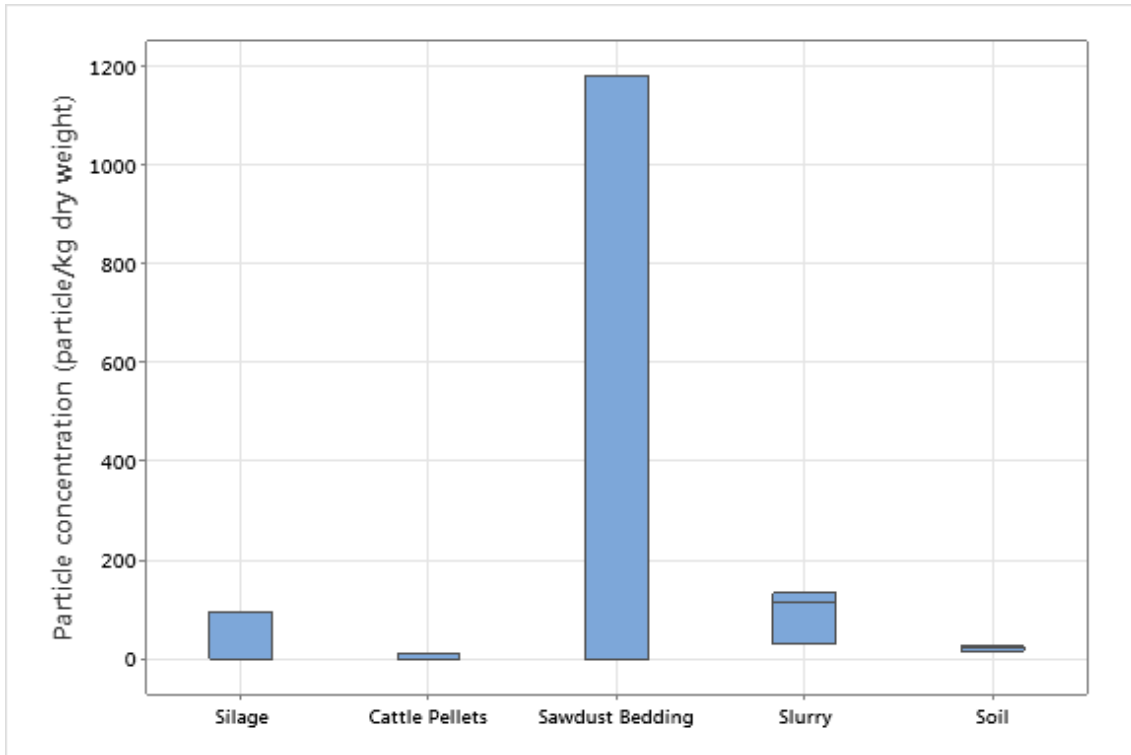


FIGURE 4.I. DISTRIBUTION OF FILM PARTICLES IN AGRICULTURAL SAMPLES AT ALL SITES COLLECTED OVER MONITORING PERIOD.

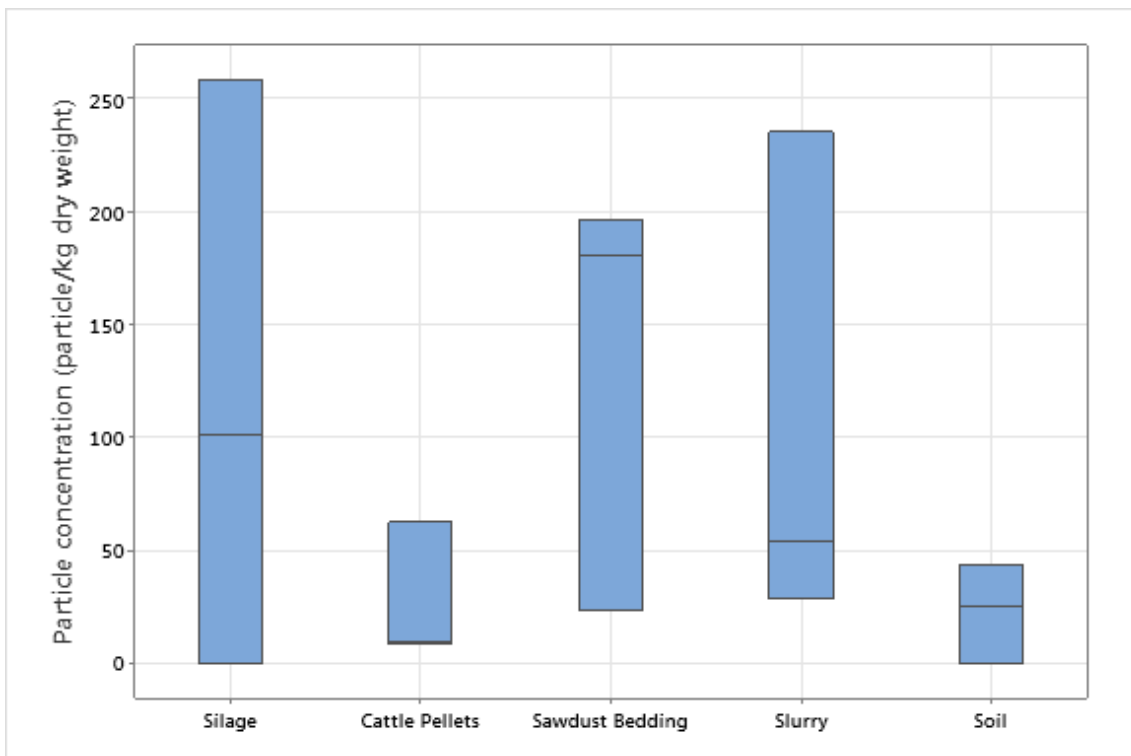


FIGURE 4.J. DISTRIBUTION OF FIBRE PARTICLES IN AGRICULTURAL SAMPLES AT ALL SITES COLLECTED OVER MONITORING PERIOD.

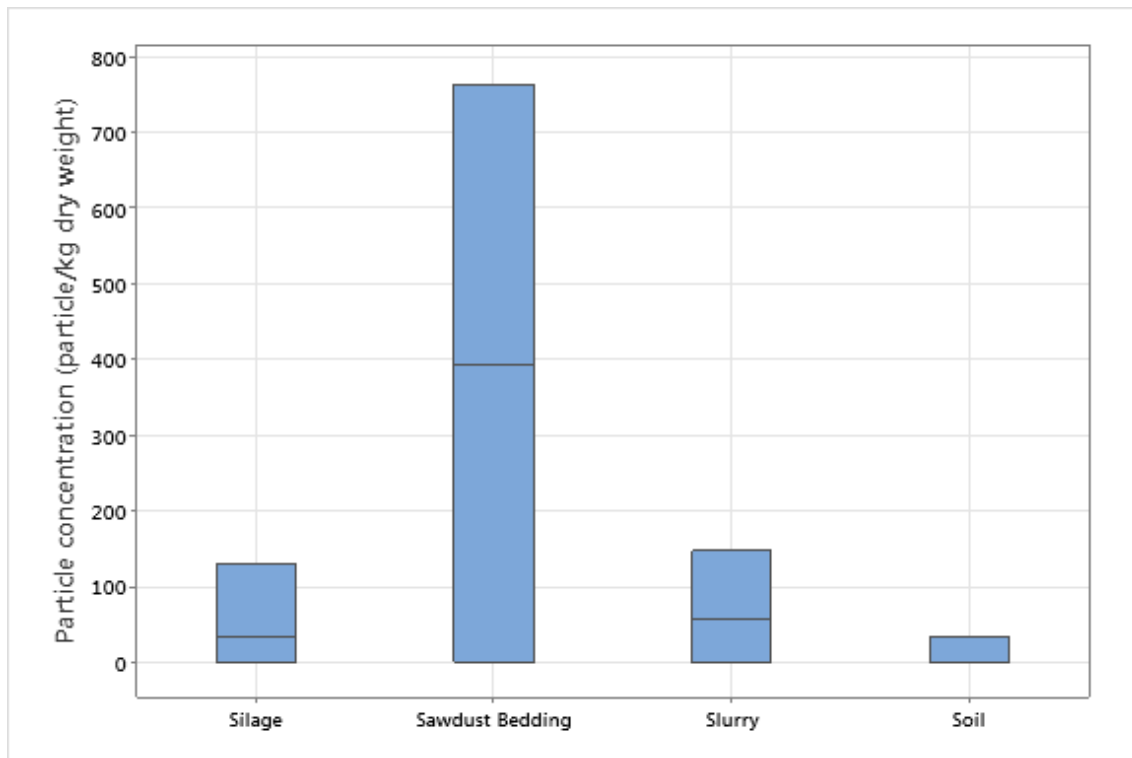


Figure 4.K. Distribution of fragment particles in agricultural samples at all sites collected over monitoring period.

4.4.4. DIFFERENCES OF MICROPLASTIC MORPHOTYPES BETWEEN SITES

Significant differences were observed in film concentrations in sawdust samples between Site A and B and between Site A and C (Dunn's test: $Z=-3.26$, $p=0.003$; $Z=-2.71$, $p=0.02$ respectively) as well as in slurry samples between Site A and B and between Site A and C (Dunn's test: $Z=-3.26$, $p=0.003$; $Z=-2.71$, $p=0.02$ respectively). Significant differences were also observed in fragment concentrations measured in sawdust bedding samples between Site A and B (Dunn's test: $Z=-3.46$, $p=0.002$) as well as fragment concentrations measured in slurry samples between Site A and B (Dunn's test: $Z=-3.46$, $p=0.002$). No differences were observed between fibre concentrations measured in agricultural samples between sample sites.

4.5. MICROPLASTIC IDENTIFICATION TECHNIQUES

4.5.1. DIFFERENCES BETWEEN MICROPLASTIC CONCENTRATIONS OBTAINED FROM FLUORESCENCE MICROSCOPY AND FTIR SPECTROSCOPY

Fluorescence microscopy was used prior to FTIR spectroscopy to differentiate between polymers and non-plastic materials extracted as potential MPs from samples. Variation in MP concentrations were observed following the application of the two MP identification techniques (mean MP concentration collected over 6-month monitoring period in Site A slurry samples: FTIR= 265 ± 185 MP/kg dw; fluorescence= 1491 ± 1898 MP/kg dw) (Figure 4.L-4.Q). No significant differences in MP concentrations derived using these techniques were observed.

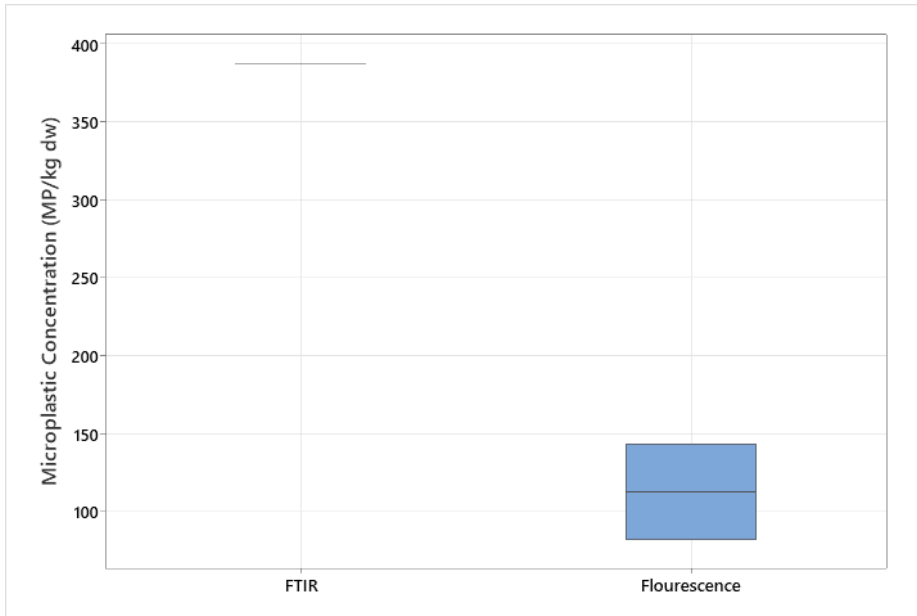


Figure 4.L. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from silage samples at Site A over a 6-month monitoring period.

