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Applications of Biosolids to Agricultural Soils and Their Effect on Soil Biology and Chemistry

Written by

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Abstract

Approximately 1.05 million dry tonnes of biosolids are produced each year in the UK. Their application to land has the potential to cause not only environmental pollution but result in the bioaccumulation of heavy metals in the food chain. Nevertheless, they are a viable option for crop fertilisation and could contribute to relieving the issues associated with hidden hunger. This thesis attempts to determine if the use of Bestways Fertiliser (granular biosolids) impacts on soil biology or chemistry, through the use of contrasting soils at Broxton Cheshire and Harper Adams University between 2007 and 2014, across a range of combinable and forage crops.

Initial investigations focused on the changes biosolids had to metal fractionation within the soils. Sequential extraction procedures were designed to remove elements bound to different factions within the soil. Results demonstrated that biosolids did not increase heavy metals in any fraction of either soil type but did increase concentrations of phosphorus by 214% in the iron/manganese bound fraction.

Long-term leaching studies evaluated elemental mobility following biosolids applications. Results showed that long term applications of biosolids increased Copper concentrations in Broxton soils by 110% and 315% in Harper soils.

To assess changes in plant-available elemental concentrations, mesocosm studies were investigated together with a long-term field experiment. Mesocosm investigations demonstrated that biosolids could produce similar yields to conventional fertilisers and concentrations in plant tissues (grain) were not raised above those grown with conventional fertilisers.

To determine the effect biosolids had on microorganism populations, a twelve-month soil respiration study was investigated. It was concluded that biosolids increased respiration at 100% and 200% application rates, but decreased respiration at 400%.

Overall conclusions suggest that the risk to the food chain from heavy metals is low, but the impact of heavy metal leaching rates into ground water must be studied further.

ACKNOWLEDGEMENTS

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Introduction

1.1. Land and crop productivity

Agriculture is at the interface of human society and the environment (Olesen and Bindi, 2002). The world population currently stands at 7.6 billion, with a projected growth to 11.2 billion by 2100 (United Nations, 2017). Food production must increase by 60-110% to meet the needs of this global population increase (Alexandratos and Bruinsma, 2012; Pradhan *et al.*, 2015; Tilman *et al.*, 2011), with crop production supplying multiple food chains. Whilst grain has always been produced for human and animal consumption, it is now also increasingly being used for biofuels. This is adding additional pressure to a system which is already under considerable stain. Whilst the demand for food increases, agriculture must either increase the area of land suitable for farming or increase the productivity of land already in production. Creating more land for agricultural production raises environmental problems through increased carbon dioxide (CO_2) emissions, with the equivalent of 4 billion tonnes of CO_2 being released annually due to deforestation (FAO, 2017). Since the 1960's, the amount of grain produced has more than doubled, whilst there has only been a 9% increase in land made available for crop production (Godfrey *et al.*, 2012; Pretty, 2008). Improving land productivity can only be achieved sustainably by enhancing its quality. Generally, agricultural land can be split into two farming systems, intensive and extensive. Intensive systems focus on high inputs and outputs with the aim to produce as much food as possible. Extensive farming is thought to be more sustainable, whilst also remediating land originally used by intensive systems (Biala *et al.*, 2007).

Efficient crop fertilisation is a key agronomic feature to attaining the increased yields required to support a growing population. Approximately 91.8 million tonnes of nitrogen fertiliser are applied to land to support crop growth globally. Crops require nutrients at various rates depending on a number of factors, including soil type, yield potential and previous crop demands. Currently, the production or mining of inorganic fertilisers consumes large amounts of fossil fuels, making production unsustainable. However, fertiliser applications currently need to be increased by 45-75% for N, 22-46% for P_2O_5 and more than double for K_2O, compared to 2010 (Pradhan *et al.*, 2015). Fertiliser production can be very expensive, for example, 37.88 million Kilojoules are required, on average, to produce one tonne of nitrogen urea (Sawyer *et al.*, 2010). This is due to the main source of energy being natural gas. Due to this direct economic link, as fossil fuels become less common and increasingly difficult to source, the costs of fertilisers will also increase. In addition, many soils require phosphate enriched fertilisers to produce optimum yields.

1.2 Limitations to crop yield improvements

Conventional, and more recently, genetically modified breeding of crops, has enabled agriculture to increase its yields to the levels we recognise today. For example, developments such as reducing wheat crop height, which enables greater nitrogen application rates without the crops lodging, has resulted from such advances (Hawkesford *et al.*, 2013). Regardless of yield projections required to sustain population growth, merely creating more agricultural land and applying additional fertiliser will not ensure sustainability.

With increasing pressure on agriculture to produce enough food for a growing population, it is not only the quantity of food being produced that is of great concern, but the quality. Whilst 800 million people globally are affected by insufficient food for their needs, an estimated 2 billion suffer from hidden hunger (Welch and Graham, 2002). Hidden hunger, a deficiency in essential micronutrients, increases the risk of early mortality and morbidity, impacting on the quality of life. Iron (Fe) and Zinc (Zn) are two of the main elements that are considered part of the Hidden Hunger problem. Zinc deficiency has been strongly linked with stunting and immunity problems in children (MacDonald, 2000), with 17% of the global population at risk of deficiency (Black *et al.*, 2013). In 2013 a Hidden Hunger Index (HHI) was developed to help document the global distribution and incidence of this problem. It was anticipated that this tool would stimulate investment and a targeted response to help tackle the problem (Muthayya *et al.*, 2013). Ruel-Bergeron *et al.*, (2015) developed the HHI further to account for changes over time. They observed that, overall, hidden hunger improved, effecting 6.7% less people from 1995 to 2011, with Zn and vitamin A deficiencies showing the greatest improvements. It was noted that the HHI may be unreliable when monitoring Zn deficiencies in 0-5-year olds, which may be the result of multiple other causes.

Zinc deficient soils are generally the cause of cereal grain Zn deficiencies. Not only is the grain deficient in the element, but crop yield can also be halved (Hussain *et al.*, 2010). It is thought that up to a third of the population could be suffering from Zn deficiency, with that value varying between 4 and 73% depending on the country (Alloway, 2008b). Poor soils are often the cause of this deficiency, and issues such as extremes in pH, salinity and high phosphorus concentrations can exacerbate the problem. Diets which contain a high proportion of cereals present a problem due to their high phytate (inositol hexaphosphate) content, which binds with Zn, reducing its ability to be absorbed in the gastrointestinal tract (Alloway, 2008b). Conversely, phytate is thought to bind more readily with calcium (Ca), inhibiting its absorption, yet, its ramifications are less pronounced (Nissar *et al.*, 2017). Currently the main approach to overcome Zn deficiency in humans is biofortification in wheat (Hussain *et al.*, 2010). Whilst other management methods exist, for example, supplementation and food diversification, these are less sustainable in the long-term and/or not possible. The absence of complications resulting from the inhibition of Ca absorption

may be compensated through the addition of Ca to wheat-based products such as bread. The Food Standards Agency (FSA) in the UK require all wheat flour (with certain exceptions) to be fortified with iron and calcium carbonate to counteract this interaction (FSA, 1998).

1.3 Sustainable fertiliser sources

Whilst nitrogen can be produced via the Harbour-Bosch process (Takahashi, 2004), new phosphorus (P) and potassium (K) sources are only available through mined resources, unless recycled. With the projected necessity to increase the quantity of fertilisers available to meet crop requirements, these natural resources are becoming increasingly depleted. Globally, P availability has been well documented in the last decade due to depletion of rock P (Koppelaar and Weikard, 2013; Cooper *et al.*, 2011; Cordell *et al.*, 2009). Once converted into phosphate, it is no longer possible to convert back into its original elemental form, therefore it has limited recyclability. Concern regarding 'peak phosphorus' resulted in an 800% increase in the price of P in 2008 (Cordell and White, 2011). Peak Phosphorus is a term originally generated from Peak Oil or Peak Fossil Fuel. It is used to describe when the maximum output of phosphorus has been reached, thus implying that all phosphorus extraction after this point in time will diminish. Whilst there is a large amount of debate with regards to the validity of such a claim (IFDC, 2010) it is still having a financial impact upon the P market. Increasingly poor-quality P will be extracted from the earth to meet fertiliser demands, contaminating the soil and posing health risks via the food chain (Grant, 2011). Rock P is often associated with cadmium contamination, which can bioaccumulate in organs such as the kidneys and liver, causing irreversible damage (Bernard, 2008). Cadmium concentrations in P fertilisers have recently been under debate by the European Union with regard to a universal limit due to these health concerns (Gilbert, 2018)

Whilst inorganic fertilisers are more popular in agriculture compared to organic manures, they provide no additional benefits to the soil other than those presented in the form of nutrients. Organic fertiliser provides the additional benefit of adding organic matter (OM) to the soil. Organic matter supports microbial life by providing a food source which is eventually degraded into humus. Humus is plant and animal material that has undergone humification, creating high molecular weight compounds, that when combined, form hydrophilic polyelectrolytes called humus. The active humus pool is the most important as it supplies energy and mineral requirements for soil microbes, consequently supporting plant growth (Banwart *et al.*, 2015).

Although organic fertilisers are widely used, they are often spread inaccurately, with varying nutrient contents and transport costs are dictated by farm location; the high water content of organic manures makes them costly to transport. For example, a high density of dairy farms in the west of the UK means cattle slurry is relatively common, whereas in the

east, its availability is limited as the majority of farms are arable. Nevertheless, this problem goes beyond cost, as it has substantial impacts on the OM content of the soil. Organic matter is essential for reducing erosion, increasing water holding capacity and ensuring a sustainable microbial population. Soils with low organic matter often have issues with soil erosion, flooding, and poor yields (FAO, 2005). Soils containing high levels of OM often have a lower bulk density, improved aggregation and a higher cation exchange capacity (the ability of a soil to hold exchangeable cations). Other notable benefits from increasing OM are an increase in nitrogen content and improvement in crop yield (Whitmore *et al.*, 2017; Song *et al*, 2015). With an ever-increasing population, desiring a diet richer in meat (Revell, 2015), the prevalence of organic manures will only continue to increase. Whilst organic manures may be less predictable in terms of their nutrient release, they are still widely used, with 65% of UK farms using organic manures in 2016 (DEFRA, 2017). Cattle, pig and poultry manures make up 93% of all manure applications with 23% of cereal and oilseed rape crops and 85-90% of maize crops receiving manure annually (AIC, 2015).

Another organic source of nutrients comes from the treatment of human sewage sludge, specifically biosolids. These are less widely used, being applied to approximately 1.5% of all UK agricultural land (Water UK, 2013). Sewage sludge is one of the earliest forms of fertiliser used by humans (Kalavrouziotis, 2017). It is a bi-product of waste water treatment and has the potential to be a valuable, renewable resource. It contains the macronutrients nitrogen (N) and P along with a range of micronutrients such as copper (Cu), zinc (Zn) and manganese (Mn). Nevertheless, depending on their end use, different management practices must be applied prior to their application, by either treating them conventionally or through enhanced treatment. Conventionally treated biosolids are suitable for crops that are combined, used for animal feed or harvested grass and forage crops. They cannot be used on fruit, vegetable, horticultural or grazed crops without time restrictions due to pathogen risks associated with biosolids (Table 1.1). Conventionally treated sewage sludge must first be treated to decrease the pathogen load. Pathogens include, *Echerichia coli* (0157:H), listeria, norovirus and microsporidia (spore-forming unicellular parasites) (Smith *et al.*, 2004). However, the reduced pathogen level of enhanced biosolids means they are suitable for all the previously mentioned crops and can therefore be used in a wider range of situations (ADAS, 2001). Whilst their use is permitted legally, under specific contracts they may not be approved. This is evident from growers producing grains for whiskey production (Lewis, 2012. Pers Comm. Mr P. Lewis is a lecturer at Harper Adams University).

A typical 23 Mg ha^{-1} application of biosolids (cake) to a field would add 3.5 Mg of OM (Geneco, not dated). Its influence on crop yield potential is substantial enough that a temperate field with a Soil Organic Matter (SOM) content below 3.4% is thought to not meet its yield potential (Loveland and Webb (2003).

Biosolids produced from diverse Waste Water Treatment Works (WWTW) differ due to their treatment methods and ultimately, vary in the type of product being applied to land. Whilst WWTW's follow the same general treatment structure, the machinery and chemicals used affect the end product. Warman and Termeer (2005) proposed that chemical flocculants used in some waste water treatment facilities can decrease P mineralisation and therefore crop availability. Common flocculants include ferric sulphate, (used in large WWTW's) ferrous chloride (used in smaller WWTW's) and aluminium sulphate (used when iron based products don't work) (Gray, 2010). Such flocculants therefore increase element concentrations (e.g. iron and aluminium) in resulting sludge.

Adding further to their variability, biosolids also contain other heavy metal(loid)s including arsenic (As), lead (Pb) and silver (Ag). Historically, biosolids have been treated with caution due to their heavy metal contamination and are under regulation to limit the risk posed by their use. Permissible limits for heavy metal contamination in soil are monitored to reduce the risk of bioaccumulation and environmental damage (Table 1.2), but evidence suggests that the level of contamination in biosolids has decreased over time (Table 1.3). It is now an offense to allow or knowingly permit a water course to be contaminated, which is the likely cause encouraging the decrease in heavy metal load. Whilst this decrease is beneficial, biosolids still pose an environmental risk, which must be monitored. One problem created by producing a drier biosolids product (e.g. granules instead of cake) is that these contaminants will be further concentrated, increasing the risk they pose.

The wastewater treatment industry is strictly monitored for the quality of water being released into the environment, but historically, little concern has been given to the quality of the biosolids being produced as a by-product of this process. Whilst there are many guidelines and regulations regarding the use of biosolids, The Safe Sludge Matrix, (ADAS, 2001), Sewage Sludge Use in Agriculture Regulations (1989) and the Biosolids Nutrient Management Matrix (ADAS, 2014), are examples of regulations applied for its safe use. The Biosolids Assurance Scheme (2014) was created to further improve confidence in the industry, whilst reassuring producers and consumers, that biosolids were safe. Aimed at sludge recyclers and processors, the scheme became compulsory at the end of 2017, requiring all 11 UK water and sludge processing companies to meet a minimum quality standard. The scheme is funded through levy payers and managed by Assured Biosolids Limited. Audits are completed by a third-party company before a NSF Certificate can be awarded. The expectation is to increase confidence in biosolid usage due to the positive impact they can have on soils physical properties. Studies by Pascual *et al.* (2009), Koutroubas *et al.* (2014) and Lloret *et al.* (2016), have all demonstrated soil improvements such as electrical conductivity, cation exchange capacity and organic matter content. Kumpiene *et al.* (2008) revealed that the addition of biosolids may also have the potential to decrease the bioavailability of heavy metals in soils.

Table 1.1. Application restrictions for conventionally and enhanced treated sludge's (ADAS, 2001).

Crop	Sludge treatment	
	Conventionally treated	Enhanced treated
Fruit	No	10 month harvest interval applies
Salad	30 month harvest interval required	10 month harvest interval applies
Vegetable	12 month harvest interval required	10 month harvest interval applies
Horticulture	No	10 month harvest interval applies
Combinable and animal feed crops	Yes	Yes
Grass and forage (grazed)	Deep injection or ploughed in only. 3 week no grazing and harvest interval	3 week no grazing and no harvest interval applies
Grass and forage (harvested)	No grazing in the same season as application. 3 week no harvest interval	3 week no grazing and no harvest interval applies

1.4 Effect of biosolids on soil properties

A review by Sharma *et al.* (2017) outlined the positive effects of biosolids application on soil properties and is summarised in Table 1.4. Adair *et al.* (2014) showed that biofuel crops (*Brassica napus* and *Camelina sativa*) could reach similar or greater yields when grown using biosolids as a nutrient source compared with conventional fertilisers. This study used two application rates, 316 kg ha^{-1} N and 158 kg ha^{-1} N with the higher application rate producing almost double the seed yield of any other treatment. Conversely, seed oil content was greatest when the crop was grown with urea as the only source of nutrients. Fernadez *et al* (2009) revealed that biosolid applications caused a reduction in crop yield with higher and more frequent applications rates. Whilst the results from each of these experiments may show contrasting conclusions, each study has one commonality, their biosolids differed.

The application of biosolids to land used for crop production has been well documented. Deeks *et al.* (2013) used the same biosolids used in this experimental series and showed, that when supplemented with additional N and K to meet a crops nutrient requirements, biosolids produced similar yields to that of commercially available, inorganic

fertilisers. Whilst this study was carried out over several years, it was only completed at one field site, with biosolids being supplemented with additional fertiliser on each occasion. Thus, there was no definitive conclusion as to whether biosolids could produce similar yields when used on their own as the additional inorganic N and K additions may have hidden the true potential of the biosolids. Additional inorganic N and K would have been utilised by the plant differently and therefore may have been favourably used and may have also affected the uptake of other nutrients.

Table1.2. Permitted levels of heavy metals in soil at the respective soil pH (DEFRA, 2006).

PTE	Maximum permissible concentration of PTE in soil (mg kg^{-1} dry solids)				Maximum permissible average annual rate of PTE addition over a 10 year period (kg ha^{-1})
	pH 5.0<5.5	pH 5.5<6.0	pH 6.0-7.0	pH(^{1}) >7.0	
Zinc	200	200	200	300	15
Copper	80	100	135	200	7.5
Nickel	50	60	75	110	3
For pH 5.0 and above					
Cadmium	3		0.15		
Lead	300		15		
Mercury	1		0.1		
*Chromium	400		15		
*Molybdenum	4		0.2		
*Selenium	3		0.15		
*Arsenic	50		0.7		
*Fluoride	500		20		

^{1} Must contain >5% $CaCO_3$

*Not listed under Directive 86/278/EEC

Table 1.3. Chronological decrease in sewage sludge heavy metal concentrations (mg kg^{-1}) from 1983 to 1996.

Element	Year			Reduction between 1983 and 1996 (%)
	1983	1990	1996	
Zn	1319	922	792	40
Cu	703	574	568	19
Ni	107	65	57	47
Cd	14	5	3.3	76
Pb	462	201	221	52
Hg	5	3.5	2.4	52
Cr	312	208	157	50

Table 1.4. Physical, chemical and biological changes recorded following biosolids applications (adapted from Sharma *et al.* (2017).