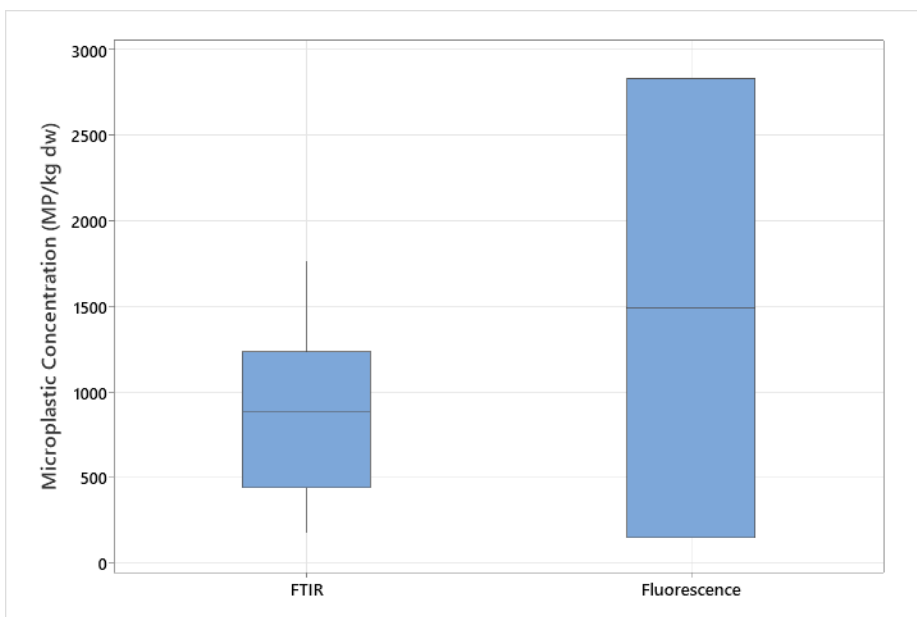


Figure 4.M. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from slurry samples at Site A over a 6-month monitoring period.

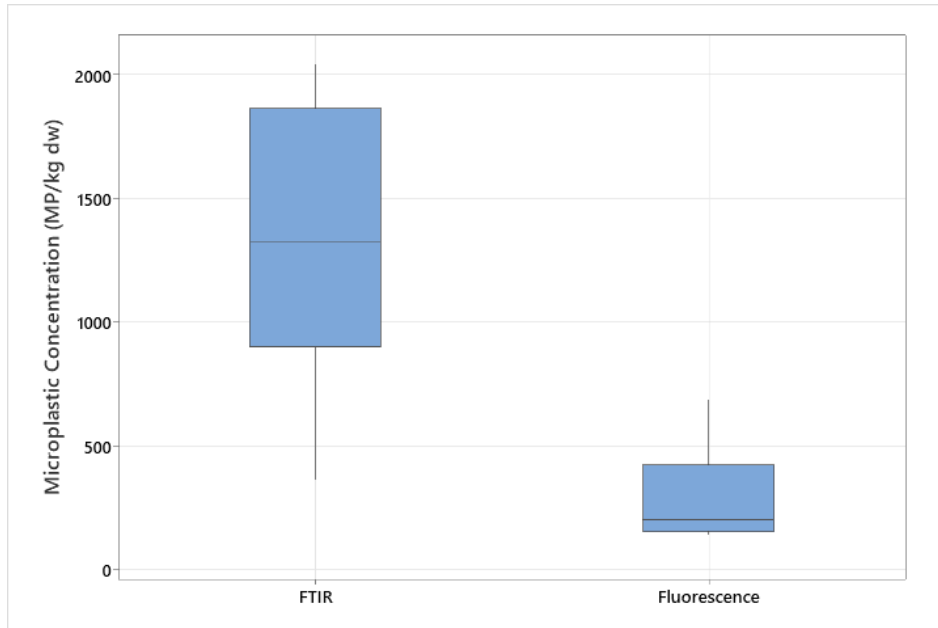


Figure 4.N. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from cattle pellet samples at Site B over a 6-month monitoring period.

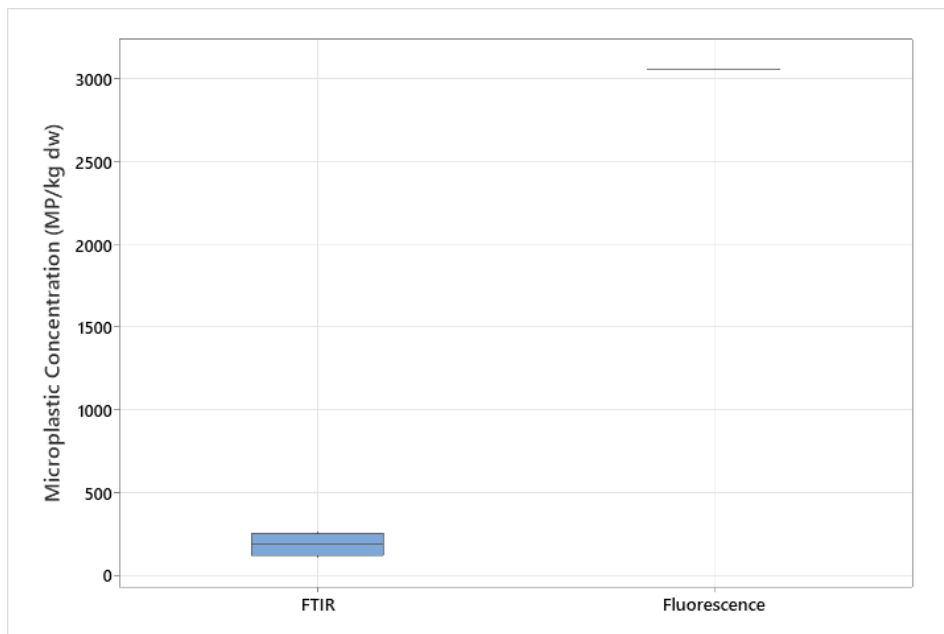


Figure 4.O. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from sawdust bedding samples at Site B over a 6-month monitoring period.

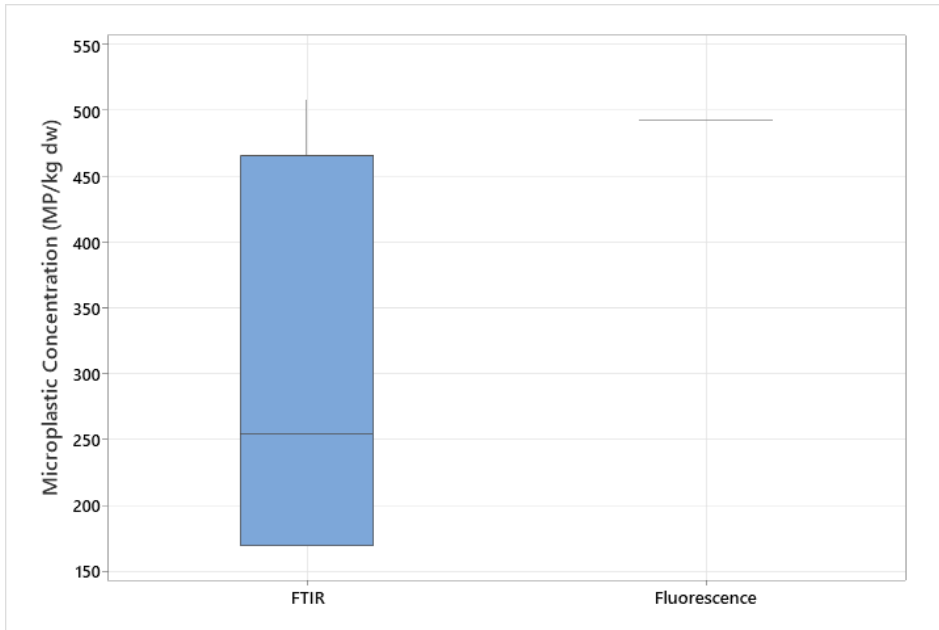


Figure 4.P. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from slurry samples at Site B over a 6-month monitoring period.

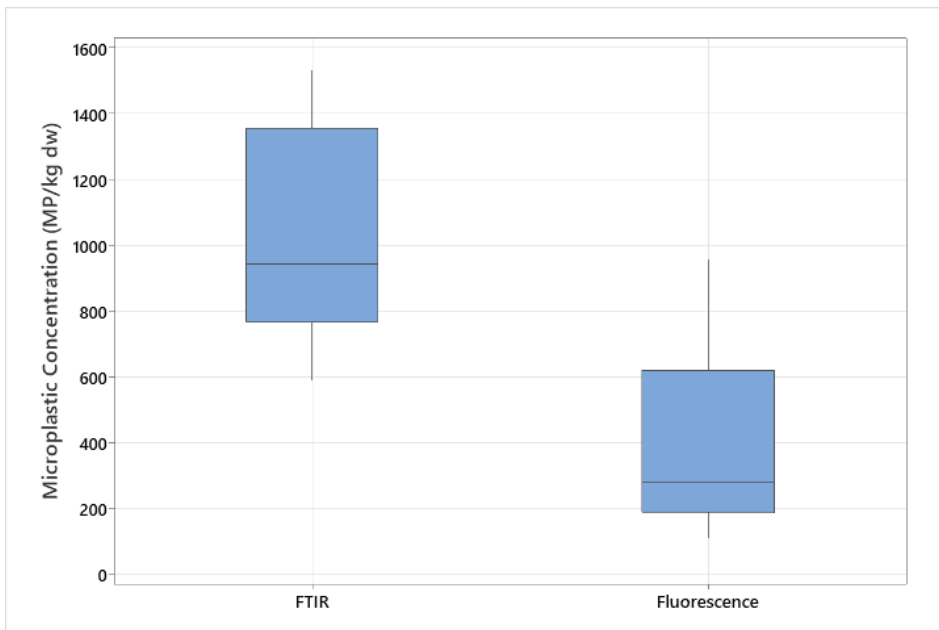


Figure 4.Q. Difference in microplastic concentration results following identification using fourier-transform infrared spectroscopy and fluorescence microscopy after extraction from sawdust bedding samples at Site C over a 6-month monitoring period.

5. DISCUSSION

5.1. MICROPLASTIC CONCENTRATIONS IN AGRICULTURAL SAMPLES

Plastics are omnipresent in an agricultural setting. This has resulted in MP contamination throughout agricultural samples due to the use of plastics in the storage and preservation of feeds to rubber mattresses in the housing process of cattle (NFU, 2021; Piehl et al., 2018; RSPCA, 2017). The inadvertent decomposition and distribution of plastic materials through the agricultural system allows for the contamination of plastics in these environments due to difficulties surrounding the recycling of agricultural plastics (Tovey, 2020; Wu et al., 2021). Based on results of this study livestock bedding has the largest impact of the introduction of MPs into the agricultural sector leading to the potential transfers of these into livestock slurries which are later transferred into soils through the spreading of these slurries, partly supporting H₁. However, the transfer pathways allow for only small quantities of MPs to be transferred between each matrix suggesting that MPs are also stored in these matrices. The UK dairy and beef industry produce the greatest volume of slurry in Great Britain (DEFRA, 2021). Therefore, it is imperative for studies such as this to ensure that these manures and slurries can be applied to agricultural soils without the risk of causing pollution through the transfer of MPs into these environments.

5.1.1. LIVESTOCK FEED

To date there is very little research on the contamination with MPs of cow feeds with results in this study making a significant contribution to field. Microplastics were measured in livestock feed (silage and cattle pellets) at all sites (50-201 MP/kg dw). Higher concentrations were measured in this study than previously reported, e.g. concentrations in cow feeds of 36-110 MP/kg wet weight (Wu et al., 2021). Cow feeds have been suggested to contain the lowest quantity of MP contamination in comparison to pig feeds with concentrations between 139-333 MP/kg wet weight which still remain lower than concentrations measured in cow feeds in this study (Wu et al., 2021). Disparity between results may be caused by potential variation in laboratory analysis and the use of wet samples in other studies. Lower concentrations of MPs in cow feeds in comparison to pig feeds may be due to the use of fewer plastic products during the farming process of cows with the main use of plastics being during the storage of feeds through polymer storage bags, bale and silage wrap, bale twine and silage sheeting (NFU, 2021; Wu et al., 2021).

A large proportion of polymers identified in livestock feed samples were PUF microfibrils. PUF has not been suggested to be commonly used in the feeding process of pastoral agriculture however, this material is commonly used in the production of rubber mattresses used in the housing process at all sites in this study (Easyfix, 2021). The degradation and distribution of this material within the cattle barn may have resulted in inputs of PUF into the livestock feed. PE was also found as was the case in a previous study of cow feeds which is a widely used polymer in the agricultural sector due to its cost-effectiveness and high durability making it beneficial for silage wrap (Business Wales, 2022; Wu et al., 2021). However, due to the presence of PE in cattle feed pellet samples rather than silage samples it can be assumed that silage wraps were not the source of contamination in feed samples.

Suggested pathways of MPs into livestock feeds include contamination from storage bags or plastic residue from bale film and twine that remains in silage causing the ingestion or inhalation of smaller MP particles (Food and Agricultural Organisation of the United Nations, 2021; Wu et al., 2021). However, this transport pathway is unlikely in this study as feed storage bags at the sites assessed were composed of PP which was not measured in feed samples. The UK Food Standards Agency allows for contamination of food grade plastic packaging waste in livestock feed at quantities of 0.15% by weight (Waste and Resource Action Programme, 2016). Food waste that can be reused for

livestock feed is often sent to a de-packaging plant where the contents are removed from the packaging (Waste and Resource Action Programme, 2016). However, there are limited numbers of these facilities in the UK making it difficult for the complete removal of plastics, therefore livestock feed can be expected to contain MP contamination (Balkenhoff, 2009; Waste and Resource Action Programme, 2016). From results of this study, it can be assumed that MPs present in livestock feeds are transferred through the system, however, this is not the sole source of MPs in the agricultural system (Beriot et al., 2021).