Soil property	Impact	Reference
Physical		
pH	Decreased	Sastre *et al.* (1996), Roca-Pérez *et al.* (2009), Xue and Huang (2013) and Lloret *et al.* (2016)
Aggregate stability	Increased	Roca-Pérez *et al.* (2009)
Bulk density	Decreased	Veeresh *et al.* (2003)
Water holding capacity	Increased	Veeresh *et al.* (2003)
Chemical		
Heavy metals	Increased	Latare *et al.* (2014), Koutroubas *et al.* (2014); Marguí *et al.*, 2016 and Lloret *et al.* (2016)
Micronutrients	Increased	Latare *et al.* (2014), Koutroubas *et al.* (2014) and Lloret *et al.* (2016)
Macronutrients	Increased	Sastre *et al.* (1996), Singh and Agrawal (2007, 2009, 2010b,c),Roca-Pérez *et al.* (2009), Xue and Huang (2013), Latare *et al.* (2014) and Lloret *et al.* (2016)
Electrical conductivity	Increased	Singh and Agrawal (2007, 2009, 2010b,c), Roca-Pérez *et al.* (2009), Xue and Huang (2013), Latare *et al.* (2014) and Lloret *et al.* (2016)
Cation exchange capacity	Increased	Singh and Agrawal (2007, 2009), Xue and Huang (2013), Latare *et al.* (2014) and Lloret *et al.* (2016
Biological		
Microbial biomass	Increased	García-Gil *et al.* (2004), Sanchez-Monedero *et al.* (2004), Xue and Huang (2013) and Lloret *et al.* (2016)
Enzyme activity	Increased	Carbonell *et al.* (2009), García-Gil *et al.* (2000), Sastre *et al.* (1996), Xue and Huang (2013) and Carbonell *et al.* (2009) and Xue and Huang (2013)
Microbial populations	Increased	Sastre *et al.* (1996)
Microbial activity	Increased	Debosz *et al.* (2002), García-Gil *et al.* (2004), Carbonell *et al.* (2009), Xue and Huang (2013), Lloret *et al.* (2016) and Sánchez-Monedero *et al.* (2004)

This thesis aims to study the impact of Bestways fertiliser (enhanced treated biosolids produced by Southern Water in 2011) on both the environment and crop production. A range of laboratory, glasshouse and field experiments were established to meet the following objectives:

1. Detail the chemical properties of the biosolids: The investigations reported within this thesis have used a single batch of biosolids that have been analysed to determine their physicochemical properties (further details can be found in Chapter 2).

2. Understand the bioavailability of metal(loid)s present in the biosolids and determine their potential to cause contamination.

3. Quantify the impact on soil biology following short and long-term biosolid applications.

4. Assess the effect of biosolids on crop growth and quantify their impact on crop metal(loid) uptake.

1.5 Chapter Details

Chapter 2. Background information.

The chapter quantifies the biosolids used throughout the experiments, together with details relating to previous applications at a field site in Broxton, Cheshire, where biosolids were applied annually between 2008 and 2014.

Chapter 3. Sequential extraction investigations.

The chapter investigates metal partitioning in two contrasting soil types following biosolids incorporation. The chapter investigates where heavy metals (Ag, As, Cu, Fe, Mn, Pb and Zn) from biosolids are potentially bound in soils and their probability to cause contamination.

Chapter 4. Long-term leaching investigations.

The chapter details the potential of biosolids to leach heavy metal contaminants (Ag, As, Cu, Fe, Mn, Pb and Zn) following their addition to soils over the long-term. Leaching of total organic carbon (TOC) is also investigated in this chapter and the role it plays in metal ion mobility.

Chapter 5. The impact of biosolid applications on soil biology.

The chapter investigates the impact of long- and short-term biosolid applications on soil biology by assessing microbial activity through respiration (carbon dioxide evolution), microbial biomass examined by adenosine triphosphate (ATP) soil concentrations and

microarthropod populations, examined using the QBS method developed by Parisis *et al.*, (2000).

Chapter 6. The impact of biosolids on crop growth and grain nutrient concentrations.

The chapter investigates the application of biosolids on crop yield and grain metal uptake. Field and glasshouse studies investigate the effect of biosolids applications on a range of cereals and the translocation of Ag, As, Cu, Fe, Mn, Pb and Zn into the grain.

Chapter 7. Conclusions and future considerations.

The study is analysed for its practical implications on the use of biosolids in agriculture, assessing their impact on the soil environment and plant growth and providing recommendations for their future use by the industry.

The central null hypothesis for the work within this thesis is that the application of biosolids to soil does not have a detrimental effect on soil biology, chemistry or resulting crop yield.

Due to the nature of the work contained in this thesis, no single literature review is presented; instead, a review of the relevant literature is covered at the start of each individual chapter.

Due to the term heavy metals being ambiguous in the literature, its use in this thesis will refer to both heavy metals and heavy metalloids.

Chapter 2.

Background to the research.

2.1 The Wastewater Treatment Process

As with all organic manures, their chemical composition is very variable. Biosolids are no exception, resulting from the changes in daily input (influent) to the Waste Water Treatment Works (WWTW). In contrast to Water Treatment Works (WTW) who use ground and surface waters, its main constituents are industry wastewater, household waste and storm surges (Southern Water, no date). Whilst most WWTW follow, broadly the same treatment process (Figure 2.1), each may employ different chemical techniques across the different treatment stages, adding further to their variability.

Preliminary Treatment

↓

Primary Treatment (sedimentation)

↓

Secondary Treatment (biological)

↓

Tertiary Treatment

↓

Sludge Treatment

↓

Disposal of Water to Rivers
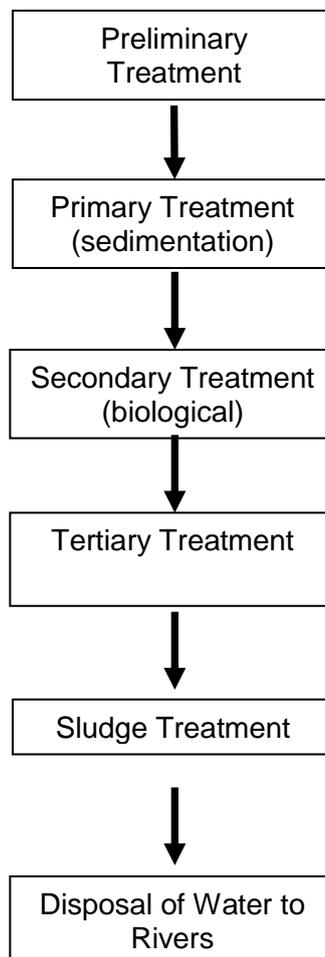
Figure 2.1. Generalised flow of wastewater treatment.

Each treatment step is established with specific aims (Gray, 2010);

1. Preliminary treatment - aiming to remove large solids and grit.

    Water with low levels of contamination and debris may be directly released following preliminary treatment via long sea outfalls.

2. Primary treatment – removal of settleable solids.

    Addition of chemicals such as iron (III) sulphate ($Fe_2(SO_4)_3$) or iron (III) chloride ($FeCl_3$) are added to aid coagulation.

3. Secondary treatment - organic matter is oxidised by microorganisms.

    Applied to all effluent being discharged to inland waters

4. Tertiary treatment – removal of residual BOD, contaminants and nutrients.

    Used for higher quality water due to eutrophication where discharge is made to a lake or groundwater.

5. Sludge treatment – dewatering, stabilisation and disposal of sludge.

    Sludge treatment differs depending on the end disposal route.

The implementation of the Urban Waste Water Treatment Directive (91/271/EEC) resulted in a change to sludge disposal aims (EC, 1991);

1. Sludge must be reused where possible, whilst limiting the impact on the environment

2. No disposal is permitted at sea

3. Total toxic, persistent and bioaccumable contaminants must be reduced with time.

Overall, the aim of the directive was to produce clean water as it was returned to the environment. Until this directive was introduced, 27% of all sludge was disposed at sea (25% via boats and 2% via pipelines). With this no longer being an option, the disposal of biosolids to agricultural land was deemed as the Best Practical Environmental Option (BPEO) (Bacon *et al.*, 2001).

2.2. Southern Water Biosolids

Southern Water produced granulated biosolids for a short period of time, marketed under the name Bestways Fertilisers. The batch used for the work presented in this thesis originated from the Ashford Wastewater Treatment Works at Bybrook (Grid reference TR 02080 43327). This WWTW currently services a population of approximately 104,000. It is described as a large filter works, containing six primary sedimentation tanks, 16 rock filter beds and eight radial humus tanks (for secondary treatment) with three plastic media nitrifying tertiary filters, alongside six deep bed sand filters. The only chemical addition to the WWTW was iron (III) sulphate, acting as a coagulant for phosphorus (P) (Veesam, 2017. Pers Comm, Ms M. Veesam, a Senior Process Scientist at Southern Water).

The site also supported a large sludge treatment centre where the resulting sludge was mixed with imported sludge from smaller WWTW's before being thickened. Following mixing, the sludge underwent Mesophilic Anaerobic Digestion (MAD) at 35ºC for 14 days to allow bacteria to decompose organic matter. Methane gas and carbon dioxide (CO_2) resulting from this process were then recycled to a combined heat and power plant on site. Following MAD, the sludge was dewatered in a decanter centrifuge, decreasing the water content, with the extracted water returning back to the WWTW for treatment. The resulting sludge cake was then stored for four months to decrease bacterial load (Kent County Council, 2005). Finally, the biosolids were processed through a Drum Dryer creating granulated biosolids (Figure 2.2) (Andritz Separation, 2012). The resulting product contains approximately 5% water, reduced from 96% (Gray, 2010).

Figure 2.2. Bestways Fertiliser produced by Southern Water.

Thermal treatment of biosolids for the production of granules is an optional step, not often utilised by WWTW's. It has the added benefit of allowing farmers to apply biosolids through a conventional fertiliser spreader at a time suitable to them, rather than relying on

contractors. It also decreases the odour associated with their application. Whilst it produces a product with a higher treatment level than sludge cake (enhanced and conventional respectively), which can be used on a wider range of crops (ADAS, 2001), the process involves heating sludge to 260ºC for 30 minutes in some cases (Gray, 2010). Where anaerobic digestion is part of the sludge treatment process, the resulting methane gas can be used as fuel, although large capital and operational costs are often the most influential factors when this additional treatment is considered. The cost in 2011 for drying biosolid was thought to add an approximately £150 per tonne to the costs of incineration (Per comms. K. Chaney, Harper Adams University). Importantly for agriculture, up to 65% of the OM can be oxidised during this process, reducing its value to soils compared with sludge cake (Gray, 2010).

2.3 Four year biosolids experiment

Prior to this study commencing in 2012, four years of biosolid applications were made to a field site in Cheshire. Field experiments investigated biosolid effect on yield at Fields Farm, Broxton, Cheshire, UK (53° 5' 7.3896'' N 2° 46' 47.5824'' W). The trial (1.4 ha arable block and 0.1 ha grassland plots) was situated in a 7.4 ha field and had received applications of biosolids annually since 2008, with harvest data being collected from 2009 under two different projects; KTP Organo-Mineral Fertiliser (OMF) (2007-2010) and END-O-SLUDG (2011-2014) (Table 2.1).This site was selected due to its poor soil condition (e.g. poor drainage) therefore providing the potential to show improvements in soil quality following annual applications of biosolids.

Table 2.1. Chemical analysis of the Bestways biosolids fertiliser (*n=30*).

Element	mg kg^{-1}
Al	7743.01
P	3.09
Cr	200.29
Mn	841.07
Fe	56012.57
Ni	587.08
Cu	676.56
Zn	916.78
As	5.77
Mo	7.18
Ag	6.18
Pb	197.66
OM%	61.40

The 1.4 ha arable block was split into 4 subsections (Figure 2.3), each being planted with a different crop annually, simulating a rotation scenario observed in commercial environments (Table 2.2). Grass plots were cut for silage, three times annually to simulate that of commercial crops (Figure 2.4).
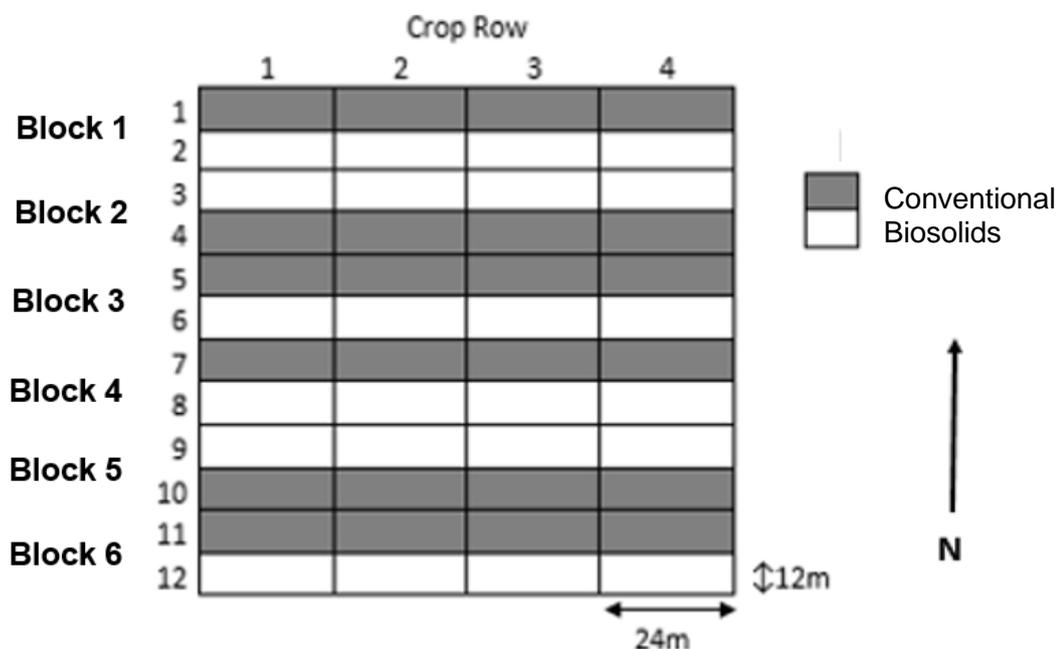
Figure 2.3 Arable field plan at Broxton, Cheshire, under the KTP OMF, and END-O-SLUDGE projects.
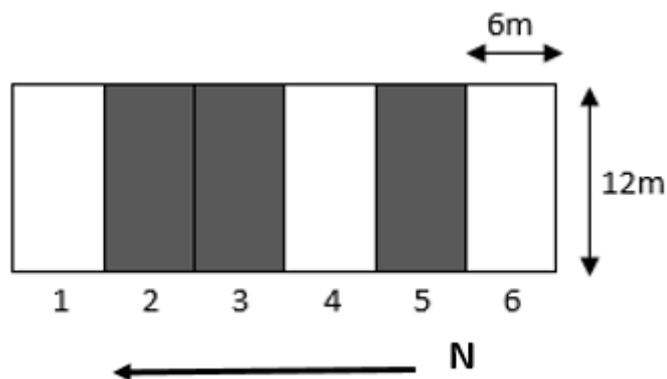
Figure 2.4. Grass field plan at Broxton, Cheshire.

Table 2.2 Crop rotation history at Broxton, Cheshire.

Crop row	2008/2009	2009/2010	2010/2011	2011/2012
1	Winter wheat	Forage maize	Winter wheat	Winter oats
2	Winter wheat	Spring wheat	Winter Barley	Triticale
3	Oilseed rape	Winter wheat	Spring beans	Winter wheat
4	Forage maize	Spring Oilseed rape	Oilseed rape	Winter wheat

Two treatments were applied throughout the experimental series, biosolids and conventional fertiliser (Table 2.4). Nitrogen (N), phosphorus (P) and potassium (K) were adjusted according to crop requirements using RB209 (DEFRA, 2011) (Table 2.2). All fertiliser applications were made using a commercial Kuhn Aero Spreader. The pneumatic spreader ensured an even spread of fertiliser across the width of the boom, reducing variability. During the 2008/2009 and 2010/2011 seasons, conventional applications were made with no phosphate as soil analysis indicated no additional P was required to meet the crops growth demands (P index 3). From 2011/2012 onwards, phosphate was applied at the same rate as biosolids to ensure that this was not a limiting factor, and consequently affecting the results.

Full details of the trial series up to 2010/2011 are available in Deeks *et al.* (2013). Using a One-way ANOVA to test for statistical significant, overall no significant differences (p>0.05) were observed between yields, with the exception of winter wheat harvested between 2008/2009. In this instance the conventionally treated crop yielded 20% higher than that of the crop receiving biosolids. Crop yield was lower than the UK average, but this was attributed to local weather and soil conditions (Deeks *et al.* 2013). The significant difference observed between 2008/2009 was thought to be due to the low number of grains produced as Thousand Grain Weight (TGW) was not significantly different between the two

treatments (41.1g for Conventionally treated and 42.0g for Biosolids treated) indicating that the weight of the grains produced were similar.

Soil cores were collected from each plot, taking 20 to a depth of 15cm, from 2008 onwards to assess the impact of the treatments on soil parameters (Table 2.5). Analyses conducted by NRM laboratories revealed that macronutrient concentrations were adequate to support crop growth, in accordance with Defra 2010.

Table 2.3 Soil nutrient status for each year, following the respective treatments.

	2008	2009		2010		2011	
	Baseline	Conventional	Biosolids	Conventional	Biosolids	Conventional	Biosolids
pH	7.10	6.88	6.88	7.18	7.08	6.85	6.85
P (mg kg^{-1})	24.00	28.00	25.25	21.00	18.50	20.75	20.50
K (mg kg^{-1})	115.00	131.75	145.00	117.00	98.75	98.00	94.00
S (mg kg^{-1})	5.00	8.50	12.00	4.75	4.00	6.75	7.75
Ca (mg kg^{-1})	2204.00	2228.50	2290.50	2284.00	2215.50	2107.50	2008.50
Mn (mg kg^{-1})	39.00	31.75	30.75	42.00	38.75	37.50	37.50
Cu (mg kg^{-1})	N/A	N/A	N/A	N/A	N/A	4.73	4.80
Zn (mg kg^{-1})	N/A	N/A	N/A	N/A	N/A	3.83	3.78
CEC (meq 100g^{-1})	14.1	N/A	N/A	N/A	N/A	N/A	N/A

The experimental work detailed in this thesis builds on previous investigations (Deeks *et al.*, 2013; Smith *et al.*, 2015). Due to biosolids high variability, all experiments detailed in this thesis have used the same batch produced by Southern Water. On arrival, the biosolids were re-mixed, sub-sampled and subsequently analysed (Table 2.1). Chemical analysis was completed via microwave digestion (Mars 6, CEM, UK) and metal(loid)s analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Organic Matter percentage (OM%) was determined by Loss on Ignition (LOI).