5.1.2. LIVESTOCK BEDDING

This is the first study to measure MP contamination in livestock bedding therefore comparison with other studies is not possible. Largest concentrations of MPs were recorded within livestock bedding samples (concentrations up to 1083 MP/kg dw) with polymers identified including PUF, PS and PE and morphotypes of fibres, film and fragments observed. Livestock bedding in this study has been identified as a key source of MP contamination into UK dairy farms.

MP concentrations may be elevated at Site B and C due to the origin of the bedding materials. All three sites use a rubber mat flooring in livestock cubicles topped with sawdust bedding. However, differences in sawdust supplier between sites may have led to variation in the MP concentration observed. Site A contained the lowest MP concentration in bedding samples with this product obtained from a by-product of timber from a kitchen manufacturer. The supplier for sawdust bedding at Site B and C was the same with this product obtained from offcuts from waste kitchen counter tops. This is likely to have resulted in the presence of MPs observed in livestock bedding samples collected at these sites with similarities between polymer types and concentrations. Polymers are common materials used in the construction of kitchens with identified materials including laminated (contain polycarbonates, acrylic and urethane which may be comprised of a layer of PUF), PVC, polyester, high impact PS and MDF (Castrucci, 2020; Eagle Plastics, 2022; Gallagher et al., 2014; Kelly, 2018; Nangalia, 2022). The reuse of waste products such as these has often been considered a vital stage in our movement towards a more circular economy, however, pollutant levels recorded in these waste products raise questions surrounding the sustainability of the use of these materials within industries such as agriculture (Ellen Macarthur Foundation; 2017). Despite being considered as a considerable input of MP contamination into UK dairy farms the ability for these MPs to be transferred into slurries and soils cannot be certain due to differences in polymer compositions throughout agricultural matrices with all MPs observed in limited concentrations in slurry and soil samples. This could suggest alternative exposure routes for plastics to enter these matrices. However, following the identification of large MP concentrations in sawdust bedding, particularly at Site B and C where waste materials are used, it is important to consider if the benefits of reusing waste products in the agricultural sector outweigh the potential negatives associated with the potential input of MPs into these environments. In order for by-products such as waste kitchen offcuts to be used sustainably in the agricultural sector it is vital that further research is done assessing MP concentrations in various sources of livestock bedding to develop understanding of potential exposure rates to the environment as a result of the use of these products.

5.1.3. LIVESTOCK SLURRY

Slurry samples collected from all sites were observed to contain MP contamination ranging from 162-530 MP/kg dw which were much greater than those currently reported in the literature. A similar study measured concentrations in manure from cows at levels of 74 ± 63 MP/kg wet weight with other studies measuring no MP contamination in cow manures (Wu et al., 2021; Zhang et al., 2022a). Similar to other agricultural samples collected, differences between results may be due to

variation in laboratory methods chosen (see chapter 5.2). MPs measured in slurries in this study were identified as PUF and PE with dominant morphotypes being film and fragments. These polymer types were not observed in other studies with PP microfibrils measured in cow manures (Wu et al., 2021).

It has been suggested that the entrainment of MPs through the digestive tracts of livestock following ingestion of contaminated feeds results in MP contamination in manures (Wu et al., 2021; Zhang et al., 2022b). In this study MP contamination present in slurries is not likely due to contaminated feeds due to few MPs measured in silage and cattle feed pellets throughout sites. However, there is the potential of MPs present in contaminated livestock bedding to be ingested or inhaled by livestock or transported via wind dispersion within the livestock barn leading to MP contamination in slurries. Disparity between MP concentration between livestock types may be due to differences in digestion processes (Zhang et al., 2022a). The lengthy digestion process of a cow may lead to the more thorough breakdown of plastics (Zhang et al., 2022a).

Additional routes of contamination can include the use of a manure scraper or through contamination of plastic piping used to transport feed and water through the farmyard (Wu et al., 2021). MP contamination from the use of a manure scraper is unlikely in this study as all sites used a stainless-steel scraper. Therefore, it is important to consider alternative MP exposure routes such as the use of plastic materials throughout the agricultural system with future studies conducting a more in-depth analysis of all plastic materials used in a farmyard such as storage bags and plastic piping for the transport of feeds. The observation of smaller MP concentrations in cow manures may allow for these to be used as a safer alternative to organic fertilisers that use other livestock manures such as pig (Zhang et al., 2022a).

5.1.4. AGRICULTURAL SOIL

MP concentrations were observed in agricultural soils exposed to the application of slurries at concentrations up to 49 MP/kg dw which is within the range of concentrations in agricultural soils presented in the literature at 0.34-18,800 MP/kg dw (Piehl et al., 2018; Zhang and Liu, 2018). This large variation can be due to the range of agricultural practices applied including the application of fertilisers, use of plastic mulches and films alongside proximity to urban settlements which will increase atmospheric deposition of MPs into soils (Kumar et al., 2020; Piehl et al., 2018; Zhang and Liu, 2018; Zhang et al., 2020a). Negligible MP concentrations were measured in soils where no livestock slurries were applied highlighted by particles in control soil samples composed of cardboard and wood. Similarities in polymer type and morphotype of MPs recorded in slurries and soils suggest the application of livestock slurries is a key exposure route to soils and thus supports H₂.

PUF was the dominant polymer identified in this study which has not been observed in other studies (Zhang et al., 2020a). Common polymers identified in the literature include PP (59% of identified polymers), PE (18% of identified polymers) and PET (18% of identified polymers) with only PUF and PE observed in this study (Zhang et al., 2020a). PE MP fragment concentrations in soils were measured at 49 MP/kg dw at Site A with this morphotype also observed in similar studies (Zhang et al., 2022b). This has been suggested to be due to the presence of MPs in organic fertilisers obtained from the recycling of sewage sludges that are applied to agricultural soils as well as the use of plastic mulches and films which commonly use PE (Guce, 2018; Piehl et al., 2018). However, as soils used in this study were not exposed to the application of plastic mulches PE MP concentrations were expected to be lower. Fibres were observed as the dominant MP morphotype in agricultural soils (99 MP/kg dw), likely due to atmospheric deposition or their presence in sewage sludge (Dris et al.,

2016; Zubris and Richards, 2005). Due to no application of sewage sludge onto soils in the current study it can be assumed that other sources are dominant such as the atmospheric deposition.

Elevated MP concentrations observed at Site A may be a resulting factor from the application of digestate fertilisers, a nutrient-rich compound created through the anaerobic digestion of waste materials (NNFCC, 2022). Concentrations in soils have been measured between 546 – 3500 MP/kg in soils after the application of fertilisers with differences observed due to variation in quantity of fertiliser applied and period of application (Corradini et al., 2019; Zhang et al., 2020a). Observations of larger MP concentrations in soils have been suggested to be due to elevated periods of time of application of organic fertiliser with MP concentrations measured at 3.5 MP/g after fertiliser was applied to soils for five years, however, concentrations were measured at 2.5 MP/g after application for two years (Corradini et al., 2019; Zubris and Richards, 2005). The introduction and retention of MP particles in soils due to the application of composts and sludges have been highlighted through studies measuring MP concentrations in soils up to 15 years after initial application which may be exacerbated by the fragmentation of MPs over time through physical and mechanical processes allowing for incorporation and redistribution of MPs in soils over time (Piehl et al., 2018; Zhang et al., 2022b; Zubris and Richards, 2005).

5.1.5. OTHER MATERIALS MEASURED

In addition to the observation of polymers in agricultural matrices materials including cardboard and ammonium hydroxide solution were measured in large concentrations at sites. Cardboard is a widely used material and is expected to have no significant impact on the agricultural system due to its ability to biodegrade (Radu et al., 2021). Ammonium hydroxide solution is a common chemical used in the agricultural sector in the production of chemical fertilisers leading to large concentrations measured in this study (Wang et al., 2017). However, very little research on the impacts of ammonium hydroxide has been complete highlighting the need for further studies in order to gain full understanding of potential impacts on the environment.

5.2. MICROPLASTIC CONCENTRATIONS IN AGRICULTURAL SAMPLES

5.2.1. EVALUATION OF MICROPLASTIC EXTRACTION METHODS

5.2.1.1. DENSITY SEPARATION

Elevated MP concentrations in agricultural samples observed in this study in comparison to similar studies could be due to variation in MP extraction methods caused by a lack of standardised methods (Rolsky et al., 2020). Based on results obtained during a preliminary study identifying high quantities of polycarbonate MPs in samples, monosodium phosphate (NaH_2PO_4) was chosen as a solid chemical for density separation due to higher densities of polycarbonate (1.22 g/cm^3) (British Plastic Federation, 2022). However, during the monitoring period of this study, no polycarbonate was identified with the dominant polymer being polyurethane foam. The application of higher density solutions during density separation for MP extraction has been suggested to elevate MP recovery rates by 10-17% with commonly used salts including NaCl (density 1.2 g/cm^3), ZnCl_2 (density $1.5\text{-}1.9 \text{ g/cm}^3$), NaI (density $1.5\text{-}1.8 \text{ g/cm}^3$) and NaH_2PO_4 (density $1.4\text{-}1.45 \text{ g/cm}^3$) (Lusher et al., 2020; Maes et al., 2017; Monteiro and da Costa, 2022; Zhang et al., 2020b). Despite benefits associated with elevated densities and recovery rates of ZnCl_2 and NaI, these saline solutions pose environmental concern due to their ability to be corrosive, react with organic matter and form a foam, therefore NaH_2PO_4 was used to achieve a high-density solution without environmental concern (Monteiro and da Costa, 2022; Zhang et al., 2020b).

NaCl has been widely used throughout MP extraction studies due to its low cost, accessibility and is not toxic to the environment, however, it is only effective in the removal of low-density polymers such as PE, PS and PP, excluding 20% of plastics in Europe such as PET and PVC which may have densities of up to 1.45 g/cm^3 (He et al., 2021; Lusher et al., 2020; Monteiro and da Costa, 2022). For polymers with a higher density, calcium chloride (CaCl_2) may be used at a density of 1.4 g/cm^3 as this is non-hazardous and relatively low cost (Lusher et al., 2020). A density of 1.37 g mL^{-1} has been suggested to provide effective MP extraction through density separation (achieved using NaH_2PO_4 at a density of 1.35 g/cm^3) whilst minimising the interference of particulates (Maes et al., 2017). However, due to the presence of predominantly low-density polymers in this study, the use of NaCl for the density separation of MPs from agricultural samples in future studies in this field may be more cost-effective.

The use of multiple saline solutions can elevate MP extraction rates from 30.2% to 88.7% when four extractions are conducted (Lusher et al., 2020). Alternative extraction methods for density separation can include the use of oil extraction with castor oil having an extraction rate of 55-100% in soil and sediment samples (Lusher et al., 2020; Monteiro and da Costa, 2022). However, a digestion process is required prior to this in order to remove OM from samples as well as the need for particle cleaning to prevent interference with identification methods (Monteiro and da Costa, 2022). Limitations with density separation include losses of MPs in the environmental matrix due to the alteration of MP density caused by attachment to OM (Lusher et al., 2020). To overcome this, samples containing high OM concentrations should undergo visual sorting of settled sediment which was applied during this study (Lusher et al., 2020).

5.2.1.1. FILTRATION AND VISUAL SORTING

After density separation, samples were filtered using vacuum filtration which has been a technique adopted in studies measuring smaller MP particles (Rolsky et al., 2020). The replacement of vacuum filtration with sieving can allow for MP extraction of particles 400 - 200 μm in size, however, this could lead to the overlooking of smaller MP particles (Rolsky et al., 2020). This may be beneficial for this study as it may have removed contamination from other material remaining in the sample after density separation and filtration. As well as this, smaller MPs were greatly overlooked during the visual observation stage of this study due to contamination of other particles causing large background noise as visual sorting is only feasible for particles $>500\mu\text{m}$ (Lusher et al., 2020).

5.2.1.2. DIGESTION

A large proportion of MP extraction studies use a digestion stage in order to remove OM from samples. However, due to limitations, a digestion process was ineffective in this study due to high OM with high levels oil and fat contents as well as potential damage (distortion and discolouration) to MPs when exposed to heat, hydrolysis or oxidation (Lusher et al., 2020; Monteiro and da Costa, 2022). However, impact on MPs is varied and can be linked to concentration and type of digestion solution used, exposure time, temperature and morphotype of the MP (Monteiro and da Costa, 2022).

Fenton's reagent coupled with 30% H_2O_2 is considered to have minimal impact on MP with 10% KOH and Fenton's reagent being beneficial for the removal of difficult OM in samples (Monteiro and da Costa, 2022). If this study were to be repeated under less time constraints the consideration of a digestive process may be beneficial due to benefits associated with the removal of biofilms on plastic surfaces elevating accuracy of identification methods which can be aided through the addition of distilled water into samples of extracted particles (Lusher et al., 2020; Rolsky et al., 2020). However, it is important to consider potential impacts an extended exposure time to digestive chemicals could have on MPs. A digestion could be beneficial after filtration to reduce OM

content remaining on filter paper, making visual observation of MPs easier as well as reducing interference with FTIR results due to the presence of residue on particle surfaces (Fan et al., 2021).

5.2.2. EVALUATION OF MICROPLASTIC IDENTIFICATION METHODS

5.2.2.1. FTIR SPECTROSCOPY

Concentrations of MPs were identified in this study between 0 – 7.94 x 10³ MP/kg dw in agricultural samples which are overall greater than concentrations observed in other studies. This may be due to laboratory methods and analysis applied in this study. The analysis of MPs in this study was conducted using ATR-FTIR spectroscopy, a widely applied polymer identification technique for MPs >50 - 10 µm in environmental samples (De Frond et al., 2022; Shim et al., 2017; Tirkey and Upadhyay, 2021). FTIR spectroscopy can be a highly advantageous method for polymer identification with accuracy levels of up to 95%, being non-destructive and its reliability in producing limited false results (De Frond et al., 2022; Shim et al., 2017; Tirkey and Upadhyay, 2021). In addition to the identification of polymer type, FTIR is highly effective at measuring non-plastic materials as demonstrated in this study where cardboard concentrations were recorded at 99 – 4710 (De Frond et al., 2022). Visual pre-sorting of MP particles is not always necessary for FTIR spectroscopy with MPs being analysed on filters, however, this was not possible in this study due to high OM concentrations remaining on filters as well as the use of hand-held ATR-FTIR which required sorting prior to analysis to allow direct contact between the particle and crystal (Käpper et al., 2018; Tirkey and Upadhyay, 2021). Accuracy of FTIR spectroscopy is high for MPs with variation observed between polymer types PS and PE (95% and 97% respectively) supporting concentrations of these polymers recorded in this study (De Frond et al., 2022). FTIR analysis has the ability to detect particle weathering through physicochemical processes by identification of oxidation intensity, however, this could not be applied to this study due to limitations of the handheld FTIR device (Tirkey and Upadhyay, 2021). This may have led to underestimations in results with suggestions that FTIR leads to underestimations of ~35% of MPs for particles <20µm (Käpper et al., 2018).

However, difficulties assessing accuracy of polymer identification existed in this study as MPs were derived from environmental samples and therefore could not be matched to a predetermined material. The use of reference materials is a common method of assessing accuracy of polymer identification with MPs matching up to previously measured spiked materials through identification of morphotype and colour (De Frond et al., 2022). Further inaccuracies may be observed due to the interference to signal intensity due to the presence of organic films on plastic surfaces, which are ubiquitous with MP degradation leading to lower accuracy of polymer identification recorded at 44% (De Frond et al., 2022; Fan et al., 2021). This may have led to inaccuracies in readings in this study and could be overcome through a digestion process (application of Fenton's reagent) applied after visual extraction of MPs to remove OM residue on plastic surfaces and reduce FTIR interference (Fan et al., 2021).

Other FTIR techniques that could be applied to future studies include µ-ATR-FTIR which is effective in the identification of smaller MPs in highly organic samples such as those in this study as well as allowing for the elimination of the need for prior visual identification of MPs which avoids potential losses of MPs (Tagg et al., 2020).

5.2.2.2. FLUORESCENCE MICROSCOPY

The use of Nile Red staining was used in this study to identify MP concentrations in agricultural samples with identified concentrations ranging 90.35 - 6717.54 MP/kg dw and has been used for the identification of polyethylene, polypropylene, polystyrene, polycarbonate, polyurethane and poly(ethylene-vinyl acetate) with high recovery rates often recorded at 96-98% (Erni-Cassola et al.,

2017; Shim et al., 2016; Maes et al., 2017). Nile Red fluorescence microscopy allows for the non-biased, cost-effective, sensitive and rapid identification of small polymers in environmental samples (Erni-Cassola et al., 2017; Liu et al., 2022; Shim et al., 2016). This process allows for the identification of smaller MPs that may be unidentifiable using FTIR and visual observation, causing these to be overlooked (Liu et al., 2022; Shim et al., 2016).

Inaccuracies relating to fluorescence microscopy include the overestimation of MPs due to the interference and staining of natural materials that may be present in MP samples if not fully removed by a digestion process prior to this analysis (Liu et al., 2022; Shim et al., 2016; Shruti et al., 2022). Whilst the staining of these particles was observed to be less intense than MP particles, it may lead to inaccuracies in results (Shim et al., 2016). However, it has been recommended that the use of Nile Red staining not be used in environmental samples due to a lack of standardised methods for the complete removal of organic materials in these samples (Shruti et al., 2022). A lack of guidelines for the confirmation of MP identification from Nile Red staining further limits the effectiveness of this method for the measurement of MPs in environmental samples (Shruti et al., 2022). This can explain larger MP concentrations measured in this study through fluorescence microscopy in comparison to FTIR spectroscopy.

Limitations such as these can be overcome by the implementation of digestive processes prior to Nile Red staining which is observed to decrease fluorescence intensity of natural materials due to reductions in hydrophobicity (Erni-Cassola et al., 2017). This allows for variation to be observed between natural materials and synthetic polymers (Erni-Cassola et al., 2017). Interference materials present in samples can be reduced due to a digestion process after sample filtration which was not complete in this study due to time limitations, however, it is important to consider potential impacts an extended exposure time to digestive chemicals could have on MPs (CSEAS Indonesia, 2020). Despite no significant difference observed in polymer extraction rate between the use of Nile Red fluorescence and FTIR, it has been suggested that combined use of these methods allows for improved MP identification (Erni-Cassola et al., 2017; Shim et al., 2016). This method can help to overcome further limitations surrounding the identification of polymer types as fluorescence microscopy is not able to do this (Erni-Cassola et al., 2017; Maes et al., 2017).

5.3.2.2. ALTERNATIVE METHODS

Debates focusing on the most reliable and accurate MP identification technique have been greatly focused on FTIR and Raman spectroscopy as they share many benefits including being a non-destructive and reliable methods (Araujo et al., 2018). However, Raman has better spatial resolution compared to FTIR allowing for the measurement of particles down to 1 μm as well as being more sensitive (Araujo et al., 2018; Ivleva et al., 2016). The use of μ -Raman allows for the analysis of particles $<20\mu\text{m}$, however, this would not have been beneficial in this study due to constraints associated with visual extraction preventing the extraction of particles of this size (Araujo et al., 2018). If Raman were to be adopted in this study purification of particles would need to be conducted prior to Raman analysis to reduce interference (Ivleva et al., 2016).

Pyrolysis gas chromatography coupled with mass spectroscopy can accurately analyse MPs $\sim 100\mu\text{m}$ (Ivleva et al., 2016). However, this technique is not always favoured due to only being able to run one particle at a time as well as difficulties associated with the handling of particles using tweezers which is necessary for this technique (Ivleva et al., 2016).

The development of new techniques such as thermoextraction and desorption coupled with gas chromatography mass spectrometry can allow for improved MP identification (Ivleva et al., 2016). This technique allows for the analysis of MPs in environmental samples without the need for visual

extraction of environmental samples, however, due to this technique being in the early developmental stages it can only identify PE, PP and PS MPs (Ivleva et al., 2016).

6. CONCLUSIONS

To date this is the first study to have identified MP concentrations in numerous agricultural matrices throughout UK dairy farms. Extensive MP concentrations have been identified throughout samples with concentrations in livestock feed, bedding and slurry observed to be greater than those previously reported in the literature (Wu et al., 2021; Zhang et al., 2022b). With the largest concentrations measured in livestock bedding it can be concluded that this is a substantial input of MPs into the agricultural system. Having originated from waste kitchen offcuts these samples contain vast MP concentrations with dominant polymers including PUF, PS and PE. Therefore, this raises questions regarding the use of by-products in the agricultural sector which has previously been considered as a key stage in moving towards a more circular economy (Ellen Macarthur Foundation, 2017). Consideration needs to be made to assess if benefits associated with the use of waste materials throughout agriculture outweigh the potential negatives of the introduction of these emerging pollutants into the agricultural system where they could have subsequent impacts on our ability to grow crops if entering agricultural soils (Mateos-Cárdenas et al., 2021).

Methods applied to this study were effective in the extraction and identification of MPs from agricultural matrices however, further work needs to be done to develop MP extraction methods from various matrices, specifically those with high OM contents. Research has largely focused on MPs extracted from water, sediment and soil matrices with limited research on MP extraction from slurries and livestock feeds with no work on extraction from sawdust bedding. The application of a multi-step density separation as well as the addition of a digestion step after filtration may allow for improved MP extraction from these challenging matrices as this will reduce OM contents in samples prior to visual analysis (Lusher et al., 2020; Rolsky et al., 2020). A multi-stage identification method using visual extraction, fluorescence microscopy and FTIR spectroscopy was beneficial for MP identification as the use of all methods cohesively allowed for the strengthening of identification results (Erni-Cassola et al., 2017; Maes et al., 2017; Shim et al., 2016).

Based on MP concentrations measured in this study, conclusions have been drawn of the input of MPs into the agricultural system through the presence of this contaminant in livestock bedding. The presence of similar polymer composition throughout matrices analysed at each site can support that once in the agricultural system they are transferred throughout. However, there is a need for future work to be done on how this enters slurries such as through ingestion, inhalation or via wind dispersion within the livestock barn. Therefore, potential pathways cannot be concluded due to a lack of evidence supporting the direct transfer of MPs between sawdust bedding and slurries and thus further work is needed to further identify MP inputs into slurries. Concentrations of MPs measured throughout this study pose potential toxicological risk to plants and biota, however, thresholds of MP concentrations need to be identified to develop understanding of the exact impact that these concentrations may pose to the wider environment. Further research is needed into potential pathways of MPs into agricultural slurries applied as organic fertilisers on soils in order to gain an in-depth knowledge of methods that can be applied to reduce MP inputs into the agricultural sector.

REFERENCES

- Abraham, J., Ghosh, E., Mukherjee, P. and Gajendiran, A. 2016. Microbial degradation of low density polyethylene. *Environmental Progress and Sustainable Energy*. **36**(1), pp.147-154.
- Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Jiménez, P.D., Simonneau, A., Binet, S. and Galop, D. 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*. **12**, pp.339-344.
- Ángeles-López, Y.G., Gutiérrez-Mayen, A.M., Valesco-Pérez, M., Beltrán-Villavicencio, M., Vázquez-Morillas, A. and Cano-Blanco, M. 2017. Abiotic degradation of plastic films. *Journal of Physics: Conference Series*. **792**, pp.1-10.
- Araujo, C.F., Nolasco, M.M., Ribeiro, A.M.P. and Ribeiro-Claro, P.J.A. 2018. Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Research*. **142**, pp.426-440.
- Balkenhoff, B. 2009. *Review of Food Waste Depackagin Equipment*. Banbury: Waste and Resource Action Plan.
- Beriot, N., Peek, J., Xornoza, R., Geissen, V. and Lwanga, E.H. 2021. Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain. *Science of the Total Environment*. **755**, p.142653.
- Biosolids Assurance Scheme. 2019. *Biosolids Agricultural Good Practice Guidance Leaflet*. [Online]. [Accessed 1st August 2022]. Available from: <https://assuredbiosolids.co.uk/wp-content/uploads/2019/01/Biosolids-Agric-Good-Practice-Guidance-January-2019.pdf>
- Blair, R.M., Waldron, S., Phoenix, V.R. and Gauchotte-Lindsay, C. 2019. Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. *Environmental Science and Pollution Research*. **26**(12), pp.12491-12504.
- Bläsing, M. and Amelung, W. 2018. Plastics in soil: Analytical methods and possible sources. *Science of the Total Environment*. **612**, pp.422-435.
- Boots, B., Russell, C.W. and Green, D.S. 2019. Effects of microplastics in soil ecosystems: Above and below ground. *Environmental Science and Technology*. **53**, pp.11496-11506.
- Brahney, J., Mahowald, N., Prank, M., Cornwell, G., Klimont, Z., Matsui, H. and Prather, K.A. 2021. Constraining the atmospheric limb of the plastic cycle. *PNAS*. **118**(16), pp.1-10.
- Brandes, E., Henseler, M. and Kreins, P. 2021. Identifying hot-spots for microplastic contamination in agricultural soils- a spatial modelling approach for Germany. **16**,p.10401.
- Büks, F. and Kaupenjohann, M. 2020. Global concentrations of microplastics in soils- a review. *Soil*. **6**, pp.649-662.
- Business Wales. 2022. *Biodegradable Plastics for Agriculture*. [Online]. [Accessed 2nd August 2022]. Available from: <https://businesswales.gov.wales/farmingconnect/news-and-events/technical-articles/biodegradable-plastics-agriculture>