Table 2.4. Nutrient content applied to the plots under their respective treatment and year.

a)

Crop row	2008/2009 Conventional (kg ha^{-1})			Biosolids (kg ha^{-1})			2009/2010 Conventional (kg ha^{-1})			Biosolids (kg ha^{-1})			2010/2011 Conventional (kg ha^{-1})			Biosolids (kg ha^{-1})		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
1	195	0	70	195	51	70	120	0	100	120	31	100	195	0	70	195	51	70
2	195	0	70	195	51	70	195	0	70	195	51	70	120	0	70	120	31	70
3	105	0	70	105	30	70	195	0	70	195	51	70	0	0	70	15	31	70
4	120	0	100	105	31	100	70	0	60	70	15	60	70	0	70	70	15	70

b)

Crop row	2011/2012 scenario A Conventional (kg ha^{-1})			Biosolids (kg ha^{-1})			2011/2012 scenario B Conventional (kg ha^{-1})			Biosolids (kg ha^{-1})		
	N	P	K	N	P	K	N	P	K	N	P	K
1	90	45	65	90	45	65	90	45	65	90	45	65
2	90	45	65	90	45	65	90	45	65	90	45	65
3	165	60	75	165	60	75	165	60	75	165	60	75
4	165	60	75	165	60	75	165	60	75	165	60	75

Table 2.5. Mean soil nutrient and pH properties between 2008 and 2011.

	2008	2009		2010		2011	
	Pre-applications	Biosolids	Conventional	Biosolids	Conventional	Biosolids	Conventional
pH	7.10	6.88	6.88	7.08	7.18	6.85	6.85
P (mg/l)	24.00	25.25	28.00	18.50	21.00	20.50	20.75
K (mg/l)	115.0	145.0	131.8	98.8	117.0	93.5	98.0
S (mg/l)	5.00	12.00	8.50	4.00	4.75	7.75	6.75
Ca (mg/l)	2204	2291	2229	2216	2284	2009	2108
Mn (mg/l)	39.00	30.75	31.75	38.75	42.00	37.50	37.50
Cu (mg/l)	4.50	-	-	-	-	4.80	4.73
Zn (mg/l)	4.60	-	-	-	-	3.78	3.83

Nutrient differences between biosolids and conventionally-treated plots were not observed over time. It is not clear from this data if the nutrients applied as biosolids were able to support growth on their own, without the addition of inorganic fertilisers, such as N. Further testing would be required to assess what impact the two fertiliser treatments had on the grain/forage produced.

2.4 Spreader tests for commercial applications

Spreader tests were completed in conjunction with Spreader and Sprayer Test Ltd. in August 2011. These tests enabled the biosolid granules to be characterised and tested for their spreadability. These results were compared with commercially available urea fertiliser (Table 2.6).

Table 2.6. Comparison of the characteristics of biosolids to urea.

	Biosolids	Urea
Density (kg L^{-1})	0.67	0.79
Strength (kg/Force)	2-10	3-5
Granule size distribution		
<2mm (%)	5	0
2-3.3mm (%)	20	10
3.3-4.74mm (%)	70	90
>4.75mm (%)	5	0

The comparison of the two materials revealed that there was a considerable difference between the two products. Biosolids are lighter and require substantially more force to crush compared to urea, demonstrating that whilst they are likely to be able to undergo the forces projected by a spinning disk spreader, they may not be able to spread as far due to their lower density. Their size distribution is also spread over a larger range than that of urea. This may result in the reduced flow of material when spreading as they are likely to form a mass. Spreader tests showed that biosolids could reach 24m (the normal spreading distance for most farmers).

The research in this thesis aims to profile the biosolids produced by Southern Water (Bestways Fertiliser). By profiling their chemical release through laboratory-based sequential extractions and leaching experiments, it is hoped that the risk posed by this product to the environment will be detailed for both short- and long-term applications.

Investigations will then focus on the impact of field applications to soil biology specifically microarthropod numbers and carbon dioxide evolution. Finally, an assessment

of how biosolids compare to conventional fertilisers in relation to crop production, showing how they may impact crop yield, will be evaluated.

The soil at the two experimental locations (Harper Adams University and Broxton, Cheshire) was profiled (Table 2.7).

Table 2.7. Soil profiles of the experimental sites at Harper Adams University and Broxton, Cheshire.

Detail	Broxton, Cheshire	Harper Adams University
Sand (2-0.063mm) (w/w%)	51	73
Silt (0.063-0.002mm) (w/w%)	26	16
Clay (<0.002mm) (w/w%)	23	11
Textural class	Sandy Clay Loam	Sandy Loam
pH (1:10)	7	7.6
P (mg kg^{-1})	23.6	60.8
K (mg kg^{-1})	156.6	350
Mg (mg kg^{-1})	111.6	82
Pseudo-total Ag (mg kg-1)	0.16	0.01
Pseudo-total As (mg kg-1)	5.5	4.74
Pseudo-total Cu (mg kg-1)	27.28	16.74
Pseudo-total Fe (mg kg-1)	5950.83	7514.94
Pseudo-total Mn (mg kg-1)	310.36	199.62
Pseudo-total Pb (mg kg-1)	39.71	15.43
Pseudo-total Zn (mg kg-1)	127.39	71.2

Chapter 3.
Metal partitioning in biosolids-amended soils.

3.1 Introduction

To understand the potential benefits and risks of any fertiliser input, it must be assessed for its effects on soil chemistry. Chemical binding of metals in soils determines their mobility and therefore influences toxicity (Ma and Rao, 1997). Chemically partitioning elements allows an understanding as to their quantity and likely reactivity, thus enabling assessments to be made regarding the short-term availability. Yet, single extractant methodologies only allow quantification of single fraction groups. No single extractant can currently be used to indicate what may replenish such fractions if they were removed from the system and total metal concentrations (the concentration of all of the element found in the soil) are not truly representative of what is bioavailable (available to be absorbed by a living system) and thus the hazards they pose to animals and humans through the soil-plant pathway.

Sequential extractions are multi-step techniques used to investigate such metal partitioning in soils. A range of extractants, with increasing extraction strength, are shaken with soil, partitioning metals bound with decreasing mobility at each stage. Such partitioning allows each fraction to be assessed individually, whilst also providing details as to the quantity of an element that could replenish concentrations should they be removed from the soil via translocation or leaching. Tessier *et al.* (1979) developed the original heavy metal sequential extraction procedure, with similar methods such as the Community Bureau of Reference (BCR) being developed later. The Tessier, BCR and other techniques broadly follow the same procedure, with the Tessier methodology being split into five stages;

1. Exchangeable fraction; removes metals adsorbed to the surface of sediment through the alteration of its ionic composition.
2. Carbonate bound fraction; removes metals through changes in pH
3. Fe/Mn oxide fraction; removes metals though reduction
4. Organically bound fraction; removes metals bound or organic matter through oxidation.
5. Residual fraction; removes metals through the breakdown of silicate structures.

BCR methodology combines the first two stages of the Tessier method and follows a general protocol.

The exchangeable fraction (stage 1 and 2 of the Tessier method) is assessed using acetic acid, which affects the adsorption of heavy metals in the exchangeable fraction.

Acetic acid displaces ions from the exchange sites, affecting a soils sorption-desorption processes.

The reducible fraction (Stage 3 of the Tessier method) is extracted using hydroxyl ammonium chloride, which interferes with the heavy metals bound to iron and manganese oxides. These oxides form nodules and concretions under alternating oxidizing – reducing conditions (Lichthouse *et al*, 2012).

The organically bound fraction (stage 4 of the Tessier method) uses hydrogen peroxide and ammonium acetate to fractionate metals bound to organic matter and sulphides. Organic matter (in the form of humic and fluvic acids) bind to metals due to its peptisation and complexation properties. Organic matter can be broken down in a natural environment to releases heavy metals into soil solution in oxidising conditions and therefore it is an important fraction to assess (Tessier *et al.*, 1979).

The final fraction (residual fraction) is normally extracted using hydrochloric acid (HCl), nitric acid (NHO$_3$) and hydrofluoric acid (HF). These acids release heavy metals bound to the soils silicates and minerals within the soils crystal structures – a fraction all other extracts were not able to release. This is the only fraction that would not be released in to soil solution under normal environmental conditions (Hartley, 2004).

Fractions can then be analysed using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Extractants are not able to fractionate individual elements, instead they extract by a binding group.

Kim and McBride (2006) proposed that the extraction order undertaken when conducting sequential extractions using the BCR methodology should not impact on the concentrations of metals extracted. This implies that the extractants have a degree of selectivity. It was determined that the reverse extraction did not produced results consistent with an independent extraction technique (citrate-bicarbonate-dithionite extraction), specifically being unable to quantify the concentration of zinc (Zn) and cadmium (Cd). It was suggested that the extractants were too strong to be selective in nature. Yet, copper (Cu) concentrations were similar regardless of the technique used. The experiment concluded that the reliability of the BCR technique was dependent on the reagents used, the order of the extraction and the metals under investigation.

Sequential extractions are normally used on soil to assess their heavy metal contents, especially where organic amendments are added as they have the potential to increase heavy metal concentrations. The extent to which these heavy metals are available to a plant are influenced by immobilisation, reduction and evaporation processes, along with a plants ability to modify its rhizosphere (Grobelak and Naproa 2015). Such applications often result in changes to soil pH. Perez-Esteban *et al.* (2013) suggested that Cu concentrations were reduced in the bioavailable fractions (water extractable, exchangeable, carbonate bound and Fe/Mn bound) through increases in pH and organic matter using compost amended

with horse and sheep manure. Results also indicated that these applications decreased the translocation of Cu and Zn to *Atriplex halimus* (Fodder Shrub), thus reducing the potential for bioaccumulation (accumulate in the cells of living tissues) in the food chain. This is important as heavy metals that bioaccumulate in tissues have the potential to increase to such levels where they may become toxic.

Shober (2007) analysed the effect of biosolid applications on heavy metal concentrations across five farms using the BCR methodology. Results indicated a significant increase in Cu and Zn, in all fractions when compared with an untreated control. However, the untreated control soil was taken from a separate field, with the assumption that heavy metal concentrations were similar prior to any biosolids being applied. Each field received a different application rate and at different frequencies and intervals. The experiment also provided no details as to which isotope of Cu was used for analysis. Whilst Cu with the atomic mass of 63 is more abundant, it has a polyatomic interference with phosphorus (P) and therefore may show falsely elevated levels. This interference is substantially lower with [65]Cu. Yet, Nogueira *et al.* (2010) concluded that no significant increase in Cd or lead (Pb) concentrations occurred in field soil receiving a range of application rates (ranging from 0 to 127.5 Mg ha^{-1} dry solids per annum) following nine years of biosolid applications. However, concentrations of Zn did increase overall.

Two hypotheses have been proposed regarding the application of biosolids to soils in which sequential extractions are key. The Plateau and Time-bomb hypotheses contrast in theory whereby biosolids will prevent the excessive uptake of heavy metals into plant tissues due to the addition of organic matter which is also found in their composition (Plateau hypothesis), compared with the mineralisation of organic matter which will release heavy metals in the soil into readily available forms, following biosolids applications. McBride (1995) suggests that the organic matter provides protection against the release of heavy metals into solution, through it being resistant to decomposition, yet the exact details regarding OM bring resistant to decomposition are not given. This would suggest that the heavy metal and OM content of the biosolids being added to the soil were always consistent, or that the OM applied could always compensate for any increase in heavy metal concentrations. We know this hypothesis to be incorrect through studies where biosolid applications have increased soil heavy metal contents (Malinowska, 2017; Sanchez-Martin *et al.*, 2007; Parkpain *et al*, 2000) and increases in plant tissue contents (Evanylo *et al.*, 2006).

In contrast, the Plateau hypothesis states that the addition of biosolids to soil increases the amount of heavy metals taken up by plants initially, however this is only a short term reaction until the exchange sites in the biosolids become dominant, causing a plateau effect. This reaction is only possible under the following conditions;

1. If the plateau is not reached and the biosolids applications are stopped, then the plant tissue concentrations will not be affected
2. If the plateau is reached and applications continue to be made, the tissue concentrations will not exceed those found at the plateau.

Chang *et al.* (1997) found that following 10 years of biosolids applications, no plateau was ever reached. It showed a continual increase in both plant and soil tissue concentrations for Cd.

The Time-Bomb hypothesis relies on the heavy metals binding irreversibly to the OM/sulphide fraction, whilst the Plateau hypothesis relies on the heavy metals in the biosolids accumulating in the more bioavailable fractions.

Sequential extractions have been used on biosolids directly. Fuentes *et al.* (2004) assessed BCR sequential extractions on a range of sewage sludge types e.g. non-stabilised and anaerobically digested) to quantify if the heavy metal contamination levels were greater than those permitted by European Union (EU) legislation. It was determined that the metal content did not exceed these limits, however, such data could have been gained through total digestion techniques. This data itself does not give any indication as to how the biosolids might react in soil as characteristics such a pH and organic matter could have a substantial influence on the release of heavy metals from each type of biosolid. In addition to this, no detail is given regarding how applicable these biosolids are to other batches produced making recommendations that the anaerobic sludge should not be applied due to its high Cr content, unclear to interpret.

Elements may also readsorb to the soil once in solution. Due to these issues there is some debate as to the reliability of sequential extractions. The Tessier methodology (Tessier, 1979) has been shown to result in the redistribution of heavy metals, even through the use of weak extractants at stage one (Quan and Bi (1993); Ajayi and Vanloon (1989); Kheboian and Bauer (1987)). Other research found that changes in the volume of reagent used resulted in different concentrations of elements being extracted. Rauet *et al.* (1999) found that increasing the volume of reagent used from the normal 8 ml and 20 ml (following the Tessier methodology), to 50 ml, a significant increase to the concentration of the metals was recorded. However, despite these concerns Ho and Evans (2000) found that the amount of reabsorption was lower than originally thought and therefore should not invalidate any results obtained using such extraction techniques. Kim and McBride (2006) suggest that lowering the pH of all reagents to 1.5-2 may prevent reabsorption. Although sequential extraction techniques have been broadly used, they cannot be compared to single extraction procedures.

The addition of biosolids to soils by Grobelek and Napra (2015) showed a decrease in Cd concentration in the exchangeable fraction, indicating that biosolid applications may decrease the availability of some heavy metals in the more available fractions. However,

bioavailability was decreased most significantly though the addition of biosolids with triple super phosphate (TSP) when observing Zn, Cd and Pb concentrations. Whilst such additions may show a positive effect when considering environmental and food chain contamination, their application together in a real agricultural environment is very unlikely. Biosolids provide excess P to plant requirements. Adding large quantities of biosolids to soils over time is likely to increase soil concentrations, possibly resulting in environmental pollution. Antille *et al.* (2014) indicated the availability of phosphorus in the same biosolids as used in this thesis were substantially below expectations. Under current guidelines provided by DEFRA in RB209, it is estimated that 50% of P is available within the first year post application (DEFRA, 2008). Antille *et al.* suggested that this may actually be as low as 6.5%. This methodology involved the incubation of soil for several months at 25ºC. Whilst this may provide a more realistic approach in assessing plant available P, compared with microwave digestion, it does not represent any detail with regards to what pools the P is found in, nor is the incubation temperature representative of the climatic conditions for most temperate countries.

Sanchez-Martin *et al.* (2007) looked at applying two application rates of biosolids (20 g and 200 g kg^{-1} soil) to two differing soil types. Their experiment involved incubating soil at 25ºC for 18 months before heavy metals were analysed using BCR. Overall, an increase in heavy metal concentrations were recorded, irrespective of the soil type used, however, the regulatory limits for heavy metal content in soils were never reached. It was noted that the OM content of the soils increased between 3.5 and 7 times in the soils receiving 200 g/kg of biosolids. Heavy metal concentrations only increased above untreated soils in the residual fraction, indicating that following a single application of biosolids to soil, they did not pose an environmental risk but could be advantageous in terms of their effect on OM. Whilst this experiment provides an insight into the release of heavy metals from biosolids, it would not be representative of soil temperatures in the UK and therefore may not be indicative of what would happen in UK soils.

Contrasting results regarding heavy metal concentrations (e.g. Perez-Esteban *et al.*, 2013 and Shober, 2007) make comparing biosolid applications difficult to apply to different situations. Whilst many studies show the effect of biosolid applications, often these biosolids are in different forms (e.g. cake, liquid and granules). The aim of this chapter was to establish the partitioning of heavy metal within two contrasting soil types treated with biosolids, using the same methodology on each. It is essential to understand the chemical bonding and thus the potential for environmental contamination and bioaccumulation that the biosolids pose in order to understand how these biosolids may best be applied to field soils in the UK. Heavy metal sequential extractions using the BCR method were carried out on Harper Adams University Farm soil and Broxton Cheshire soil, following one application of biosolids. The BCR methodology was created in 1987 to try to eliminate such variation in

analytical techniques for metal detection in soils, therefore, this technique was selected for analysing the biosolid treated soils in this chapter (Fuentes *et al*., 2004). The BCR methodology only differs from Tessier in the first two stages; with BCR combining stage one and two into a single step. The BCR technique was compared with the Tessier methodology by Zhang *et al*. (1998). They concluded that there was no significant difference between the results from the two techniques. This was further supported by Rosado *et al*. (2016).

A further sequential extraction was carried out on biosolids treated soil from Broxton to assess the accumulation of P following 8 successive years of applications to assess whether inorganic P fertilisers may be appropriate to apply alongside biosolids to decrease heavy metal availability.

3.1.1 ICP-MS analysis

Heavy metals are often found at the parts per billion or parts per trillion level. Whilst these concentrations are low, they have the potential to bioaccumulate in tissues. This therefore requires accurate analytical equipment to enable their detection and monitoring. Several pieces of equipment are available to monitor their concentrations, however no single methodology is suitable for all scenarios (Soondan *et al*., 2014). Atomic absorption spectrometry (AAS), inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) are some of the more commonly used analytical instruments for environmental monitoring (ThermoFisher Scientific, no date).