- Calhoun, A. 2016. Polypropylene. In: *Plastics Design Library. Multilayer Flexible Packaging*. Second Edition. United States: William Andrew Applied Science Publishers, pp.35-45.
- Castrucci, G. 2020. *Pros & Cons of Kitchen Cabinet Materials*. [Online]. [Accessed 10th August 2022]. Available from: <https://www.laurysenkitchens.com/blog/pros-cons-kitchen-cabinet-materials/>
- Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J.H., Abu-Omar, M., Scott, S.L. and Suh, S. 2020. Degradation rates of plastics in the environment. *ACS Sustainable Chemistry and Engineering*. **8**, pp.3494-3511.
- Chen, Y., Awasthi, A.K., Wei, F., Tan, Q. and Li, J. 2021. Single-use plastics: Production, usage, disposal, and adverse impacts. *Science of the Total Environment*. **752**, pp.141772.
- Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E. and Geissen, V. 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Science of the Total Environment*. **671**, pp.411-420.
- Cowger, W., Gray, A., Christiansen, S.H., DeFrono, H., Deshpande, A.D., Hemabessiere, L., Lee, E., Mill, L., Munno, K., Ossmann, B.E., Pittroff, M., Rochman, C., Sarau, G., Tarby, S. and Primpke, S. 2020. Critical review of processing and classification techniques for images and spectra in microplastic research. *Applied Spectroscopy*. **74**(9), pp.989-1010.
- Crossman, J., Hurley, R.R., Futter, M. and Nizzetto, L. 2020. Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. *Science of the Total Environment*. **724**, p.138334.
- CSEAS Indonesia. 2020. *Microplastic Analysis Introductory Course*. [Online]. [Accessed November 21st 2021]. Available from: [Microplastic Analysis Introductory Course](#)
- De Frono, H., Hampton, L.T., Kotar, S., Gesulga, K., Matuch, C., Lao, W., Weisberg, S.B., Wong, C.S. and Rochman, C.M. 2022. Monitoring microplastics in drinking water: An interlaboratory study to inform effective methods for quantifying and characterising microplastics. *Chemosphere*. **298**. p.134282.
- De Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S. and Rillig, M.C. 2019. Microplastics can change soil properties and affect plant performance. *Environmental Science and Technology*. **53**, pp.6044-6052.
- De Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R. and Rillig, M.C. 2018. Impacts of microplastics on the soil biophysical environment. *Environmental Science and Technology*. **52**(17), pp.9656-9665.
- De Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S. and Rillig, M.C. 2017. Microplastics as an emerging threat to terrestrial ecosystems. *Global Change Biology*. **2018**(24), pp.1405-1416.
- Demirors, M. 2011. The History of Polyethylene. In: Strom, E.T. and Rasmussen, S.C. eds. *100+ Years of Plastics Leo Baekeland and Beyond*. Washington: American Chemical Society, pp.115-145.
- Department for Environment, Food and Rural Affairs. 2021. *British Survey of Fertiliser Practice. Fertiliser use on farm for the 2020 crop year*. London: Home Office.
- Dris, R., Gasperi, J., Saad, M., Mirande, C. and Tassin, B. 2016. Synthetic fibres in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin*. **104**, pp.290-293.

Eagle Plastics. 2022. What is High Impact Polystyrene Used For? [Online]. [Accessed 10th August 2022]. Available from: <https://www.eagleplastics.co.uk/what-is-high-impact-polystyrene-used-for/>

Easyfix. 2021. *Jupiter F/F45*. [Online]. [Accessed 13th September 2022]. Available from: <https://easyfix.com/uk/product/jupiter-f/>

Eerkes-Medrano, D., Thompson, R.C. and Aldridge, D.C. 2015. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Research*. **75**, pp.63-82.

Ellen Macarthur foundation. 2017. What is the circular economy? [Online]. [Accessed 15th September 2022]. Available from: <https://archive.ellenmacarthurfoundation.org/circular-economy/what-is-the-circular-economy>

Engineering ToolBox. 2017. *Densities of Aqueous Solutions of Inorganic Sodium Salts*. [Online]. [Accessed 17th January 2022]. Available from: https://www.engineeringtoolbox.com/density-aqueous-solution-inorganic-sodium-salt-concentration-d_1957.html

Enyoh, C.E., Shafea, L., Verla, A.W., Verla, E.N., Qingyue, W., Chowdhury, T. and Paredes, M. 2020. Microplastics exposure routes and toxicity studies to ecosystems: An overview. *Environmental Analysis Health and Toxicology*. **35**(1), pp.1-10.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C. and Christie-Oleza, J.A. 2017. Lost, but found with Nile Red: A novel method for detecting and quantifying small microplastics (1 mm to 20 μ m) in environmental samples. *Environmental Science and Technology*. **51**(23), pp.13641-13648.

Fan, C., Huanh, Y.Z., Lin, J.N. and Li, J. 2021. Microplastic constituent identification from admixtures by Fourier-transform infrared (FTIR) spectroscopy: The use of polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and nylon (NY) as the model constituents. *Environmental Technology and Innovation*. **23**, p.101798.

Food and Agricultural Organisation of the United Nations. 2021. *Assessment of Agricultural Plastics and their Sustainability, A call for Action*. Rome: Food and Agricultural Organisation of the United Nations.

Gallagher, M.J., Humphrey, W., Sailsbury, W.C. and Syphers, D.W. 2014. *Method of manufacturing an interior trim panel using polyurethane powder*. US5580501A.

Geyer, R., Jambeck, J.R. and Law, K.L. 2017. Production, use and fate of all plastics ever made. *Science Advances*. **3**(7), pp.1-5.

Gong, J. and Xie, P. 2020. Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere*. **254**, p.126790.

Guce, M. 2018. *Pros and Cons of Using Plastic Mulch*. [Online]. [Accessed 10th August 2022]. Available from: <https://ecogardener.com/blogs/news/pros-and-cons-of-using-plastic-mulch>

Guo, J.J., Huang, X.P., Wang, Y.Z., Li, Y.W., Li, H., Cai, Q.Y., Mo, C.H. and Wong, M.H. 2020. Source, migration and toxicology of microplastics in soil. *Environment International*. **137**, p.105263.

- Harms, I.K., Diekötter, T., Troegel, S. and Lenz, M. 2021. Amount, distribution and composition of large microplastics in typical agricultural soils in Northern Germany. *Science of the Total Environment*. **758**, p.143615.
- He, D., Luo, Y., Lu, S., Liu, M., Song, Y. and Lei, L. 2018. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends in Analytical Chemistry*. **109**, pp.163-172.
- He, D., Zhang, X. and Hu, J. 2021. Methods for separating microplastics from complex solid matrices: Comparative analysis. *Journal of Hazardous Materials*. **409**, p.124640.
- Heinze, W.M., Mitrano, D.M., Lahive, E., Koestel, J. and Cornelis, G. 2021. Nanoplastic transport in soil via bioturbation by *Lumbricus terrestris*. *Environmental Science and Technology*. **55**, pp.16423-16433.
- Hengstmann, E. and Fischer, E.K. 2019. Nile red staining in microplastic analysis- proposal for a reliable and fast identification approach for large microplastics. *Environmental Monitoring and Assessment*. **191**, pp.1-9.
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M. 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environmental Science and Technology*. **46**, pp.3060-3075.
- Hodson, M.E., Duffus-Hodson, C.A., Clark, A., Prendergast-Miller, M.T. and Thorpe, K.L. 2017. Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates. *Environmental Science and Technology*. **51**, pp.4714-4721.
- Horton, A.A. and Dixon, S.J. 2017. Microplastics: An introduction to environmental transport processes. *WIREs Water*. **5**(2), p.1268.
- Hu, J., He, D., Zhang, X., Li, X., Chen, Y., Gao, W., Zhang, Y., Ok, Y.S. and Luo, Y. 2022. National-scale distribution of micro(meso)plastics in farmland soils across China: Implications for environmental impacts. *Journal of Hazardous Materials*. **424**, p.127283.
- Hurley, R.R., Lusher, A.L., Olsen, M. and Nizzetto, L. 2018. Validation of a method for extracting microplastics from complex organic-rich, environmental matrices. *Environmental Science and Technology*. **52**, pp.7409-7417.
- Ivleva, N.P., Wiesheu, A.C. and Niessner, R. 2016. Microplastic in aquatic ecosystems. *Materials Science and Polymers*. **56**(7), pp.1720-1739.
- Käpper, A. Fischer, M., Scholz-Böttcher, B.M., Oberbeckmann, S., Labrenz, M., Fischer, D., Eichhorn, K.J. and Voit, B. 2018. Comparison of μ -ATR-FTIR spectroscopy and pu-GCMS as identification tools for microplastic particles and fibres isolated from river sediments. *Analytical and Bioanalytical Chemistry*. **410**, pp.5313-5327.
- Kelly, C. 2018. *What is Laminating*. [Online]. [Accessed 10th August 2022]. Available from: <https://www.plasticsintl.com/blog/what-is-laminating/#:~:text=Plastic%20laminates%20are%20usually%20various,to%20the%20base%20plastic%20material.>
- Khalid, N., Aqeel, M. and Norman, A. 2020. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. *Environmental Pollution*. **267**, p.115653.

- Koutnik, V.S., Leonard, J., Alkidim, S., DePrima, F.J., Ravi, S., Hoek, E.M.V. and Mohanty, S.K. 2021. Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modelling. *Environmental Pollution*. **274**, p.116552.
- Kumar, M., Xiong, X., He, M., Tsang, D.C.W., Gupta, J., Khan, E., Harrad, S., Hou, D., Ok, Y.S. and Bolan, N.S. 2020. Microplastics as pollutants in agricultural soils. *Environmental Pollution*. **265**, p.114980.
- Laing, J., Mytton, A. and Sutherland, S. 2022. Why plastic recycling doesn't work with Sian Sutherland- Part 2. [Podcast]. [Accessed 11th July 2022]. Available from: <https://podcasts.apple.com/gb/podcast/private-parts/id1208435573?i=1000569204460>
- Lei, L., Liu, M., Song, Y., Lu, S., Hu, J., Cao, C., Xie, B., Shi, H. and He, D. 2018. Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in *Caenorhabditis elegans*. *Environmental Science: Nano*. **5**(8), pp.2009-2020
- Li, J., Song, Y. and Cai, Y. 2020. Focus topics on microplastics in soil: Analytical methods, occurrence, transport, and ecological risks. *Environmental Pollution*. **257**, p.113570.
- Liu, L., Xu, M., Ye, Y. and Zhang, B. 2022. On the degradation of (micro)plastics: Degradation methods, influencing factors, environmental impacts. *Science of the Total Environment*. **806**, p.151312.
- Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X. and He, D. 2018. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. *Environmental Pollution*. **242**, pp.855-862.
- Löder, M.G.J. and Gerdt, G. 2015. Methodology used for the detection and identification of microplastics- A critical appraisal. In: Bergmann, M., Gutow, L. and Klages, M. eds. *Marine Anthropogenic Litter*. Switzerland: Springer International Publishing.
- Lu, Q., He, Z.L. and Stoffella, P.J. 2012. Land application of biosolids in the USA: A review. *Applied and Environmental Soil Science*. **10**, pp.1-11.
- Lusher, A.L., Munno, K., Hermabessiere, L. and Carr, S. 2020. Isolation and extraction of microplastics from environmental samples: An evaluation of practical approaches and recommendations for further harmonization. *Applied Spectroscopy*. **74**(9), pp.1049-1065.
- Lwanga, E.H., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A. and Geissen, V. 2016. Microplastics in the terrestrial ecosystem: Implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). *Environmental Science and Technology*. **50**, pp.2685-2691.
- Lwanga, E.H., Vega, J.M., Quej, V.K., de los Angeles Chi, J., del Cid, L.S., Segura, G.E., Gertsen, H., Salánki, T., van der Ploeg, M., Koelmans, A.A. and Geissen, V. 2017. Field evidence for transfer of plastic debris along a terrestrial food chain. *Scientific Reports*. **7**, p.14071.
- March, M.D., Haskell, M.J., Chagunda, M.G.G., Langford, F.M. and Roberts, D.J. 2014. Currents trends in British dairy management regimes. *Journal of Dairy Science*. **97**(12), pp.7985-7994.
- Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D. and Rogers, D.L. 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environmental Pollution*. **218**, pp.1045-1054.

Mateos-Cárdenas, A., van Pelt, F.N.A.M., O'Halloran, J. and Jansen, M.A.K. 2021. Adsorption, uptake and toxicity of micro- and nanoplastics: Effects on terrestrial plants and aquatic macrophytes. *Environmental Pollution*. **284**, p.117183.