Atomic absorption spectrometry can be used to detect up to 70 different elements. It measures elemental concentrations by passing a light source through the atoms of a sample, whereby the atoms absorb energy. Differences in the amount of light emitted verses the amount of light reaching the detector can then be converted in to concentrations of an element. Different forms of AAS exist, with accuracy differing depending on the type used (e.g. flame atomic absorption spectrometry detects to parts per million and graphite furnace atomic absorption spectroscopy detects to parts per billion) (Helaluddin *et al*., 2016). This method of detection has few problems with interferences between elements but may require a large sample volume. Its sample throughput is also very slow when analysing multiple elements (Helaluddin *et al*., 2016).

Inductively coupled plasma-optical emission spectrometry works by generating photons from the sample, whereby the specific wavelengths can be measured. Element concentrations can then be calculated as the number of photos are directly proportional to the concentration of the element (ThermoFisher Scientific, no date). Whilst ICP-OES can detect to the parts per billion level, it is often used for samples containing high total dissolved solids and samples with higher elemental regulatory limits. Its sample throughput is superior

to that of AAS, with approximately 60 elements being analysed in just one minute (Helaluddin *et al.*, 2016; Soondan *et al.*, 2014 and ThermoFisher Scientific, no date).

Inductively coupled plasma mass spectrometry allows the detection of elements at levels too low for atomic absorption or inductively coupled plasma-optical emission. Its ability to detect such low concentrations has resulted in it being known as the most powerful technique (Soondan *et al.*, 2014). With the potential to detect elements at the parts per trillion level, its isotopic analysis allows data to be collected to detect low levels of environmental contamination. When compared with ICP-OES, it has few elemental interferences (when using a collision cell) and can quantify the concentration of all elements in less than one minute. Its cost in comparison to AAS and ICP-OES is normally substantially higher and samples may require further dilution in order to be processed when compared to ICP-OES (Helaluddin *et al.*, 2016).

The ICP-MS can be broken up into the following nine sections (PerkinElmer Inc., 2011);

1. Sample induction system – take up the sample into the ICP-MS via the nebuliser and spray chamber. The sample forms an aerosol here.
2. Torch and RF coil – the coil encircles one end of the torch, generating a plasma when a radio frequency passes through the coil. Plasmas are generally between 6000 and 8000°C. The plasma creates and ion of the elements in the sample
3. Sample interface – the interface between the high temperature in the plasma and the rest of the operating system
4. Vacuum system – produces a vacuum in the system to allow the ions to travel.
5. Collision cell – Not present on the ICP-MS at Harper Adams University, but allows the user to remove interference of other elements in the ICP-MS which may distort the results.
6. Ion optics/ ion deflector – guides the ions into the quadrupole where neutrons and photos pass are discarded
7. Mass spectrometer – filters ions based on their mass-to-charge ratio
8. Detector – counts the ions via dynodes
9. Data handling – interprets the output from the dynodes and allows the instrument to be controlled.

Whilst the ICP-MS is able to detect substantially lower levels of elements that other instruments, it has some drawbacks.

The largest problem with element detection when using an ICP-MS is interference. This can occur in two different forms. Isobaric interferences are those which result from different isotopes sharing a similar mass (e.g. Fe and Ni both having an isotope with a mass of 58). This type of interference can be avoided through the use of a different element.

Polyatomic interferences are more difficult to avoid, without the use of a collision cell. They result from the collision of two or more elements (e.g. argon (Ar) and chlorine (Cl)). Argon and Cl combine to for $ArCl^+$ which has the same mass as arsenic 75. Therefore, without the use of a collision cell to separate the combined ions from the elemental ones, it is likely that the concentration data produced would be incorrect. In instances such as this, the Cl can be omitted from the ICP-MS, by not introducing it in the first instance, for example using nitric acid instead of hydrochloric acid. However, element such as calcium (Ca) are found in large concentrations in soil and have the potential to cause a polyatomic interference with nickel (Ni) due to the presence of oxygen (O) in the sample. In such instances where a collision cell is not available, a different isotope for Ni should be selected. A full list of polyatomic interferences was produced by May and Wiedmeyer (1998).

Null-hypotheses:
1. The application of biosolids to soil does not increase heavy metal contamination within any fraction, irrespective of soil type applied to.
2. The application of biosolids to soil has no impact on the P content of the soil in any fraction.

Chapter objectives;
1. Determine if a single application of biosolids to contrasting soil types has changed the concentrations of Ag, As, Cu, Fe, Mn, Pb and Zn and if there has been any effect on the fractions in which these elements are found (water extractable, exchangeable, Fe/Mn bound, organic matter/sulphide bound, residual).
2. Determine if long term applications of biosolids to the Broxton field site have increased the concentration of P in soils and investigate which fractions (water extractable, exchangeable, Fe/Al bound, Ca bound, residual) phosphorus is associated with.
3. Determine if long term applications of biosolids to the Broxton field site have increased the concentration of P in soils and investigate which fractions (water available, labile, Fe/Al bound, Ca bound and residual) phosphorus is associated with to suggest whether additional P applications could be beneficial for controlling heavy metal release.

3.2 Methodology

3.2.1 Preparation of soil samples

Bulk (500 g) untreated surface soil samples (0-20 cm) were collected using a stainless-steel auger from Broxton, Cheshire and Four Gates Field, Harper Adams University Farm. Further bulk samples were collected using the same methodology for the P sequential extractions from the treated plots detailed in Chapter 2. The bulked samples were formed from taking multiple sore cores from the untreated area before being subsampled for experimental purposes. All sample were returned to the laboratory, air dried (30 ºC) and subsequently ground with a pestle and mortar before being passed through as <2 mm sieve, to ensure uniformity of soil particle size. The sieve was cleaned between samples using sand and a high-pressure air gun to ensure no cross contamination between samples. Each soil was then treated with the respective treatment (Table 3.1) and left to equilibrate for one month.

All reagents were sourced from Fisher Scientific (Loughborough, UK) and were of analytical reagent grade. To each sequential extraction, an additional extraction step was incorporated to establish the quantity of metals would be water extractable. Harper Adams University Farm soils consisted of 3 treatments, 4 replicates and 5 extractants whilst Broxton soil analysis was completed in triplicate (3 treatments, 3 replicates and 5 extractants).

Table 3.1. Sequential extraction treatments applied to Broxton and Harper Adams University Farm soils.

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Untreated	0	0	0
Conventional	160 (ammonium nitrate)	55 (triple Super Phosphate (TSP))	45 (muriate of Potash (MOP))
Biosolids	160 (urea and available N from biosolids)	55 (biosolids)	45 (muriate of Potash (MOP))

3.2.2 Sequential extraction procedure for heavy metals

Sequential extractions of heavy metals was carried out using the modified Community Bureau of Reference (BCR) methodology developed by Sungur *et al.* (2014). All extractions were carried out in Nalgene polypropylene centrifuge tubes (50 ml).

Stage 1: Water extractable

Soil (1.00 g) was mixed with deionised water (40 ml), then shaken on an orbital shaker for 2 hours (170 rpm). Samples were then centrifuged at 3000 rpm for 10 minutes to separate the aqueous extract from the soil. The supernatant was then decanted into a separate centrifuge tube and stored at 4°C until further analysis.

Stage 2 – Exchangeable metals

0.11 mol L^{-1} acetic acid (40 ml) was added to the soil residual from stage 1. Samples were placed on an orbital shaker for 16 hours (170 rpm). Subsequently, samples were centrifuged for 10 minutes (3000 rpm). The supernatant was decanted into separate tubes and stored at 4°C.

Stage 3 – Fe and Mn bound

0.5 mol L^{-1} hydroxylammonium chloride (40 ml) was then added to the soil residual from stage 2. This was then shaken on an orbital shaker for 16 hours. The aqueous solution was then separated from the residue as described in stage 1 and the supernatant stored at 4°C.

Stage 4 –Organic matter and sulphide bound

8.8M hydrogen peroxide (H_2O_2) (10 ml) was added to the residue from stage 3. This was shaken for 1 hour (170 rpm). Samples were then placed in a water bath (85°C) until nearly dry and allowed to cool. A further 10ml of 8.8M H_2O_2 was added to each sample, shaken then evaporated again under the same conditions. Ammonium acetate (50 ml) was then added to the near dry soil residue and shaken for 16 hours. Each sample was then centrifuged following the procedure in stage 1 with the resulting supernatant being separated and stored at 4°C.

Stage 5 – Residual fraction

Soil (0.20 g) was placed into Teflon digestion vessels. Concentrated nitric acid, 14M (10 ml) was then added following the procedure outlined in section 2.3, stage 5.

3.2.3 Phosphorus sequential extraction procedure

A sequential extraction procedure developed by Kostyanovsky *et al.* (2015), specifically for P fractionation, was used for this study. Air dried soil samples (2.00 g) were weighed into Nalgene polypropylene centrifuge tubes (50 ml), and all extractions were carried out in the tubes to minimise soil loss

Stage 1: Water extractable

To each tube, purite water (18.2 MΩ) (30 ml) was added. They were then placed on an orbital shaker for two hours (170 rpm), at room temperature. Samples were then centrifuged at 3000 rpm for 10 minutes. The supernatant was then decanted into a separate centrifuge tube and stored at 4°C until further analysis.

Stage 2: Exchangeable P

1M KCl (30 ml) was then added to the soil residue from stage 1. This mixture was then shaken for 2 hours, at room temperature. The aqueous solution was then separated from the residue as described in stage 1. The supernatant was stored at 4°C.

Stage 3: Fe and Al Bound

0.1M NaOH (30 ml) was added to the soil residue from stage 2. This was then left to shake for 17hrs, at room temperature. The aqueous solution was separated from the residue, as previously described. The supernatant was stored at 4°C.

Stage 4: Ca bound

0.5M HCl (30 ml) was mixed with the soil residue from stage 3. This was then shaken for 24 hours, at room temperature. The resulting aqueous solution was separated as described above, the supernatant stored at 4°C.

Stage 5: Residual fraction

The residual fraction from stage 4 was then allowed to air dry. This was then crushed to a fine powder in the centrifuge tube using a glass rod. Soil (0.20 g) was then added to Teflon digestion vessels and HNO_3 (10 ml) added. Soils were then digested with concentrated (14M) nitric acid (HNO_3) in a MARS 6 microwave digester (CEM, UK) (section 2.6).

3.2.4 Microwave digestion of soils, biosolids and Certified Reference Material

Samples were digested using a CEM Mars Xpress Microwave digestion instrument. Soil was weighed (0.20 g) into Teflon digestion vessels (120 ml) to which analytical grade 14 M HNO_3 (10 ml) was added. The digestion procedure was as follows;

- Ramping to 180 °C (20 minutes)
- Holding at 180 °C (20 minutes)
- Vessel cooling (20 minutes)

Samples were filtered through Whatman 42 filter paper into acid washed volumetric flasks (25 ml). Once filtered, each sample was made to volume using purite water (18.2 MΩ) and

stored in centrifuge tubes (50 ml) at 4ºC prior to analysis. Certified Reference Material (CRM) of Bowens Kale (1974) was digested to determine recoveries.

3.2.5 Elemental analysis

Samples were quantified using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (X-series 2 ICP-MS, Thermo Scientific MA, USA).

Nitric acid digest samples (1 ml) were diluted with purite water (9 ml) before being prepared for ICP-MS analysis using the following sample preparation method;

- 4.85 ml sample solution
- 100 µl HNO_3 (14M)
- 50 µl internal standard solution (1 ppm gallium (Ga))

Samples were vortex mixed for 5 seconds to ensure they were mixed uniformly.

The following isotopes of each element were analysed, [107]Ag, [75]Ag, [65]Cu, [57]Fe, [55]Mn, [208]Pb, [66]Zn and [31]P. The ICP-MS plasma was running under vacuum at $6000^{\circ}C$. Whilst each of these elements may not be the most abundant, they were chosen to avoid polyatomic and isobaric interferences which may have skewed the results. Hydrochloric acid was not used during the microwave digestion to reduce the risk of isobaric interference. Phosphorus has the potential to result in an isobaric interference due to the mass of Ag and P forming [49]Ti, however, Kunze et al. (1998) found this to be undetectable.

Lake Ontario Water (CRM TMDA 64.2) was used as a water reference material (Table.3.2). This was to ensure that the values being produced by the ICP-MS were within the accepted range of the reference sample. TMDA is universally available reference material for liquid samples with heavy metal contamination.

Table 3.2.TMDA recoveries using ICP-MS.

Element	Recovery (ppm)	TMDA 64.2 reference value (ppm)	Tolerance (+/-)	Recovery %
Mn	0.2746	0.295	0.0223	93.085
Fe	0.282	0.306	0.0271	92.157
Cu	0.2712	0.274	0.0241	98.978
Zn	0.2856	0.310	0.0265	92.129

Sequential extraction samples were prepared using the supernatant, without any additional dilution. For NaOH extractions, an additional dilution step was necessary due to precipitation of humic acids.

3.2.6 Statistical analysis

All sequential extractions were analysed using Genstat (VSN International, 18th edition). Pseudo-totals were analysed using a one-way analysis of variance (ANOVA), with interactions between treatments and extractants being analysed using a split-plot ANOVA, using the treatment as the whole plot and extractants as the sub-plot. A post-hock Bonferroni test, with a significance level of 0.05, was used to determine statistical differences for interactions. Significance was deemed to occur if $p < 0.05$, however results with a $p > 0.05 < 0.1$ were deemed to be of interest.

3.3 Results

3.3.1 Heavy Metal Sequential Extractions

3.3.1.1 Harper Adams University Farm

Sequential extractions were carried out on both the Harper Adams University Farm soils (sandy loam) and the Broxton, Cheshire soils (clay loam).

No difference in pseudo-total heavy metal concentrations was recorded for any treatment in the Harper Adams University Farm soils ($p > 0.05$), thus indicating that the addition of any fertiliser did not affect the overall concentration.

No elements showed a treatment-extractant interaction indicating that the biosolids did not increase the concentration of As, Ag, Mn, Fe, Pb or Zn following one application. Coefficient of variations varied substantially with some cv% being as high as 218%, which could be due to natural variation in the soil or interferences on the ICP-MS (Figure 3.1). Such interferences as P with Cu detection and calcium (Ca) with Fe may have increased the concentrations of some elements detected. Only ^{31}P can be analysed on an ICP-MS.
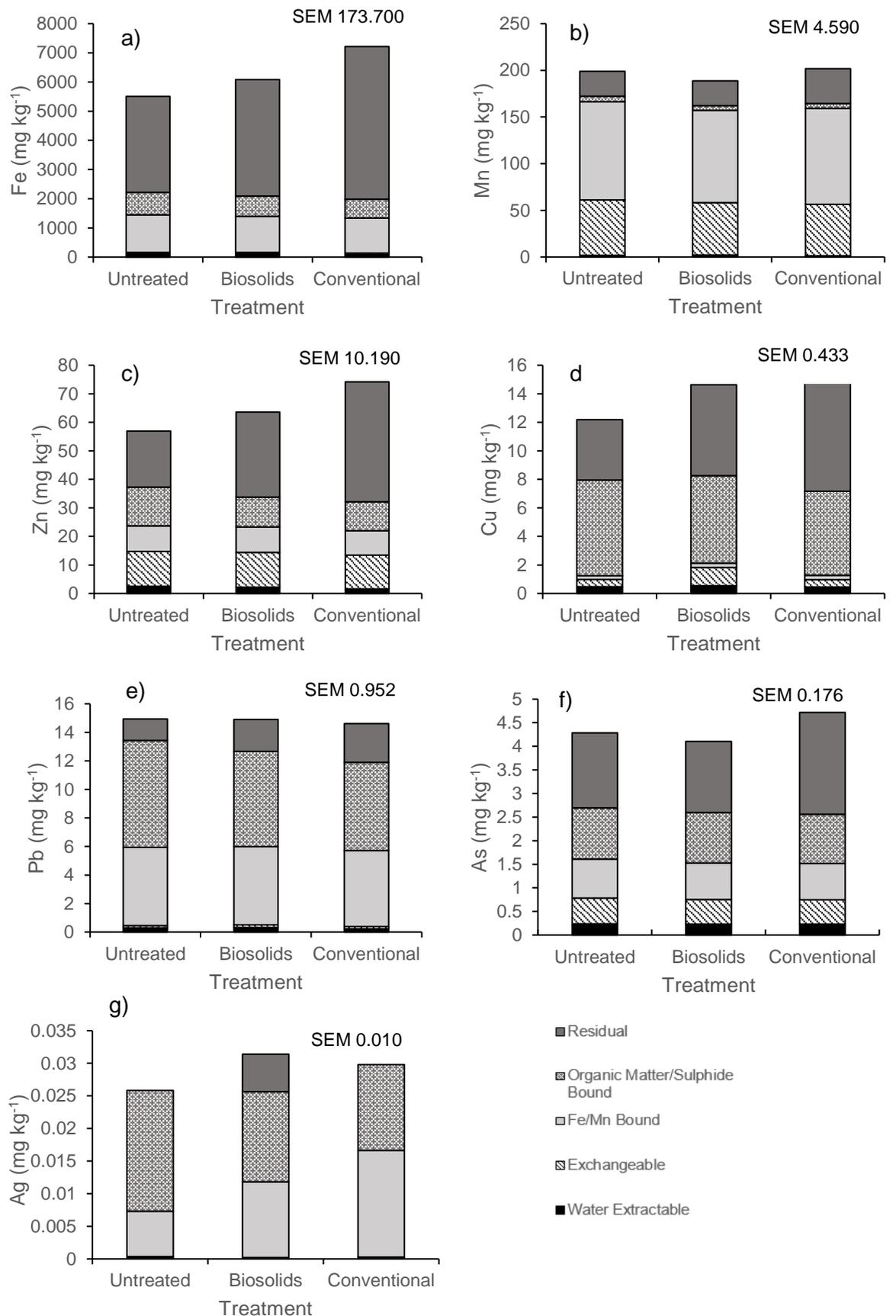
Figure 3.1 Partitioning of pseudo-total elemental concentrations, a) Fe, b) Mn, c) Zn, d) Cu, e) Pb, f) As, g) Ag extracted using Sungur *et al.* (2014) on Harper Adams University Farm soil, following one application of biosolids. Note: graphs are presented with different mg kg scales due to the large variation in concentrations recovered. Residual d.f =24

3.3.1.2 Broxton, Cheshire sequential extractions

Significant differences were recorded between treatments in the Broxton, Cheshire soils, although none of these increases were recorded in biosolids treated soils when compared to the untreated soils (Figure 3.2). Pseudo-total concentration differences were recorded for Mn (p=0.005) with biosolids soils being significantly lower on average than both conventionally treated and untreated soils. Iron, Pb and As concentrations were also found to be close to significant (p=0.062, 0.069 and 0.075 respectively). However in each instance the biosolids treated soil had the lowest concentration.

Interactions were recorded between extractant and treatment in the Broxton soils. In the residual fraction, untreated soils were higher in As concentration (4.72 mg kg^{-1}) compared with the biosolids treated soil (2.81 mg kg^{-1}). Conventionally treated soil concentrations were not statistically different from either biosolids treated or untreated soils (3.76 mg kg^{-1}). Biosolids treated soils recorded lower Fe concentrations (p<0.001) compared with the untreated and conventionally treated soils (2811 mg kg^{-1}, 4637 mg kg^{-1} and 3835 mg kg^{-1} respectively). Lead concentrations were also lower (p<0.001) in biosolids treated soils with 17.82 mg kg^{-1} compared with 27.99 mg kg^{-1} for untreated soils and 23.54 mg kg^{-1} in conventionally treated soils.

The only fraction showing significant differences between treatments outside of the residual fraction, was the Fe/Mn bound fraction with Mn. Untreated soils had the highest concentration of Mn (161.37 mg kg^{-1}) when compared with conventionally treated soil (130.12 mg kg^{-1}). The lowest concentration was again found in the biosolids treated soil (75.85 mg kg^{-1}). No other elements showed a significant difference between treatments at any fraction.

Further analysis comparing the two sites heavy metal content showed significant differences for all elements, except for Zn (Table 3.3). However, different inputs make such comparisons difficult to attribute directly to the treatments used.

Table 3.3. Heavy metal statistical differences between two soil types.

Element	p value	Site with higher concentration	cv%	Residual d.f.
Ag	<0.001	Broxton	51.7	24
As	0.018	Harper	54.9	24
Cu	0.002	Broxton	24.7	24
Fe	<0.001	Harper	61.5	24
Mn	<0.001	Broxton	11.3	24
Pb	<0.001	Broxton	27.5	24
Zn	0.782	N/A	73.9	24

Overall, the lower concentrations of heavy metals in Broxton, Cheshire soils would suggest that a single application of biosolids does not increase the concentration of any of the heavy metals considered above those seen in conventionally treated or untreated soils.
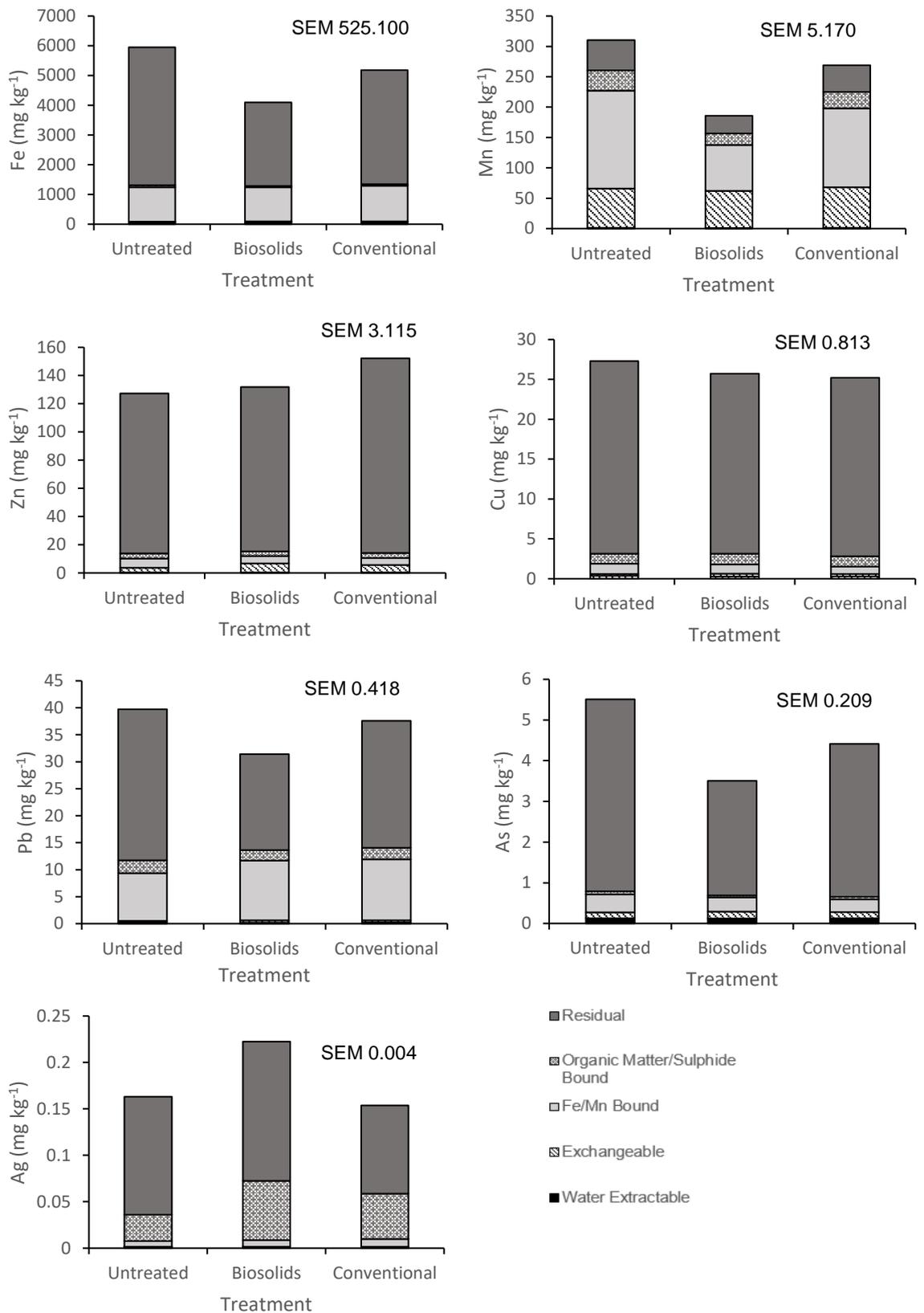
Figure 3.2. Pseudo-total elemental concentrations a) Fe, b) Mn, c) Zn, d) Cu, e) Pb, f) As, g) Ag extracted using Sungur *et al.* (2014) on Broxton, Cheshire soils, following one application of biosolids. Note: graphs are presented with different mg kg^{-1} scales due to the range of concentrations recovered. Residual d.f. 36.

3.3.2 Phosphorus sequential extractions (Broxton, Cheshire soil).

Following the results from Grobelek and Napra (2015) suggesting that the application of P in addition to biosolids may decrease their mobility, a phosphorus sequential extraction was carried out on the Broxton Cheshire soils to assess what impact repeated applications had on the P concentration of soil from the treated plots (Figure 3.3.). The analytical protocol for this experiment is presented in sections 3.2.3, 3.2.4 and 3.2.5. Biosolids treated soils were higher in P in the Fe/Al bound fraction (836.3 mg kg^{-1}) compared to both the untreated and conventionally treated soils (355.6 mg kg^{-1} and 391.1 mg kg^{-1} respectively). This therefore suggests that the biosolid applications are increasing the concentration of P within one fraction of the soil. No other fractions showed a significant difference between treatments. No P was removed in the exchangeable fraction indicating that all exchangeable P had already been removed in the water exchangeable fraction. This fraction represented less than 1.5% of the total P.
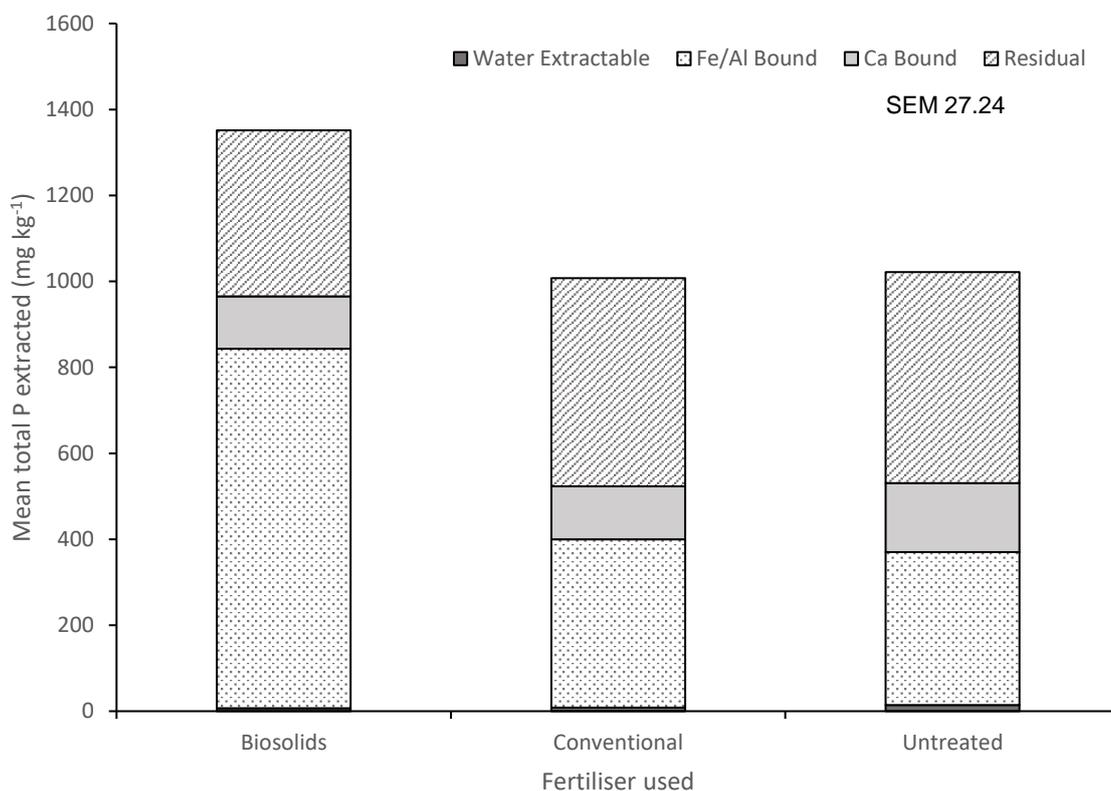
Figure 3.3 Partitioning of P extracted using Kostyanovsky *et al.* (2015) from Broxton, Cheshire soils following eight years of applications of fertilisers. Residual d.f. 30

3.4 Discussion

When considering the environmental risk posed by an organic amendment such as biosolids, many are assessed on their total elemental concentration or available fractions rather than considering the chemical partitioning of each element. Sequential extractions show both total concentration and the chemical partitioning of elements within soil. They can be used to indicate which elements pose the greatest environmental risk and therefore influence application guidelines. All fractions are considered to be bioavailable to plants with the exception of the residual fraction (Zang *et al.*, 2015). However, whilst each of these fractions may have the potential to become bioavailable, the increased bonding strength seen with each step would suggest that the OM and sulphide bound fraction is less likely to become plant available before the Fe/Mn bound fraction, yet this would be dependent on soil conditions.

Two contrasting soil types were assessed for their heavy metal contents following a single application of biosolids and conventional fertiliser to untreated soil. A direct comparison of the two soil types (Table 3.2) showed significant difference between all elements, with the exception of Zn, yet no soil type was significantly higher in all elements. Due to the site management practices being historically different, with differing fertiliser regimes and cropping histories, it is difficult to compare the two sites directly. Literature discussing the direct comparison of heavy metal partitioning following the application of biosolids on two differing soil type is minimal with only Sanchez-Martin *et al.* (2007) showing comprehensive data. However, whilst the Sanchez-Martin *et al.* (2007) study uses two different soil types (loamy sand and a sandy loam), there was only a few percent difference in the sand, silt and clay composition. A more controlled study looking at a single soil type, amended with compost may be required to enable a direct comparison, thus reducing the impact of variation in characteristics such as pH.

Individually the two sites analysed in this chapter showed contrasting results. Harper Adams University Farm showed no increase in pseudo-total heavy metal concentrations, or an interaction between treatment and extractant. This would suggest that a single application of biosolids did not increase the concentration of any heavy metal in any fraction of the soil. Whilst this suggests that the biosolids do not pose an increased risk following a single application, this would also suggest that the biosolids do not increase the more bioavailable fractions for beneficial heavy metals (Zn, Cu, Fe and Mn) and thus may not be suitable for rectifying micronutrient deficiencies.

In contrast, the Broxton soils showed differences between treatments for pseudo totals of Mn, with As, Fe, and Pb having p values less than 0.1 and thus possibly being of interest.

Interactions were recorded for As, Fe, and Pb in the residual fraction, with the untreated and conventionally treated soils being higher than the biosolids treated soil in

each instance. This fraction is considered the most stable fraction and therefore the least likely to be mobilised and either leach or enter the food chain. For all elements except Mn, the largest fraction was the residual fraction overall accounting for 69% (Ag), 84% (As), 88% (Cu), 74% (Fe), 63% (Pb), and 89% (Zn). With only 0.4% (Ag), 3% (As), 1% (Cu), 1% (Fe), 1% (Pb), and 2% (Zn) found in the water exchangeable and exchangeable fractions. Manganese was the only element to show a significant difference in a fraction that was not the residual fraction. Biosolid treated soils showed the lowest concentration of Mn in this fraction, with conventionally treated soil being higher and the untreated soil being the highest.

The residual fraction for Mn only accounted for 16% of the total, with the water exchangeable and exchangeable fractions accounting for 14%. This difference in fraction proportions may be explained by the largest fraction being the Fe/Mn bound fraction yet does not explain Fe being most concentrated in the residual fraction. Iron was added in the form of ferric sulphate as part of the waste water treatment process and therefore should increase Fe concentrations in biosolid amended soils. The methodology used in this chapter does not use HF acid as required for the total extraction of elements from soil due to health and safety restrictions. Therefore, the lack of Fe concentration increase observed, may be due to Fe being bound in the residual fraction that was not digested into solution. Whilst the recovery values for the TMDA were within the tolerance limits presented in the certificate of the certified reference material, Mn, Fe and Zn were all below 95% recovery. Whilst there is no official guidance for acceptable percentage recovery, it is believed that recovery should be above 95%. The addition of a collision cell to the ICP-MS may improve the recovery values for further research, improving confidence in the data collected. It is therefore likely that the values presented are below the true concentrations in the samples. The data collected from the two soil types contrast with much of the data currently available. Shober (2007) found a significant increase in Cu and Zn concentrations in all fractions following biosolids applications. With Parkpain *et al.* (2000) also finding that Cu, Zn and Mn all increasing in the bioavailable fractions following biosolid applications. However, much of this work has been done on biosolids in a cake or liquid form and so differences may be attributed to the type of product being used.

The P sequential extractions indicated that total P extracted was significantly higher overall in the biosolids treated soil than the untreated or conventionally treated soils. This therefore indicates that the P additions made via the biosolid applications are accumulating in the soil. From this experiment it is not possible to explain whether this is due to too much P being applied over time, or a lack of P uptake into the plants. It may be that the addition of P in the conventionally treated soil was not large enough for it to oversupply the crops demands, or that the applications had not been made over a long enough period for the conventionally treated soil to record significantly greater concentrations compared to the

untreated. The Fe/Al bound fraction was the largest fraction for the biosolids treated soil and significantly larger than the untreated and conventionally treated soils. This can be explained by the addition of ferric sulphate for the extraction of phosphorus from the wastewater at the treatment works (Per. Comms. Mamatha Veesam, Senior Process Scientist, Southern Water). Ferric sulphate is used as a coagulant to remove P. It causes the P to precipitate as salts which then coagulate, reducing the P in the waste water. Whilst ferric sulphate is used in this instance, other common salts used for removing P from waste water include ferrous chloride and aluminium sulphate. Two of the influencing factors with regards to which compound is used to aid the extraction of P from wastewater are the size of the WWTW and the cost of the compound used (Gray 2011). No P was found in the exchangeable fraction, indicating that all P that would have normally been found here was removed by the water extractable stage, indicating that acid rain may not be required for P to enter groundwater. This fraction represented less than 1.5% of the total P in the soil, whilst the Fe/Al bound fraction represented 61%. No significant differences between treatments regarding the concertation of P was found in this fraction, suggesting that biosolids were not adding to this fraction. Whilst the movement of P from the Fe/Al fraction to the more bioavailable fractions would be dependent on a number of factors (including pH, water content and time), the idea of adding excess P to the soil alongside biosolid applications may not be considered good practice. Eutrophication is thought to be predominantly caused by the erosion of soil into water systems and therefore the Fe/Al fraction, would not have to become mobile and plant available in order for it to impact the environment. The exact mechanism by which this happens is still not clear and thought to be more complicated than previously thought (Ekholm and Lehtoranta, 2012), however, it would be irresponsible to make such applications, using current understanding.

It should be noted that the methodology of sequential extractions is beyond that which would be seen in the natural environment. Sahuquillo *et al.* (1999) identified that the pH of the extractants used in a sequential extraction procedure is a key factor influencing the concentration obtained. This was confirmed by Davidson *et al.* (1999). The BCR methodology requires one extractant to be altered as low as pH 2, indicating how difficult it would be for a plant to extract these metals. This therefore suggests that whilst such methods are useful for understanding the fractionation of elements, they may not be representative of the potential for being translocated into a plant. A review by Zimmerman and Weindorf (2010) suggests that sequential extractions should be used with other analytical techniques to analyse a soils metal fractionation accurately as reabsorption is likely to underestimate the more mobile fractions, whilst overestimating the oxidisable and residual fractions.

Following the sequential extraction analysis across a range of heavy metals, in contrasting soil types, both null hypotheses must be rejected as both heavy metal and P

concentrations were found to vary in different fractions of soil. These differences were predominantly recorded in the residual fraction, with the untreated soil containing higher concentrations than the biosolids treated soils and therefore the risk posed to the environment is deemed low from these results. It is evident from the biosolid analysis that they contain heavy metals. Whilst the application rates used here were suitable for a cereal crop, they were low when compared to soil volume. The dilution effect in the soil may explain why increases were not observed from these biosolid additions.