Mohajerani, A. and Karabatak, B. 2020. Microplastics and pollutants in biosolids have contaminated agricultural soils: An analytical study and a proposal to cease the use of biosolids in farmlands and utilise them in sustainable bricks. *Waste Management*. **107**, pp.252-265.

Möller, J.N., Löder, M.G.J. and Laforsch, C. 2020. Finding microplastics in soils: A review of analytical methods. *Environmental Science and Technology*. **54**, pp.2078-2090.

Molotoks, A., Smith, P. and Dawson, T.P. 2020. Impacts of land use, population, and climate change on global food security. *Food and Energy Security*. **10**(1), pp.1-20.

Monteiro, S.S. and da Costa, J.P. 2022. Methods for the extraction of microplastics in complex solid, water and biota samples. *Trends in Environmental Analytical Chemistry*. **33**, pp.1-13.

Nangalia, S. 2022. *What's the Best Material for Kitchen Cabinets?* [Online]. [Accessed 10th August 2022]. Available from: <https://www.houzz.in/magazine/whats-the-best-material-for-kitchen-cabinets-stsetivw-vs~82630988>

NFU. 2021a. *Farm plastic waste management*. [Online]. [Accessed 5th October 2021]. Available from: https://www.nfuonline.com/cross-sector/environment/waste/waste-news/environment-agency-advice-for-good-waste-management-on-farm/?utm_source=b160621&utm_medium=email&utm_campaign=bulletin

Ng, E.L., Lwanga, E.H., Eldridge, S.M., Johnston, P., Hu, H.W., Geissen, V. and Chen, D. 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. *Science of the Total Environment*. **627**, pp.1377-1388.

Nicholson, F., Bhogal, A., Taylor, M., McGrath, S. and Withers, P. 2018. Long-term effects of biosolids on soil quality and fertility. *Soil Science*. **183**(3), pp.89-98.

Nithin, B. and Goel, S. 2017. Degradation of Plastics, In: Goel, S. (eds) *Advances in Solid and Hazardous Waste Management*. India: Springer, pp.235-247.

Nizzetto, L., Futter, M. and Langaas, S. 2016a. Are agricultural soils dumps for microplastics of urban origin? *Environmental Science and Technology*. **50**, pp.10777-10779.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D. and Whitehead, P.G. 2016b. A theoretical assessment of microplastic transport in river catchments in their retention by soils and river sediments. *Environmental Science Processes and Impacts*. **18**, p.1050.

NNFCC. 2022. Digestate. [Online]. [Accessed 13th September 2022]. Available from: <https://www.biogas-info.co.uk/about/digestate/>

Okoffo, E.D., Donner, E., McGrath, S.P., Tschärke, B.J., O'Brien, J.W., O'Brien, S., Ribeiro, F., Burrows, S.D., Toapanta, T., Rauert, C., Samanipour, S., Mueller, J.F. and Thomas, K.V. 2021. Plastics in biosolids from 1950 to 2016: A function of global plastic production and consumption. *Water Research*. **201**, pp.117367.

- Picó, Y. and Barceló, D. 2020. Pyrolysis gas chromatography-mass spectroscopy in environmental analysis: Focus on organic matter and microplastics. *TrAC Trends in Analytical Chemistry*. **130**, p.115964.
- Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C. and Laforsch, C. 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. *Scientific Reports*. **8**(17950), pp.1-9.
- Prata, J.C., da Costa, J.P., Lopes, I., Andrady, A.L., Duarte, A. and Rocha-Santos, T. 2021. A One Health perspective of the impacts of microplastics on animal, human and environmental health. *Science of The Total Environment*. **777**, p.146094.
- Prata, J.C., da Costa, J.P., Girão, A.V., Lopes, I., Duarte, A. and Rocha-Santos, T. 2019. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Science of The Total Environment*. **686**, pp.131-139.
- Razali, N.M. and Wah, B.Y. 2011. Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests. *Journal of Statistical Modelling and Analytics*. **2**(1), pp.21-33.
- Rillig, M.C., Lehmann, A., de Souza Machado, A.A. and Yang, G. 2019. Microplastic effects on plants. *New Phytologist*. **223**(3), pp.1066-1070.
- Rillig, M.C., Ziersch, L. and Hempel, S. 2017. Microplastic transport in soil by earthworms. *Scientific Reports*. **7**(1362), pp.1-6.
- Rillig, M.C. 2012. Microplastic in terrestrial ecosystems and the soil? *Environmental Science and Technology*. **46**, pp.6453-6454.
- Rodrigues-Seijo, A., Lourenço, J., Rocha-Santos, T.A.P., da Costa, J., Duarte, A.C., Vala, H. and Pereira, R. 2016. Histopathological and molecular effects of microplastics in *Eisenia andrei* bouché. *Environmental Pollution*. **220**, pp.495-503.
- Rolsky, C., Kelkar, V., Driver, E. and Halden, R.U. 2020. Municipal sewage sludge as a source of microplastics in the environment. *Current Opinion in Environmental Science and Health*. **14**, pp.16-22.
- Roy, P.K., Surekha, P. and Rajagopal, C. 2011. Surface oxidation of low-density polyethylene films to improve their susceptibility towards environmental degradation. *Journal of Applied Polymer Science*. **122**, pp.2765-2773.
- RSPCA. 2017. *The welfare of dairy cows*. [Online]. [Accessed 29th September 2021]. Available from: file:///C:/Users/User/Downloads/Dairy%20Cow%20Information%20Sheet_%20Feb%202017.pdf
- Ryan, P.G. 2015. A Brief History of Marine Litter Research. In: Bergmann, M., Gutow, L., Klages, M. eds. *Marine Anthropogenic Litter*. Switzerland: Springer, pp.1-25.
- Shim, W.J., Hong, S.H. and Eo, S.E. 2017. Identification methods in microplastic analysis: a review. *Analytical Methods*. **9**, pp.1384-1391.
- Shim, W.J., Song, Y.K., Hong, S.H. and Jang, M. 2016. Identification and quantification of microplastics using Nile Red staining. *Marine Pollution Bulletin*. **113**, pp.469-476.

Shrunti, V.C., Pérez-Guevara, F., Roy, P.R. and Kuttralam-Muniasamy, G. 2022. Analysing microplastics with Nile Red: Emerging trends, challenges, and prospects. *Journal of Hazardous Materials*. **423**, p.127171.

Smith, L. 2022. *Plastic waste, Research Briefing*. London: House of Commons Library.

Song, Y.K., Hong, S.H., Eo, S., Han, G.M. and Shim, W.J. 2019. Rapid production of micro- and nanoplastics by fragmentation of expanded polystyrene exposed to sunlight. *Environmental Science and Technology*. **54**(18), pp.11191-11200.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Rani, M., Lee, J. and Shim, W.J. 2015. A comparison of microscopic and spectroscopic identification methods for analysis of microplastic in environmental samples. *Marine Pollution Bulletin*. **93**(1-2), pp.202-209.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O. and Schaumann, G.E. 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Science of The Total Environment*. **550**, pp.690-705.

Tagg, A.S., Sapp, M., Harrison, J.P., Sinclair, C.J., Bradley, E., Ju-Nam, Y. and Ojeda, J.J. 2020. Microplastic monitoring at different stages in a wastewater treatment plant using reflectance micro-FTIR imaging. *Frontiers in Environmental Science*. **8**(145), pp.1-9.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D. and Russell, A.E. 2004. Lost at sea: where is all the plastic? *Science*. **304**, p.838.

Tirkey, A. and Upadhyay, L.S.B. 2021. Microplastics: An overview on separation, identification and characterization of microplastics. *Marine Pollution Bulletin*. **170**, p.112604.

Uberoi, E. 2021. *UK dairy industry statistics*. (HC 2721). [Online]. London: House of Commons Library. [Accessed 24th August 2022]. Available from:
<https://researchbriefings.files.parliament.uk/documents/SN02721/SN02721.pdf>

Uddin, S., Fowler, S.W. and Behbehani, M. 2020. An assessment of microplastic inputs into the aquatic environment from wastewater streams. *Marine Pollution Bulletin*. **160**, p.111538.

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F. and Law, K.L. 2015. A global inventory of small floating plastic debris. *Environmental Research Letters*. **10**(12), p.124006.

Veerasingam, S., Ranjani, M., Venkatachalapathy, R., Bagaev, A., Mukhanov, V., Litvinyuk, D., Mugilarasan, M., Gurumoorthi, K., Guganathan, L., Aboobacker, V.M. and Vethamony, P. 2021. Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. *Critical Reviews in Environmental Science and Technology*. **51**(22), pp.2681-2743.

Wang, W., Yuan, W, Xu, E.G., Li, L., Zhang, H. and Yang, Y. 2021. Uptake, translocation, and biological impacts of micro(nano)plastic in terrestrial plants: Progress and prospects. *Environmental Research*. **203**, p.111867.

Wang, F., Zhang, X., Zhang, S., Zhang, S., Adams, C.A. and Sun, Y. 2020. Effects of co-contamination of microplastics and Cd on plant growth and Cd accumulation. *Toxics*. **8**(2), pp.1-12.

Wang, Z., Chen, J., Mao, S., Han, Y., Zhang, L., Li, Y. and Li, C. 2017. Comparison of greenhouse gas emissions of chemical fertilizer types in China's crop production. *Journal of Cleaner Production*. **141**, pp.1267-1274.

Waste and Resource Action Programme. 2016. *Guidance for food and drink manufacturers and retailers on the use of food surplus as animal feed*. Banbury: WRAP.

Wu, R.T., Cai, Y.F., Chen, Y.X., Yang, Y.W., Xing, S.C. and Lian, X.D. 2021. Occurrence of microplastic in livestock and poultry manure in South China. *Environmental Pollution*. **277**, p.116790.

Xu, C., Zhang, B., Gu, C., Shen, C., Yin, S., Aamir, M. and Li, F. 2020a. Are we underestimating the sources of microplastic pollution in terrestrial environment? *Journal of Hazardous Materials*. **400**, p.123228.

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Z., He, Y., Wang, H., Lu, Z., Brookes, P.C., Tang, C., Gan, J. and Xu, J. 2020b. Microplastics in the soil environment: Occurrence, risks, interactions and fate- A review. *Critical Reviews in Environmental Science and Technology*. **50**(21), pp.2175-2222.

Yang, J., Li, R., Zhou, Q., Li, L., Li, Y., Tu, C., Zhao, X., Xiong, K., Christie, P. and Luo, Y. 2021. Abundance and morphology of microplastics in an agricultural soil following long-term repeated application of pig manure. *Environmental Pollution*. **272**, p.116028.

Yazici, B. and Yolacan, S. 2007. A comparison of various tests of normality. *Journal of Statistical Computation and Simulation*. **77**(2), pp.175-183.

Zang, H., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y. and Jones, D.L. 2020. Microplastics in the agroecosystem: Are they an emerging threat to the plant-soil system? *Soil Biology and Biochemistry*. **148**, p.107926.

Zhang, S., Li, Y., Chen, X., Jiang, X., Li, J., Yang, L., Yin, X. and Zhang, X. 2022a. Occurrence and distribution of microplastics in organic fertilisers in China. *Science of The Total Environment*. **844**, p.157061.

Zhang, J. Wang, X., Xue, W., Xu, L., Ding, W., Zhao, M., Liu, S., Zou, G. and Chen, Y. 2022b. Microplastics pollution in soil increases dramatically with long-term application of organic composts in a wheat-maize rotation. *Journal of Cleaner Production*. **356**, p.131889.

Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K.H., Wu, C. and Lam, P.K.S. 2021. Understanding plastic degradation and microplastic formation in the environment. *Environmental Pollution*. **274**, p.116554.

Zhang, B., Yang, X., Chen, L., Chao, J., Teng, J. and Wang, Q. 2020a. Microplastics in soils: a review of possible sources, analytical methods and ecological impacts. *Journal of Chemical Technology and Biotechnology*. **95**(8), pp.2052-2068.

Zhang, X., Yu, K., Zhang, H., Liu, Y., He, J., Liu, X. and Jiang, J. 2020b. A novel heating-assisted density separation method for extracting microplastics from sediments. *Chemosphere*. **256**, p.127039.

Zhang, Y., Pu., S., Lv., X., Gao, Y. and Ge, L., 2020c. Global trends and prospects in microplastics research: A bibliometric analysis. *Journal of Hazardous Materials*. **400**, p.123110.

Zhao, S., Zhang, Z., Chen, L., Cui, Q., Cui, Y., Song, D. and Fang, L. 2022. Review on migration, transformation and ecological impacts of microplastics in soil. *Applied Soil Ecology*. **176**, p.104486.

Zhou, Y., Sun, Y., Liu, J., Ren, X., Zhang, Z., Wang, Q. 2022. Effects of microplastics on humification and fungal community during cow manure composting. *Science of the Total Environment*. **803**, p.150029.