3.5 Chapter Conclusions

Sequential extractions indicate that the risk of environmental contamination is low following one application of biosolids to soil. Many heavy metal concentrations were significantly lower in the residual fraction of the biosolid treated soil from Broxton indicating that there must be either increased plant uptake or leaching of these elements. No significant differences were recorded between biosolids treated and untreated soils from Harper Adams University Farm in available fractions, suggesting that single applications do not increase plant available Ag, As, Cu, Fe, Mn, Pb and Zn levels. Applications of TSP made alongside the application of biosolids has been suggested as a possible way to reduce heavy metal mobility in soils. Following seven years of applications to the soil, biosolids were found to have increased the P concentrations overall, specifically in the Fe/Mn bound fraction. This would suggest that applications of TPS alongside biosolids, would not be a responsible way of limiting heavy metal contamination. Heavy metal extraction data would suggest that such instances recorded by Grobelek and Napra (2015) are not a problem that would warrant these applications anyway. Zimmerman and Weindorf (2010) suggest that BCR may underestimate the concentration of heavy metals in the bioavailable fractions. This, along with the suggestion that the carbon in the biosolids may increase the mobility and thus leaching of heavy metals from the soil should be studied further.

Chapter 4.

The leaching potential of biosolids.

4.1. Introduction

The application of biosolids to land, not only increases soil organic matter (SOM) but adds nutrients required for plant growth yet, they also potentially add heavy metal contaminants. Whilst some of these elements are beneficial to human and animal diets, at certain concentrations they pose health and environmental risks if their concentrations become too high. Understanding the implications associated with biosolid applications would enable them to be applied more efficiently. Historically, biosolids have contained very high concentrations of heavy metals, however, they have been increasingly stabilised to improve their potential as a fertiliser (Islam *et al.*, 2012). The most mobile elemental fractions have the potential to be translocated into plant tissues (Chapter 6) or leached from soils. Leaching can be defined as the movement of nutrients held in the labile fraction, downwards through the soil profile (Lehmann and Schroth, 2003). This process removes nutrients from surface soil horizons (O, A and B) either resulting in plants needing to extend their root systems to reach the nutrients, or loss of nutrients to ground water. Generally, for nutrients to be leached from soil, field capacity of the soil must be exceeded, however, leaching may also occur in dry clay soils following heavy rain (Alloway, 2013). Whilst all soils are susceptible to leaching, some are at greater risk, for example sandy soil with a low SOM content.

Both nitrogen (N) and phosphorus (P) are the most documented elements being leached, with high nitrate levels in soil posing a risk to human health and phosphorus leaching being associated with eutrophication of freshwater systems. Whilst restrictions are in place to prevent excessive N and P applications (e.g. Nitrogen Vulnerable Zones and Biosolids Nutrient Matrix), little consideration has been given to the potential movement of heavy metals from biosolids in soils and any potential associated risks.

Currently in the UK, several documents are available with recommendations on how to apply biosolids, thereby restricting their impact on both the environment and the food chain, whilst still allowing soils to benefit from their nutrient content and OM. Nevertheless, most of these guidelines are formed from data only accounting for total heavy metal content, and not related to the labile fraction in soils (Speir *et al.*, 2003). Some metals are non-essential, posing an environmental and/or health risk, whilst others are essential to life but toxic in high concentrations. Such risk emphasises the need to understand the bioavailable fraction of soil heavy metals.

Several factors affect leaching and consequently the potential of biosolids to harm the environment and contaminate food chains.

4.1.1.Soil pH and leaching potential

Soil pH is one of the most important factors affecting the availability of heavy metals (Harter, 1983). Key factors influencing pH in soils include the decomposition of organic matter and rainfall. The production of carbon dioxide (CO_2) from the decomposition of the organic matter, in combination with rainfall, results in the release of H^+ ions acidifying soils. Organic matter decomposition through microbial activity can also decrease soil pH through the production of ammonia and hydrogen sulphide, which following oxidation, produces nitric and sulphuric acids.

Generally, research suggests that metals become increasingly available with decreasing pH. A pH decreases in soil can be caused by a number of things, including the use of ammonium fertilisers and acid rain. Blake and Goulding (2002) demonstrated that a decrease in soil pH below 4.0 mobilised Cd, whilst pH 5.0 - 6.0 mobilised manganese (Mn), cadmium (Cd), zinc (Zn), nickel (Ni) and copper (Cu). Such changes in pH affect the availability of heavy metals and their potential to reach phytotoxic concentrations, therefore impacting on the suitability of the land for growing crops. Studies have revealed conflicting results regarding the effect biosolids have on soil pH. Speir *et al.* (2003) indicated that annual additions of 100 Mg ha^{-1} of biosolids significantly reduced pH. Consequently, it was found that Cu, Ni and Zn concentrations were all higher in ground waters, as well as increased Zn concentrations in plant tissues. Subsequent liming of the treated soil reduced Zn concentrations from 27 to 0.04 mg kg^{-1}, indicating that leaching may be managed, should contamination occur. Stehouwer *et al.* (2006) coa three-year leaching study following the application of biosolids at 134 Mg ha^{-1} to a reclaimed mine site in Pennsylvania. Lysimeters were inserted to a depth of 1 m to collect water samples from biosolids-treated and untreated soils. It was observed that biosolids had caused a reduction in pH, which was most prominent in the first year following application, whilst Mn, Cu, Ni, lead (Pb) and Zn became more mobile. It was proposed that this may be a result of the decrease in pH mobilising the heavy metals. It should be noted that the applications detailed by Stenhouwer *et al.* (2006) were not clear as to the nature of the biosolid (i.e. solid or liquid) and an assumption was made that they used cake material based on the units used for their application rate. For both Speir *et al.* (2003) and Stehouwer *et al.* (2006), the applications rates are very high compared with those in the UK, where cake would normally be applied closer to 20t/ha. This therefore makes the interpretation of their results to UK farming situations less clear.

Islam *et al.* (2013) undertkook a large field scale experiment studying the effects of biosolids on heavy metal accumulation. They showed no difference in soil pH following 25 years of biosolid applications, and this may be due to the biosolids having been treated with lime to stabilise them prior to application.

4.1.2 Soil Carbon and leaching potential

Another critical factor affecting heavy metal mobility is soil carbon. Soil carbon is critical for food production, climate regulation and biodiversity. Carbon is readily lost from the soil through a range of processes (Figure 4.1) and therefore it is important to ensure adequate additions are made to compensate for such loses. Sandermand and Baldock (2010) provide evidence that global soil carbon levels are decreasing; with one of the main causes being agriculture. To maintain food production at their current levels, soil carbon must be maintained, however dissolved organic carbon can also affect heavy metal mobility in soils by the organic acids acting as a chelating agent (Alloway, 2013; Sherene, 2009). Whilst increasing heavy metal mobility will remove them from the rhizosphere, reducing the risk of bioaccumulation in plant tissue, it also increases the risk of contamination to ground water (Alloway 2013).

Fang *et al.* (2016) found that when sewage sludge compost was added to the soil (48 Mg ha^{-1}), the dissolved organic matter content increased leaching of heavy metals from soils. Subsequently, Ni and Zn concentrations leached from sewage sludge compost treated soils with a pH greater than seven, were "several orders of magnitude higher" than the untreated soil. This was directly attributed to the higher dissolved organic matter content being higher in the waters from the sewage sludge compost treated soils.

Biosolids have been shown to increase the Total Organic Carbon (TOC) of soils. Wijesekara *et al.* (2017) found that following biosolids applications (70 t/ha), there was a 45% increase in TOC. Whilst this was linked to an increase in the crop biomass produced, no soil heavy metal analysis was carried out, hence the true hazard potential of the applications was not assessed.

Ferraz and Lourenco (2004) proposed the use of a Langmuir-type model to predict the maximum amount of a contaminant in a leaching solution. The model demonstrated that by increasing organic matter (OM), an increase in heavy metal concentrations occurred. However, the methodology required to produce the data for the model uses pH solutions between 2.1 and 3. Whilst again, this shows the ultimate potential of an element to be leached, it does not indicate directly any impacts of increased OM.

Atmospheric Carbon 720Pg

**Fossil fuels
5.5Pg C year^{-1}**

**Soil microbe
respiration
60Pg C year^{-1}**

**Plant
destruction**

**Plant
respiration
60Pg C year^{-1}**

Soil Carbon 3300Pg
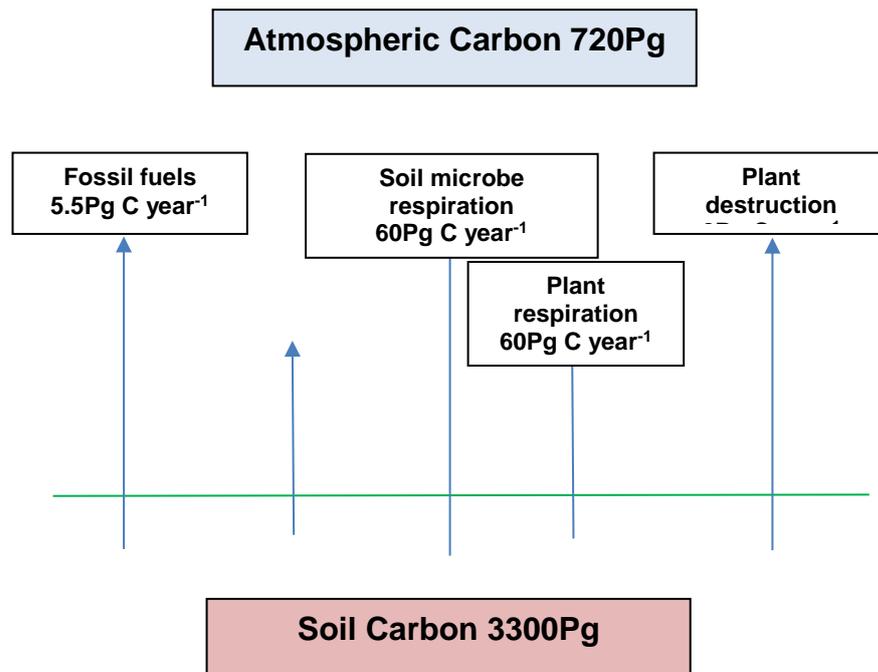
Figure 4.1. Atmospheric losses of carbon from soil

4.1.3 Techniques for assessing leaching

Historically, extraction methods such as ethylenediaminetetraacetic acid (EDTA) have provided a number that has related heavy metal extraction to bioavailability. Manouchehri *et al.* (2006) describe EDTA as a powerful chelating agent as it competes at surface sites of the solid soil phase, extracting metal cations from the soil into solution (Manouchehri and Bermond, 2006). Often work using EDTA involves using a range of concentrations indicating a lack of uniform testing using this technique (Manouchehri *et al.* 2006; Khalkhalian *et al.* 2006; Lo and Yang, 1999). Manouchehri and Bermond (2006) reviewed work using EDTA to extract metals from soil and found that the percentage removal varied dramatically between papers (e.g. 10 - 99% removal of Pb was achieved) with a range of leaching steps used across the research also (1 - 7 steps were recorded). Other techniques such as diffusive gradient thin films (DGT) have been used to monitor the diffusion of metals in soil solution with a chelating resin. The DGT probe is inserted to remove metal ions, and is designed to work similarly to a plant root, by decreasing the metal concentration locally. This allows the movement of ions, via diffusion, in the soil solution to the probe and the release of more labile metals from a different pool. The replenishment of this pool is then thought to represent what is accessible to a plant root. Dočekalová *et al.* (2012) suggest that such a method is simple and reliable for the assessment of the available metal pool, suggesting that it is a quick method that provides more accurate results than EDTA extraction techniques, yet such techniques were not detailed in the research. Parker *et al.* (2017) suggest that whilst DGT may be suitable to use as a monitoring tool for heavy

metals, especially in place of total extraction procedures which were felt to not correspond with the concentrations found in pore waters.

Hartley *et al.* (2004) utilised leaching columns to conduct a modified Dutch Leaching column test on three contaminated soils. Using acidified water (pH4) long-term leaching experiments were carried out to evaluate the leaching potential of As over time. This technique allowed a longer-term assessment for the leaching to be undertaken and thus the impact of adding any input to soil to be assessed quickly, whilst using the movement of water as the key driver of the reaction.

Although heavy metal release from biosolids has been studied, much of the work has been done using strong acids or techniques which are not applicable to what plants can readily absorb. Furthermore, the work has often been completed over a short period of time. Here, a series of experiments build on the knowledge obtained in Chapter 3, whilst attempting to explain the longer-term implications of biosolid applications to soils.

Chapter aims: Evaluate the leaching potential of heavy metals and TOC from biosolids using novel techniques.

Chapter objectives:
Quantify the leaching of heavy metals and TOC from biosolids over repeated wetting and drying periods.
Quantify the leaching potential of heavy metals and TOC from biosolid treated soils in comparison to untreated soils.

Null hypothesis: There is no difference between heavy metal or carbon contents of the leachate collected from biosolids-treated soils, when compared to untreated soil.

4.2 Methodology

4.2.1 Wet and Dry experiment

A series of wet and dry periods were enacted on biosolids suspended in purite water to assess the leaching behaviour of heavy metals over time. This study was established using water at pH 4 and 7 to assess the impact on heavy metal release.

Experimental design

Nalgene polypropylene tubes (50 ml) were filled with biosolids (5 g) taken from a 25 kg subsample. Each tube was then filled with 50 ml purite water (18.2 MΩ) and placed on a rotary shaker (170 rpm) for 24 hours. Water was tested to ensure the pH was between 6.8 and 7.2 before being placed in the tubes. Supernatants were filtered through Whatman 42 filter papers before being stored at 4 ºC. Analysis was carried out on pH (Jenway 3510), heavy metals using ICP-MS (X-series 2 ICP-MS, Thermo Scientific MA, USA) and TOC (TOC-VE/SSM-5000A, Shimadzu, Tokyo, Japan). The biosolids were then left to dry at room temperature for 48 hours before the process was repeated. In total, 10 cycles were completed. Five replicates were used for each cycle.

The experiment outlined above was repeated using purite water (18.2 MΩ) adjusted to pH 4 (HNO_3 14 M). This enabled a comparison of biosolid heavy metal leaching at contrasting pH levels.

4.2.2 Leaching column experiments

A laboratory experiment was established to assess the leaching potential of metals from biosolids at two applications rates 0 kg ha^{-1} N and 500 kg ha^{-1} total N (Table 4.1) The 500 kg ha^{-1} total N application rate was chosen due to the Biosolids Nutrient Management Matrix guidelines (ADAS, 2014), with this rate being double the recommended rate. This therefore represents where poor application accuracy occurred. Soil was collected from untreated areas of the field before being amended (they had received no fertiliser in seven years).

Table 4.1. Application details for the untreated Modified Dutch Leaching experiments; a) Broxton field soil, b) Harper Adams University Farm soil

a)

Treatment application rate	Details
0 kg ha^{-1} N	Untreated control
500 kg ha^{-1} N	500 kg ha^{-1} total N applied as biosolids (double the permitted application rate under The Biosolids Nutrient Management Matrix)

b)

Treatment application rate	Details
0 kg ha^{-1} N	Untreated control
500 kg ha^{-1} N	500 kg ha^{-1} total N applied as biosolids (double the permitted application rate under The Biosolids Nutrient Management Matrix)

The experiment was repeated using two soil types; untreated soil from Broxton, Cheshire and Harper Adams University Farm soil. Bulk, untreated soil samples were air dried at 30 ºC and then sieved (<2 mm) to remove stones. Subsamples of soil (500 g) were then mixed with the respective concentrations of biosolids. Biosolid application rates were calculated assuming applications were made to the surface before being incorporated to a depth of 20 cm (shallow plough depth). Samples were left to homogenise for 24 hours before being placed in the leaching columns. Leaching columns (XK50 Pharamacia Biotech; 20 cm length, 5 cm internal diameter) were fitted with Whatman pre-filters (pore size 1.5 µm) and sealed using Gold Label Pond Aquarium Sealer. This sealant contains no contaminants and therefore would not result in any contaminant leaching into the samples. Following application, this was left to set for 48 hours.

A reservoir of deionised water, adjusted to pH 4 using HNO_3 (14 M) was pumped using a peristaltic pump at a flow rate of 1 ml min^{-1} (Hartley *et al.* 2004). Water entered each leaching column through the base, ensuring water filled the entire column before exiting via tubing at the top (Figure 4.2). Leachate was collected in 5 litre water containers at predetermined volumes (Table 4.3). Each treatment was carried out in triplicate.

A further leaching experiment was carried out using the same methodology but using treated soils from Broxton, Cheshire, which had received eight years of biosolid and conventional fertiliser applications (Table 4.2).

Table 4.2. Treatments for the Broxton field experiment Modified Dutch leaching columns.

Treatment	Details
Untreated	Untreated control
Biosolids	Soil that had received biosolid applications for 8 years
Conventional	Soil that had received conventional fertiliser applications for 8 years.

Table 4.3. Volumes of water used in the Modified Dutch Leaching investigation and the equivalent time based on the average annual rainfall of 660 ml for Harper Adams University 1981-2010 (Met Office, no date).

Fraction (K)	Volume (ml)	Rainfall equivalent (years)
1	300	0.45
2	600	0.91
3	1500	2.27
4	3000	4.55
5	6000	9.09
6	15000	22.73
7	30000	45.45

Sub-samples (50 ml) were taken from each container at the designated volume and stored at 4ºC.

4.2.3 Elemental analysis

Samples were quantified using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (X-series 2 ICP-MS, Thermo Scientific MA, USA).

Wet and dry and all leaching samples were analysed without dilution, using the following volumes;

- 4.85 ml sample solution
- 100 µl HNO_3 (14M)
- 50 µl internal standard solution (1 ppm gallium (Ga)).

Samples were vortex mixed for 5 seconds to ensure they were mixed uniformly. Limits of detection (LOD) and limits of quantification (LOQ) are shown in Table 4.5

Lake Ontario Water (CRM TMDA 64.2) was used as a water reference material (Table.4.4). This was to ensure that the values being produced by the ICP-MS were within the accepted range of the reference sample. TMDA is a commercially available reference material suitable for water sample validation on an ICP-MS.

Table 4.4.TMDA recoveries using ICP-MS.

Element	Recovery	TMDA 64.2 reference value	Tolerance	Recovery %
Mn	0.279	0.295	0.0223	94.576
Fe	0.282	0.306	0.0271	92.157
Cu	0.271	0.274	0.0241	98.905
Zn	0.289	0.310	0.0265	93.226

Table 4.5. LOD and LOQ of ICP-MS (Garbarino and Taylor, 1996)

Element	LOD (mg L^{-1})	LOQ (mg L^{-1})
^{55}Mn	0.00006	0.00023
^{63}Cu	0.00002	0.00010
^{66}Zn	0.00008	0.00039
^{75}As	0.00060	0.00240

4.2.4 Statistical analysis

All data sets were analysed using Genstat (VSN International, 18th edition). Statistical deemed of interest.