Zhou, J., Wen, Y., Marshall, M.R., Zhao, J., Gui, H., Yang, Y., Zeng, Z., Jones, D.L. and Zhang, H. 2021. Microplastics as an emerging threat to plant and soil health in agroecosystems. *Science of the Total Environment*. **787**, p.147444.

Zhou, Y., Wang, J., Zou, M., Lia, Z., Zhou, S. and Li, Y. 2020. Microplastics in soils: A review of methods, occurrence, fate, transport, ecological and environmental risks. *Science of the Total Environment*. **748**, p.141368.

Zhu, F., Zhu, C., Wang, C. and Gu, C. 2019. Occurrence and ecological impacts on microplastics in soil systems: A review. *Bulletin of Environmental Contamination and Toxicology*. **102**, pp.741-749.

Zubris, K.A.V. and Richards, B.K. 2005. Synthetic fibres as an indicator of land application of sludge. *Environmental Pollution*. **138**, pp.201-211.

APPENDICES

APPENDIX A- PRELIMINARY METHODS AND INTERPRETATIONS

Preliminary methods were used in order to develop methodologies for this study. Digestion was trialled using Fenton's reagent as it is highly effective in removing OM whilst having minimal impact on MP degradation (Lin et al., 2021; Monteiro and da Costa, 2022). Fenton's reagent was created using 69.5 g ferrous iron (II) sulfate alongside 5 mL of concentrated hydrogen peroxide in 500 mL deionised water. 20 mL of Fenton's reagent was added to 10 g sample and heat on a hot plate at 40°C covering with foil lid. Once the mixture started to boil it was removed from the heat to allow boiling to slow before being returned to the heat for 10 minutes. Samples were covered with foil and left at ambient temperature for 18 hours to allow for digestion to be complete.

Saline solutions were tested for extraction efficiency between NaCl and NaH₂PO₄ to conduct density separation at densities of 1.25 g/cm⁻³ and 1.35 g/cm⁻³ respectively. 100 mL of saline solution was added to 10 g sample and stirred manually for 5 minutes (Monteiro and da Costa, 2022; Zhang et al., 2020b). Samples were covered with a foil lid and left to settle for 24 hours (Zhang et al., 2020b). Supernant was separated from solid material by decanting it into a clean glass beaker and covered with a foil lid.

Filtration of supernant was conducted using a vacuum filter with glass fibre filter papers GF/A 47 mm diameter. Filter papers were left for 24 hours covered in a pre-labelled glass petri dish before visual sorting using a high-powered stereo microscope.

Results from preliminary methods showed the ineffectiveness of digestion in this study due to time constraints and the high OM contents in tested samples. MP extraction rates were slightly elevated through the use of NaH₂PO₄ solution at a density of 1.35 g/cm⁻³ with 52% of extracted particles from samples where NaH₂PO₄ was applied being polymers compared to 46% when NaCl was used (Figure A1).

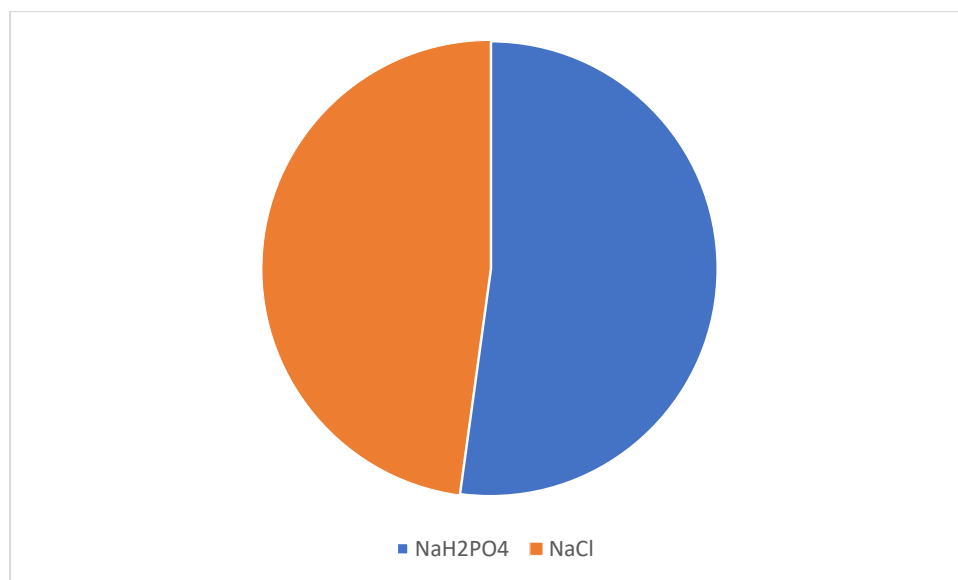


FIGURE A1. MICROPLASTICS EXTRACTED FROM SAMPLES USING DIFFERING SALINE SOLUTIONS.

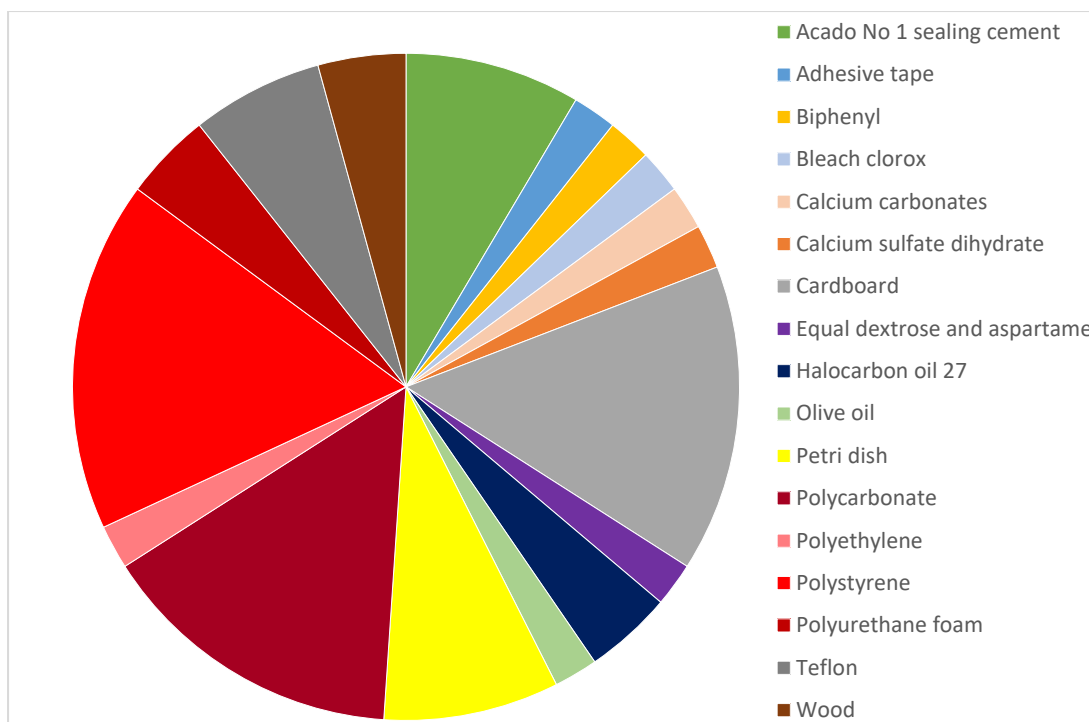


FIGURE A2. MATERIALS IDENTIFIED UNDER FTIR SPECTROSCOPY FROM AGRICULTURAL SAMPLES WITH NaCl USED DURING DENSITY SEPARATION.

APPENDIX B- MICROPLASTIC CONCENTRATION RAW DATA TABLES

Table B1. Microplastic concentrations in agricultural samples at Site A measured in MP/kg dw.

MATERIAL	Silage	Feed	Sawdust Bedding	Slurry	Soil
Polyethylene	0	0	0	353.0568	49.45486
Polyurethane foam	387.1363	398.4467	283.2703	4942.795	148.3646

Table B2. Microplastic concentrations in agricultural samples at Site B measured in MP/kg dw.

MATERIAL	Silage	Feed	Sawdust Bedding	Slurry	Soil
Polyethylene	0	0	1982.892	0	0
Polystyrene	0	0	5523.771	0	0
Polyurethane foam	201.3338	748.1078	1841.257	1185.382	52.23833

Table B3. Microplastic concentrations in agricultural samples at Site C measured in MP/kg dw.

MATERIAL	Feed	Sawdust Bedding	Slurry	Soil
Polyethylene	56.83207	353.8128	0	0
Polystyrene	0	2948.44	0	0
Polyurethane foam	113.6641	3184.315	323	0

APPENDIX C- CONCENTRATION OF OTHER MATERIALS EXTRACTED FROM AGRICULTURAL SAMPLES IDENTIFIED USING FTIR SPECTROSCOPY

In addition to the presence of polymers in agricultural samples, analysis using FTIR spectroscopy identified numerous material types (Figure 4.14 - 4.16). Throughout all samples, the most abundant material identified was ammonium hydroxide solution (mean concentrations of ammonium hydroxide solution in all samples over monitoring: 1274 particles/kg dw) making up 24% of all identified materials measured in sawdust bedding samples collected at Site C over a 6-month monitoring period (Figure 4.16b).

C1. OBSERVATIONS AT SITE A

The most dominant material identified at Site A was polyurethane foam which was identified in all samples at this site with greatest concentrations observed in slurry samples (4942.8 MP/kg dw) making up 58% of materials extracted from Site A slurry samples across monitoring (Figure 4.14d). Cardboard particles were measured in large concentrations in silage and soil samples at Site A (1548.55 MP/kg dw and 346.18 MP/kg dw respectively) making up 42% and 50% of materials in these samples respectively (Figure 4.14a and 4.14d). Slurry samples collected over monitoring showed the largest range of different types of materials with eight materials identified (Figure 4.14d)

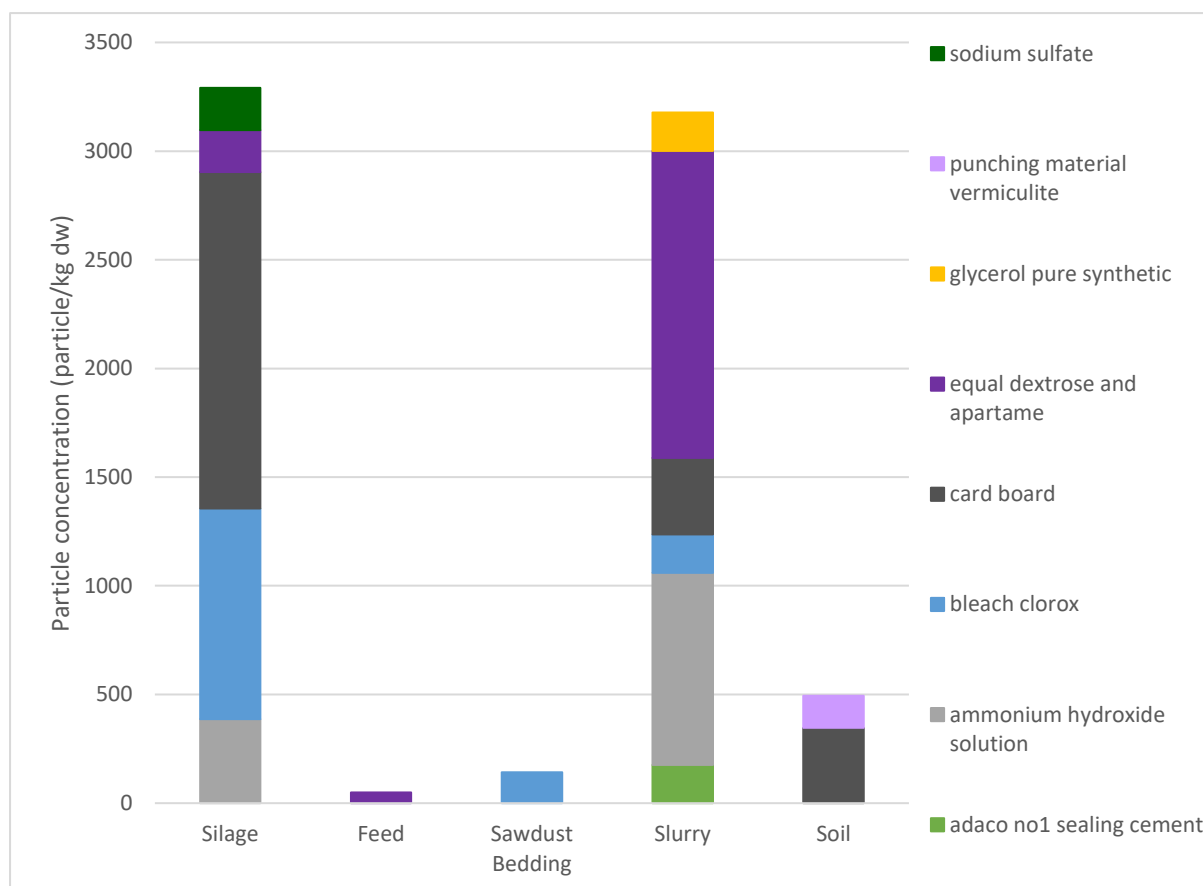


FIGURE C1. MATERIALS IDENTIFIED USING FTIR SPECTROSCOPY DURING POLYMER IDENTIFICATION OF POTENTIAL MICROPLASTICS EXTRACTED FROM AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE A.

C2. OBSERVATIONS AT SITE B

At Site B the most dominant material identified over monitoring was ammonium hydroxide solution which was identified in cattle pellet, sawdust bedding and slurry samples collected (Figure 4.15b-d). Largest ammonium hydroxide solution concentrations were measured in slurry samples (2370.77

MP/kg dw) making up 47% of materials identified in this sample (Figure 4.15d). The largest quantity of materials were measured in sawdust bedding samples with 17 materials identified (Figure 4.15c).

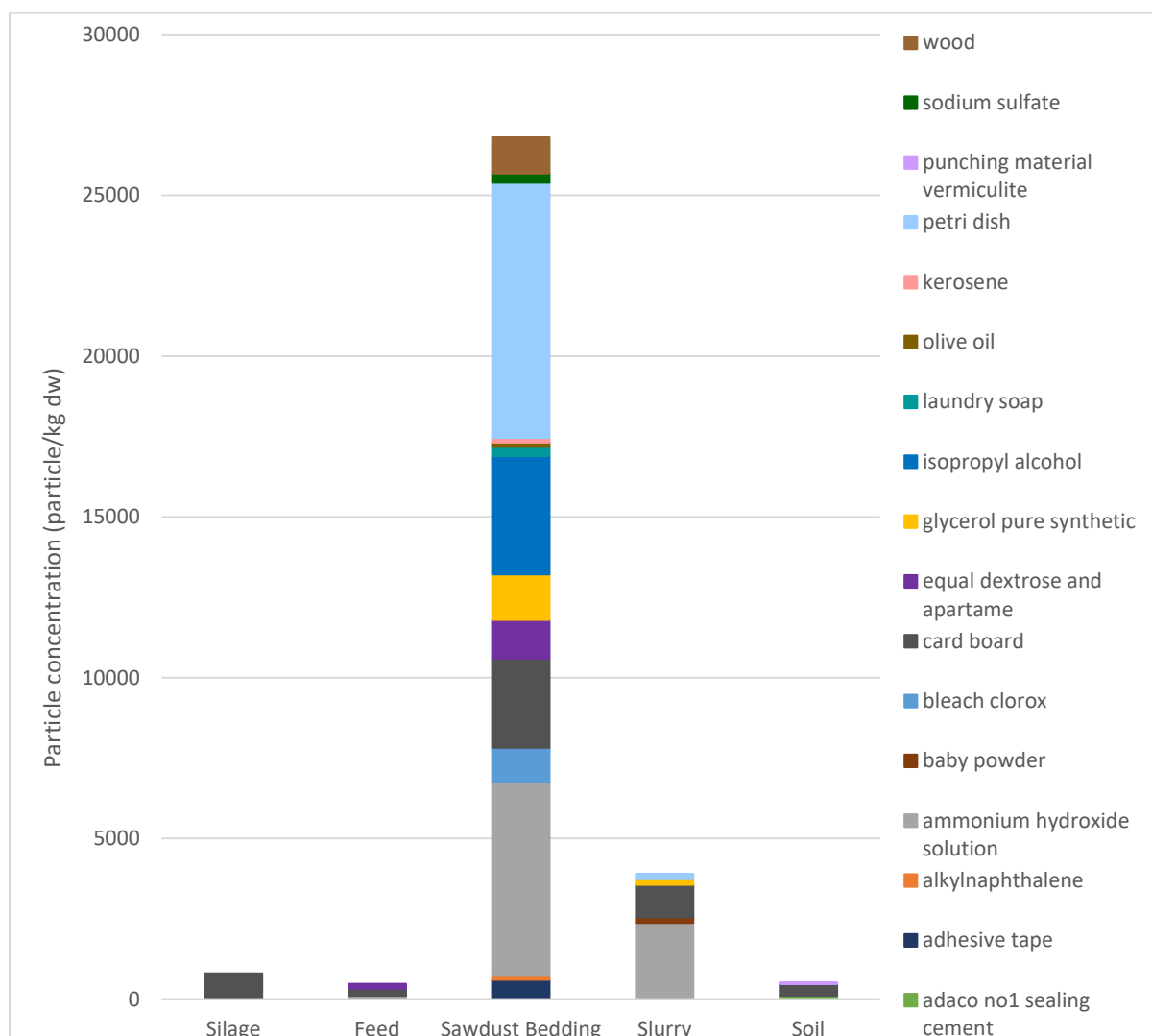


FIGURE C2. MATERIALS IDENTIFIED USING FTIR SPECTROSCOPY DURING POLYMER IDENTIFICATION OF POTENTIAL MICROPLASTICS EXTRACTED FROM AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE B.

C3. OBSERVATIONS AT SITE C

The most dominant material identified in samples collected at Site C was ammonium hydroxide solution being measured in cattle pellet, sawdust bedding and slurry samples (Figure 4.16b-d). Sawdust bedding samples contain the largest concentration of this material making up 24% of materials measured in sawdust bedding samples over monitoring (Figure 4.16b). Sawdust bedding samples contained the largest range of materials with 17 recorded (Figure 4.16b).

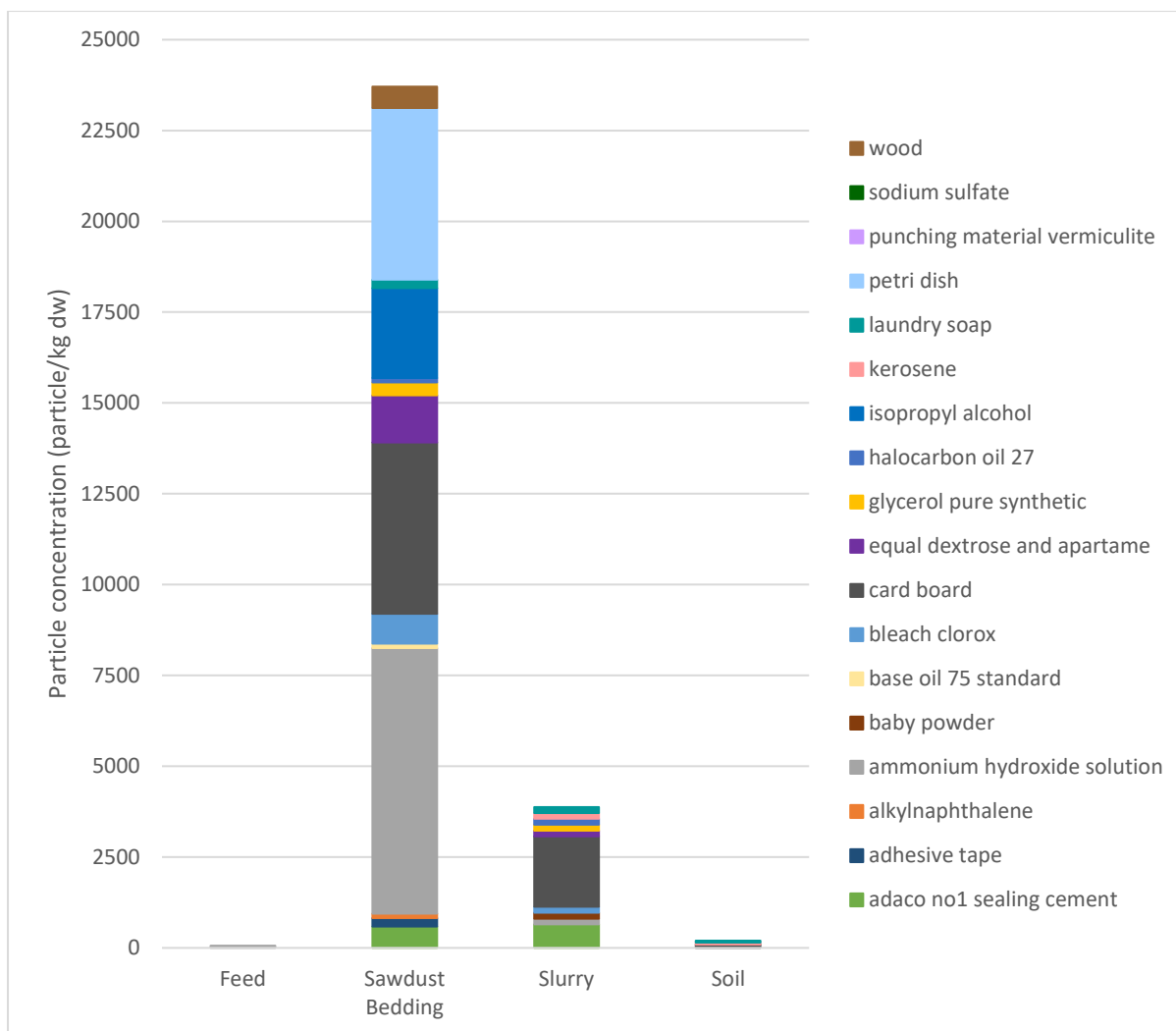


FIGURE C3. MATERIALS IDENTIFIED USING FTIR SPECTROSCOPY DURING POLYMER IDENTIFICATION OF POTENTIAL MICROPLASTICS EXTRACTED FROM AGRICULTURAL SAMPLES ACROSS A 6-MONTH MONITORING PERIOD AT SITE C.

Table C1. Material concentrations measured in agricultural samples at Site A measured in particle/kg dw.

MATERIAL	Silage	Feed	Sawdust Bedding	Slurry	Soil
adaco no1 sealing cement	0	0	0	176.5284	0
ammonium hydroxide solution	387.1363	0	0	882.642	0
bleach clorox	967.8408	0	141.6352	176.5284	0
card board	1548.545	0	0	353.0568	346.184
equal dextrose and apartame	193.5682	49.80583	0	1412.227	0
glycerol pure synthetic	0	0	0	176.5284	0
punching material vermiculite	0	0	0	0	148.3646
sodium sulfate	193.5682	0	0	0	0
Polyethylene	0	0	0	58.8428	8.242477
Polyurethane foam	32.26136	8.300972	0	235.3712	0

Table C2. Material concentrations measured in agricultural samples at Site B measured in particle/kg dw.

MATERIAL	Silage	Feed	Sawdust Bedding	Slurry	Soil
adaco no1 sealing cement	0	53.43627	0	0	104.4767
adhesive tape	0	0	601.5387	0	0
alkylnaphthalene	0	0	120.3077	0	0
ammonium hydroxide solution	0	53.43627	6015.387	2370.765	0
baby powder	0	0	0	169.3404	0
bleach clorox	0	0	1082.77	0	0
card board	805.3353	213.7451	2767.078	1016.042	365.6683
equal dextrose and apartame	0	160.3088	1203.077	0	0
glycerol pure synthetic	0	0	1416.352	169.3404	0
isopropyl alcohol	0	0	3682.514	0	0
laundry soap	0	0	283.2703	0	0
olive oil	0	0	141.6352	0	0
kerosene	0	0	141.6352	0	0
petri dish	0	0	7931.569	169.3404	0
punching material vermiculite	0	0	0	0	52.23833
sodium sulfate	0	0	283.2703	0	0
wood	0	0	1133.081	0	0
Polyethylene	0	0	220.5642	0	0
Polystyrene	0	0	421.0771	0	0
Polyurethane foam	33.55564	26.71814	20.05129	28.22339	0

Table C3. Material concentrations measured in agricultural samples at Site C measured in particle/kg dw.

MATERIAL	Feed	Sawdust Bedding	Slurry	Soil
adaco no1 sealing cement	0	589.6879	646	0
adhesive tape	0	235.8752	0	0
alkylnaphthalene	0	117.9376	0	0
ammonium hydroxide solution	56.83207	7312.13	161.5	0
baby powder	0	0	161.5	0
base oil 75 standard	0	117.9376	0	0
bleach clorox	0	825.5631	161.5	0
card board	0	4717.504	1938	99.24273
equal dextrose and apartame	0	1297.313	161.5	0

glycerol pure synthetic	0	353.8128	161.5	0
halocarbon oil 27	0	117.9376	161.5	0
isopropyl alcohol	0	2476.689	0	0
kerosene	0	0	161.5	49.62136
laundry soap	0	235.8752	161.5	49.62136
petri dish	0	4717.504	0	0
punching material vermiculite	0	0	0	0
sodium sulfate	0	0	0	0
wood	0	589.6879	0	0
Polyethylene	9.472011	19.65626	0	0
Polystyrene	0	196.5626	0	0
Polyurethane foam	0	58.96879	26.91667	0