Each of the leaching and wet and dry experiments were analysed using a one-way analysis of variance (ANOVA) to determine differences between total concentrations leached. A

repeated measures ANOVA was then used to analyse the leached concentrations over time and as an interaction between treatment and time, with significance between factors being determined by the least significant difference (LSD).

Correlations between heavy metals and TOC were assessed using Pearson's correlation on both the wet and dry and Modified Dutch Leaching Column experiments. Where means were not found to differ between pH in the wet and dry experiment, datasets were combined.

Figure 4.2 Modified Dutch leaching investigation.

4.3 Results

4.3.1 Wet and dry experiment (Figure 4.3)

Iron

Concentrations extracted using pH 4 water were greater (p=0.025) than pH7 water (12.72 mg L^{-1} and 6.12 mg L^{-1} respectively). This indicates that under acidic conditions, Fe may be more mobile. This may be due to a change in the cation exchange capacity of the biosolids, with Fe being displaced by H$^+$ ions. No difference was recorded between time points

although the p value was close to significance (p=0.058). The greatest concentration was recorded in the first sample. No interaction between pH and fraction was recorded.

Copper

Mean concentrations extracted showed no differences between pH4 and pH 7 (p=0.966) with 3.60 mg L^{-1} and 3.58 mg L^{-1} respectively extracted. A difference was recorded in concentrations extracted over time with the following statistical difference recorded between the fractions; 24>48>72>96>120=144=168=192=216=240 (p<0.001). No interaction was recorded between pH and time (p=0.282).

Manganese

Manganese concentrations did not differ between the two pH's (p=0.064) with 1.194 mg L^{-1} extracted using pH4 water and 0.801 mg L^{-1} extracted using pH 7 water. Manganese concentrations extracted over time did differ (p<0.001), with the highest concentration being extracted at 24 hours, with the following statistical differences between the time points 24>48=72=96<120>144=168=192=216=240. An interaction was recorded with the concentration of Mn being extracted from pH 4 water being more concentrated after 24 hours, when compared with pH 7.

Zinc

No differences were recorded between the total concentrations of Zn extracted between the two pH treatments (p=0.156) with pH 4 extracting 1.049 mg L^{-1} and pH7 extracting 0.906 mg L^{-1} respectively. A difference was recorded regarding the extraction of Zn over time (p<0.001), with the largest concentration being extracted in the first 24 hours (0.183 mg L^{-1}). A second peak in the concentration of Zn was found at 120 hours, being significantly higher than the times preceding and following (except at 24 hours). No interaction was recorded (p=0.108).

Arsenic

Arsenic concentrations did differ between the two pH solutions (p=0.012) with pH 4 extracting 0.078 mg L^{-1} and pH 7 extracting 0.060 mg L^{-1}. A difference in the amount of As extracted over time was also recorded with all fractions significantly decreasing in As concentration compared to the previous fraction, except 192, 216 and 240 hours which were statistically similar to each other. An interaction was recorded (p=0.003) with pH 4 recording higher concentrations of As at 24, 48 and 120 hours.

Lead

A statistical difference was recorded between the concentration of lead extracted by the two pH solutions (p=0.016), with pH 4 extracting 0.069 mg kg^{-1} and pH 7 extracting 0.046 mg L^{-1} on average. A significant change in concertation was recorded over time (p=0.026). Pb concentrations peaked between 96 and 120 hours. An interaction was recorded with pH 4 having a higher concentration of Pb at 120 hours when compared with pH 7.

Silver

No differences were recorded between treatments (p=0.329) with both pH4 and pH7 extracting very small concentrations (0.013 mg L^{-1} and 0.017 mg L^{-1} respectively). A difference was recorded between the concentrations extracted over time with the 24, 48 and 72 hour time points extracting more Ag than all the other time points (p=0.002). No interaction was recorded between pH and time.

Total Organic Carbon (TOC)

Mean concentrations of TOC leached did not differ between pH4 and 7 treatments (p=0.429) (1865 mg L^{-1} and 1627 mg L^{-1} respectively). No difference was seen regarding TOC release over time (p=0.097), No interaction (p=0.880) was recorded between pH and time (Figure 4.4).

A Pearson's Correlation was carried out to assess the relationship between TOC and elements being released over time. Due to the difference in mean concentrations extracted using pH 4 and 7 water, As, Fe and Pb were assessed at both pH's for correlations with TOC (Figure 4.5 a, b and c). The data found that As was positively correlated at pH 4 only (p<0.045). Iron and Pb were not found to positively correlate at either pH. Copper was the only element to correlate with TOC when the data sets from pH 4 and 7 were combined (p= 0.011) however this was a weak correlation as the R^2 value was just 0.269.
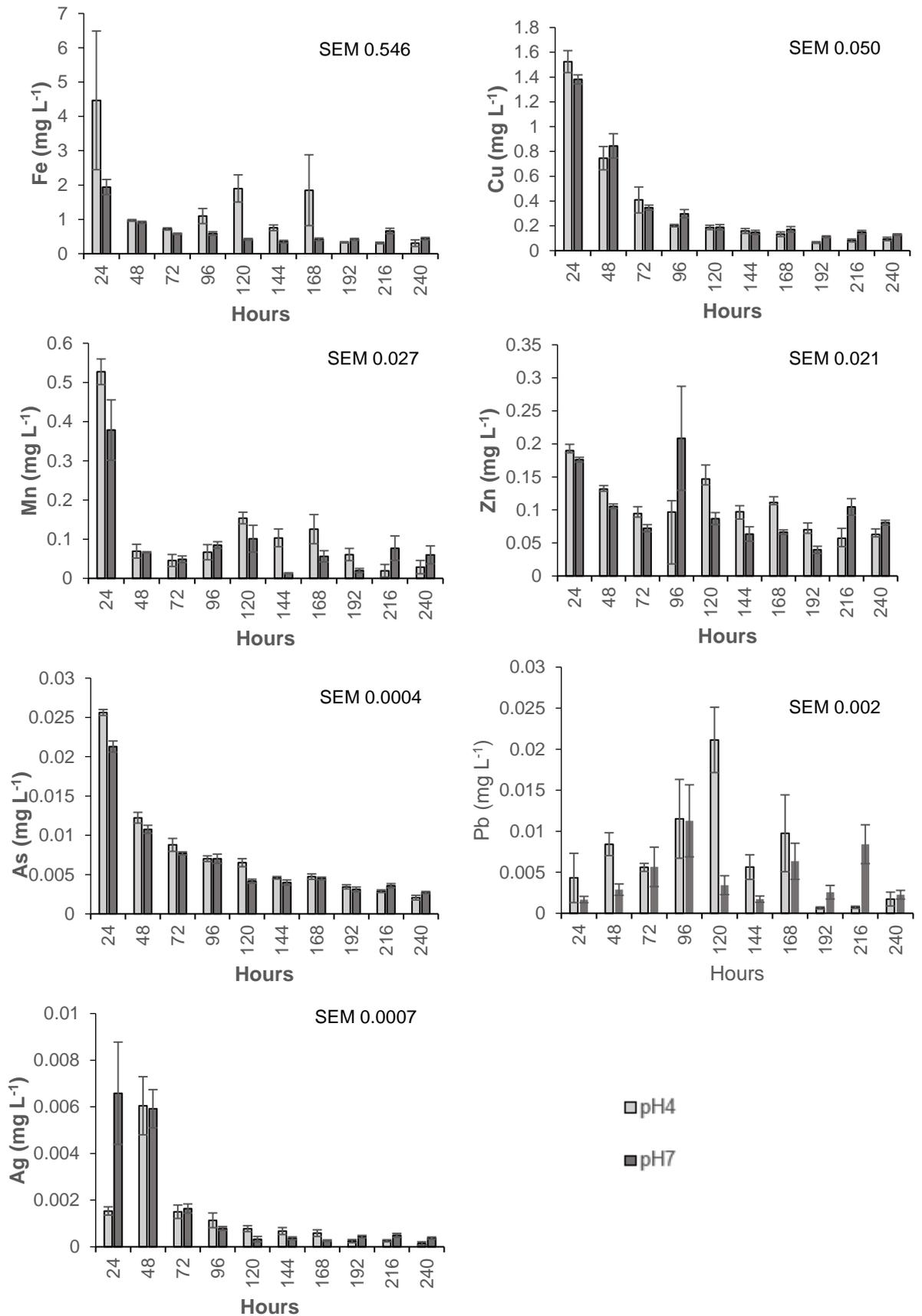
Figure 4.3. Heavy metal release from biosolids following 240 hour wetting and drying regime at pH 4 and 7. a - g are presented in order of decreasing concentrations. Residual d.f. 65.

The chart shows TOC (mg L⁻¹) on the y-axis (0 to 900) versus Hours on the x-axis (0 to 300). Two data series are shown: pH7 (dark) and pH4 (light).

pH 7; y = 5E-06x⁴ - 0.0027x³ + 0.5884x² - 53.172x + 1764.8
R² = 0.9575 R= 0.9785

pH4; y = 4E-06x⁴ - 0.0025x³ + 0.5307x² - 46.514x + 1530.5
R² = 0.9767 R=0.9882
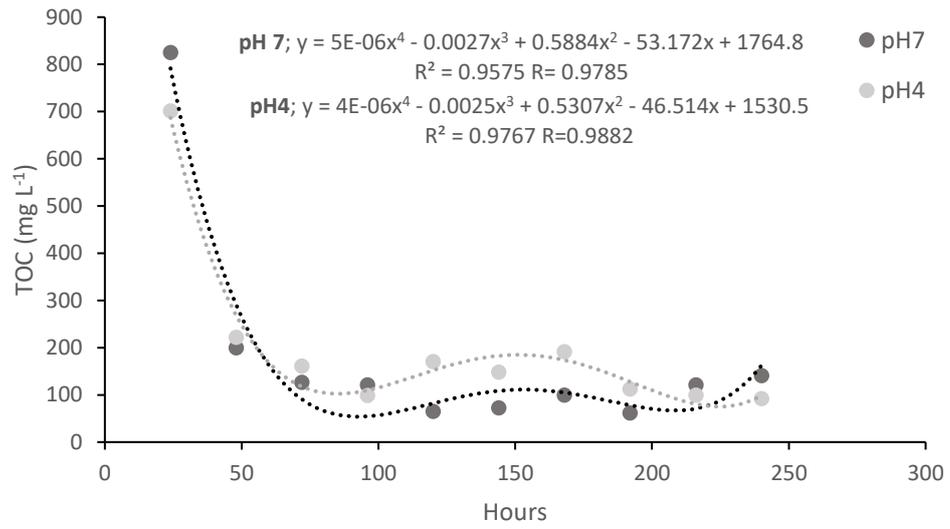
Figure 4.4 Models for the release of TOC from biosolids at pH4 and 7 following a series of wet and dry cycles (n=5).
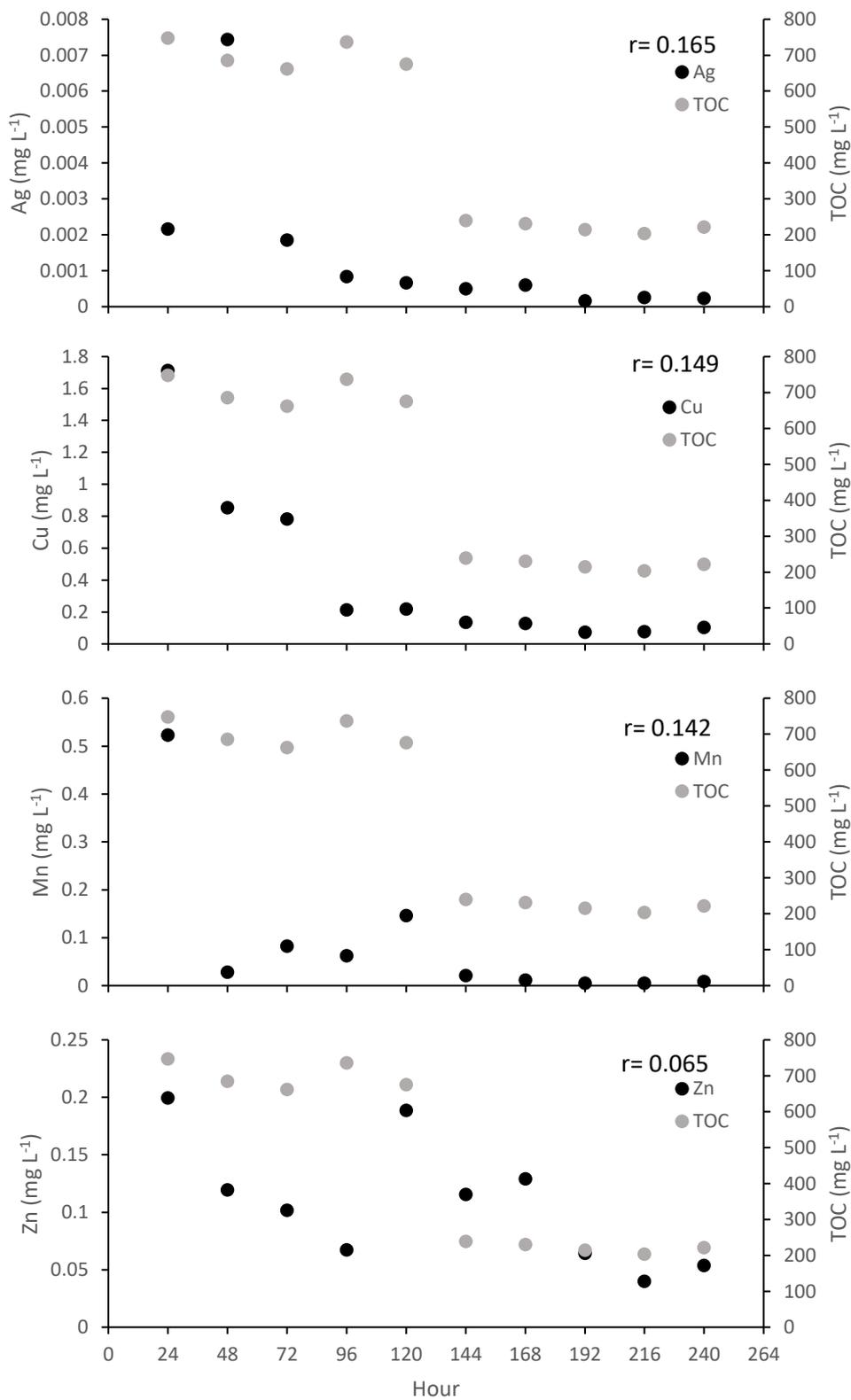
Figure 4.5a. Pearson's correlations between TOC and heavy metals where the pH was shown not to affect the concentration of heavy metal leached.
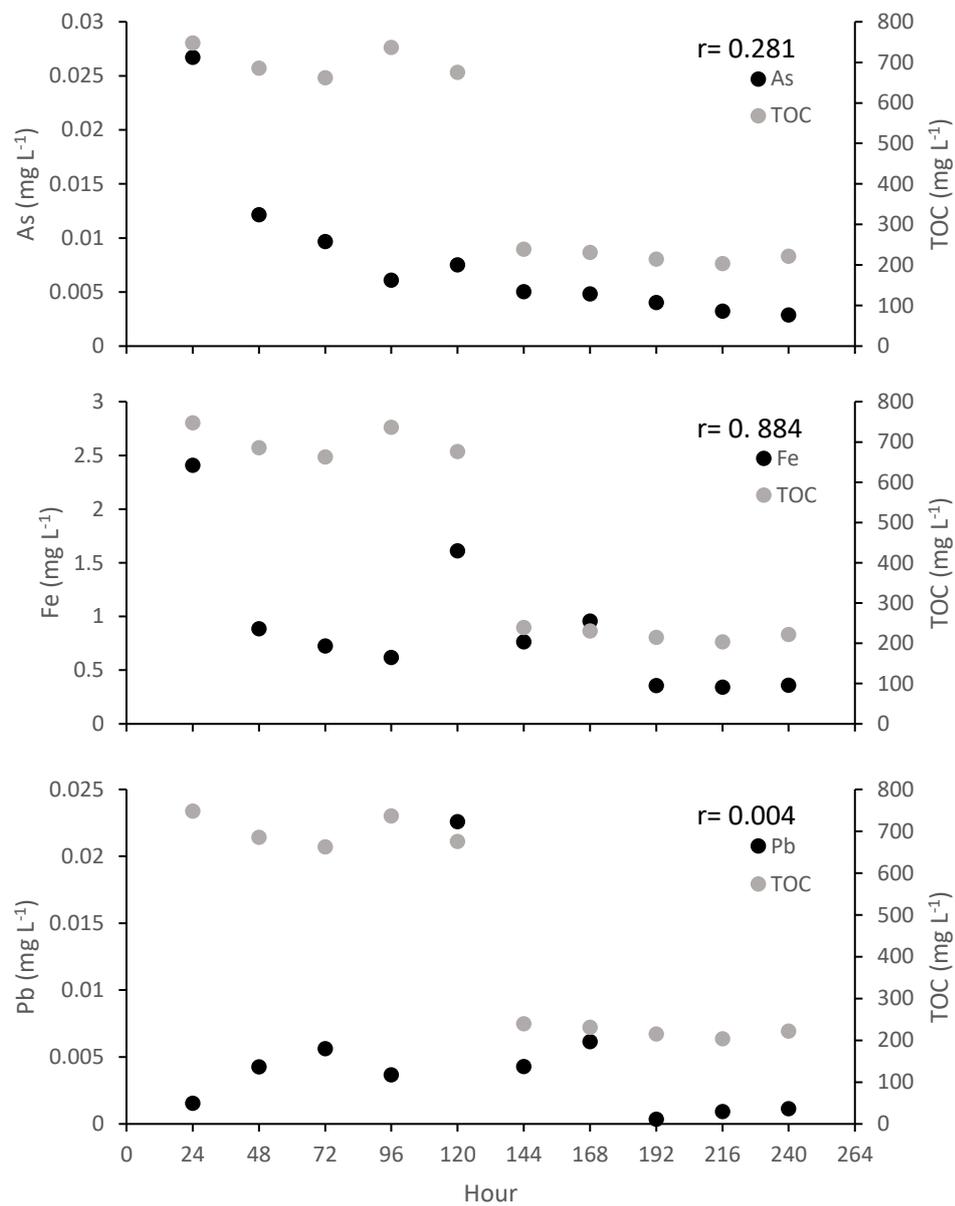
Figure 4.5b. Pearson's correlations between TOC and heavy metals, where pH was deemed to affect the concentration of the element extracted. Correlation displayed is for concentrations extracted using water adjusted to pH 4.
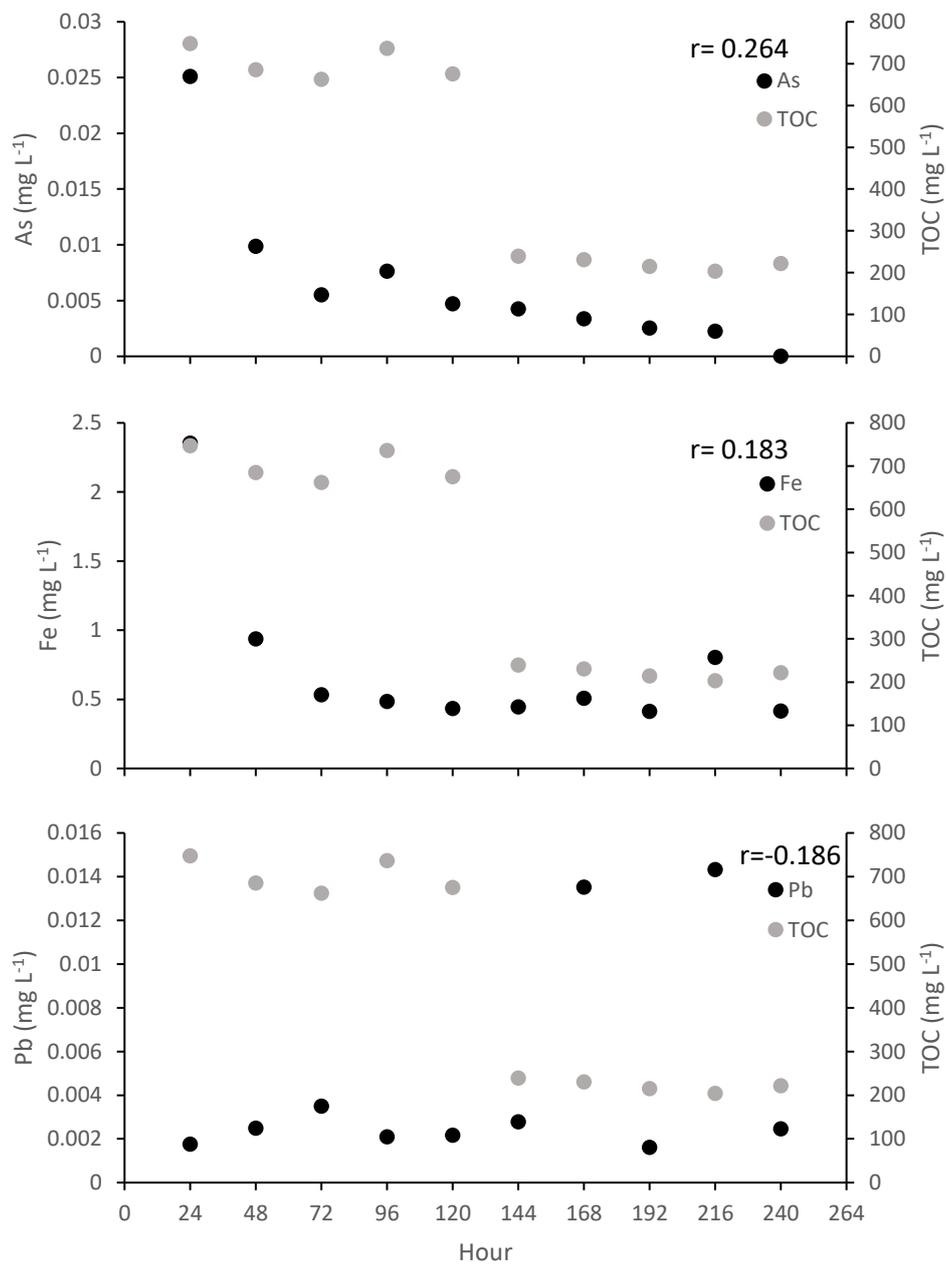
Figure 4.5c. Pearson's correlations between TOC and heavy metals, where pH was deemed to effect the concentration of the element extracted. Correlation displayed is for concentrations extracted using water adjusted to pH 7.

4.3.2 Modified Dutch Leaching Experiments

4.3.2.1 Broxton 0 kg ha^{-1} N and 500 kg ha^{-1} total Nitrogen (N) (Figure 4.6)

Iron

Total Fe concentrations extracted from the two treatments did not differ from each other (p=0.940) with untreated samples containing 47.60 mg L^{-1} on average compared to treated soils averaging 46.8 mg L^{-1}. Difference (p<0.001) in Fe extracted was recorded between the fractions, with the following differences in fractions; 1=2<3=4<5=6<7. No interaction was recorded between treatments and fractions however the p value was 0.061. The largest difference between treatments was recorded at fraction 7 with the mean concentration of Fe leached being 13.87 mg L^{-1} for biosolids treated and 20.36 mg L^{-1} for untreated.

Manganese

Total concentrations of Mn under the two treatments did not differ (p=0.987) with untreated soil producing an average of 13.30 mg L^{-1} compared to 13.40 mg L^{-1} under the biosolids treated soil. A difference was recorded for Mn concentrations from different fractions with the highest concentration being recorded in fractions 5 and 6 (3.75 mg L^{-1} and 2.98mg L^{-1} respectively). The lowest concertation was produced in the first fraction with no difference recorded between any other fractions. No interaction was recorded between treatment and fraction (p=0.179).

Arsenic

Mean concentrations of As extracted under the two treatments were not different overall (p=0.695), indicating that applying biosolids did not increase the extractable As content. The amount of As extracted did differ between fractions (p=0.120). No interactions between treatment and exposure time were recorded (p=0.515). It should be noted that soils contain large amounts of calcium (Ca) which is known to interfere with As and therefore these results may have been impacted upon due to this.

Copper

Differences in leachate Cu concentrations were recorded between treatments (p=0.029) with biosolids treated soil leaching 0.07 mg L^{-1} and untreated soils leaching 0.063 mg L^{-1}. A difference in concentrations (p=0.007) was recorded between fractions, with fractions 6 and 7 containing higher concentrations. No interaction was recorded between treatment and fraction for Cu concentrations (p=0.389).

Zinc

On average a greater (p=0.027) concentration of Zn was extracted from biosolids treated soils (0.217 mg L^{-1}) compared to untreated soil (0.055 mg L^{-1}). No difference (p=0.072) was recorded for fraction. No interaction was recorded between treatment and fraction (p=0.218).

Silver

Total concentrations of Ag extracted using the Modified Dutch Leaching Columns showed no difference between untreated and biosolids treated soil (p=0.419). Whilst there was a more than ten-fold difference between the concentrations of Ag extracted under the untreated and biosolids treated soil (0.012 mg L^{-1} and 0.0206 mg L^{-1} respectively), the dataset was very variable, recording a cv% of 572.3%. No differences were recorded between the fractions (p=0.377), nor was there an interaction recorded (p=0.402).

Lead

Mean concentrations of lead extracted overall were not different between treatments (p=0.604) indicating that a single application of biosolids did not increase the leachable Pb. The amount of lead leached over time did not change (p=0.088) with the first fraction being statistically lower than the last fraction. No interaction was recorded (p=0.280)

TOC

Mean concentrations of carbon from each treatment did not differ (p=0.434) with untreated averaging 2744 mg L^{-1} and biosolids treated averaging 3109 mg L^{-1} total TOC leached. A difference in TOC release was recorded overall with regards to fractions (p<0.001). Excluding fractions 1 and 2, each following fraction was significantly larger than the preceding fraction, with fraction 7 being the largest (1346.3 mg L^{-1}). No interaction (p=0.135) was recorded between fraction and treatment (Figure 4.7).

Positive correlations between TOC and heavy metals were recorded for Ag, As, Cu, Fe and Zn (p=0.0478, 0.0221, p<0.001, p<0.001 and p=0.02 respectively) (Figure 4.8a). Strong correlations were only found to occur with Cu and Fe (R^2=0.912 and 0.930 respectively) with all other R^2 values being less than 0.5. No correlation was found between TOC and Mn or Pb (Figure 4.8b).
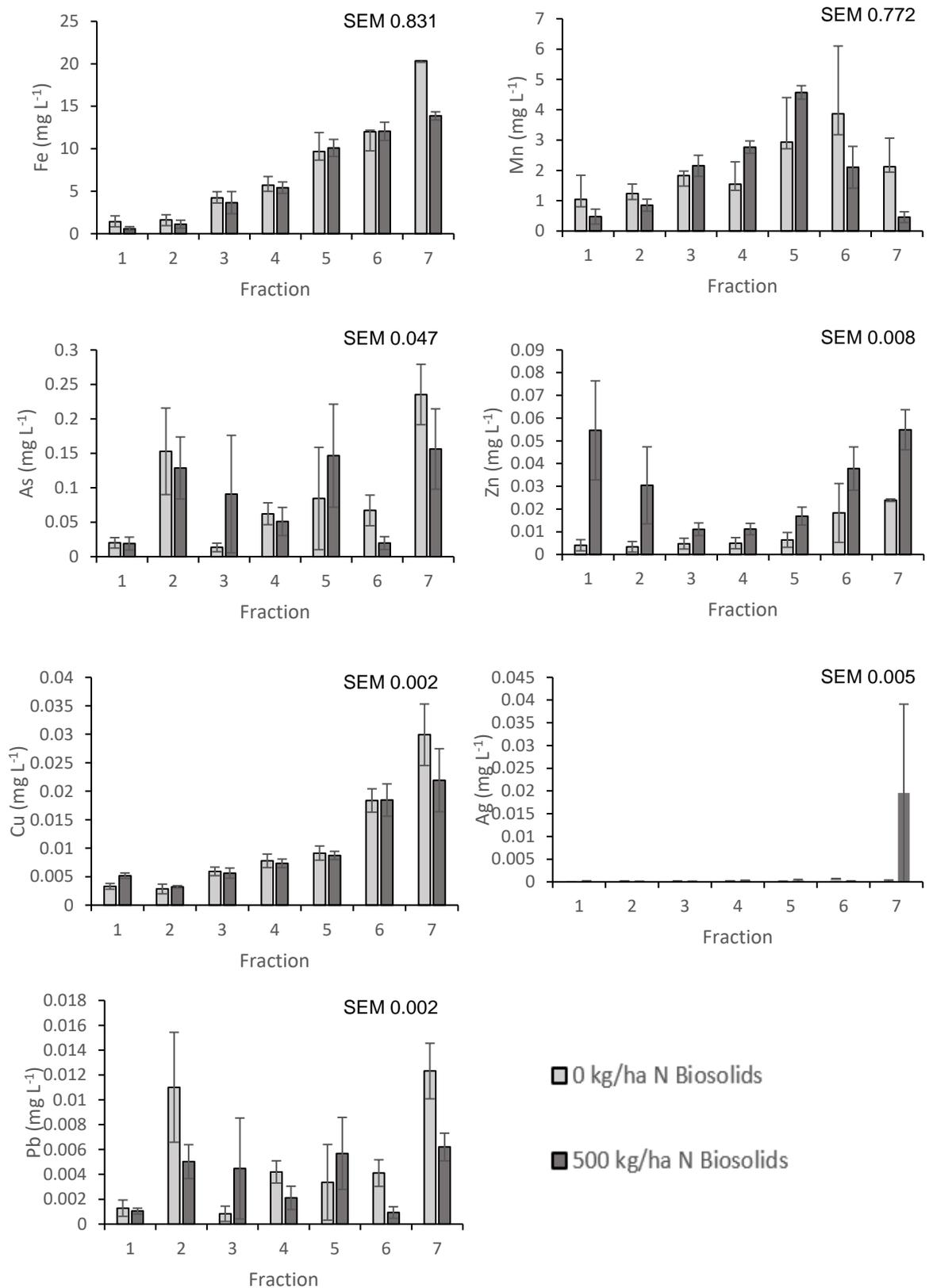
Figure 4.6. Mean elemental leachate concentrations extracted using the modified Dutch leaching experiment on Broxton soil with biosolids being applied at 500 kg ha^{-1} N. Note, graphs are presented in decreasing order of concentration due to the wide range of concentrations extracted (±SE). Residual d.f. 23. All concentrations were within the limits of detection for the ICP-MS used (Table 4.5).
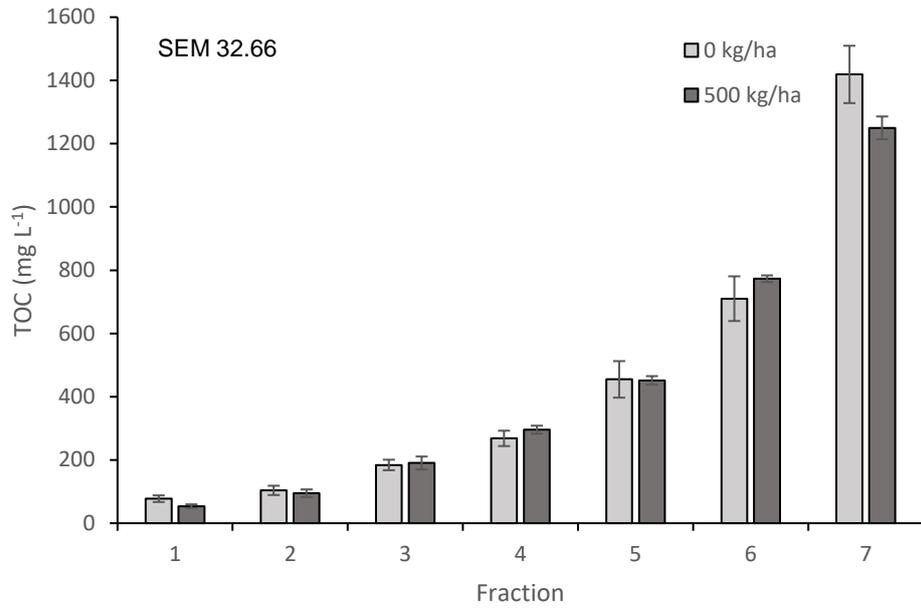
Figure 4.7. Mean TOC leached from Broxton soil following a single application of biosolids using the Modified Dutch leaching test (± S.E.). Residual d.f. 23
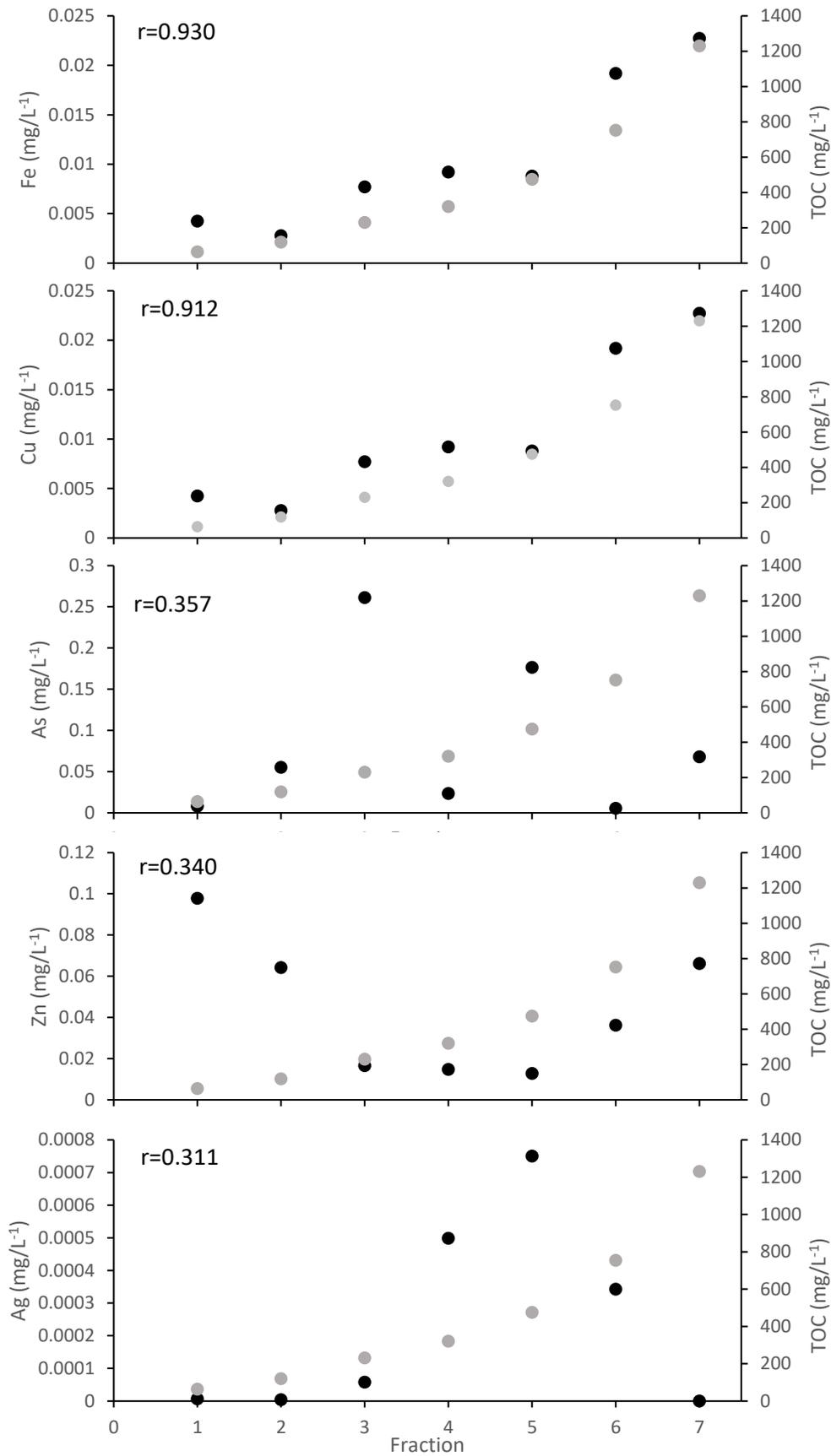
Figure 4.8a. Pearson's correlations of TOC and heavy metals that showed a significant correlation (p<0.05) from the Modified Dutch Leaching columns using soil from Broxton Cheshire.
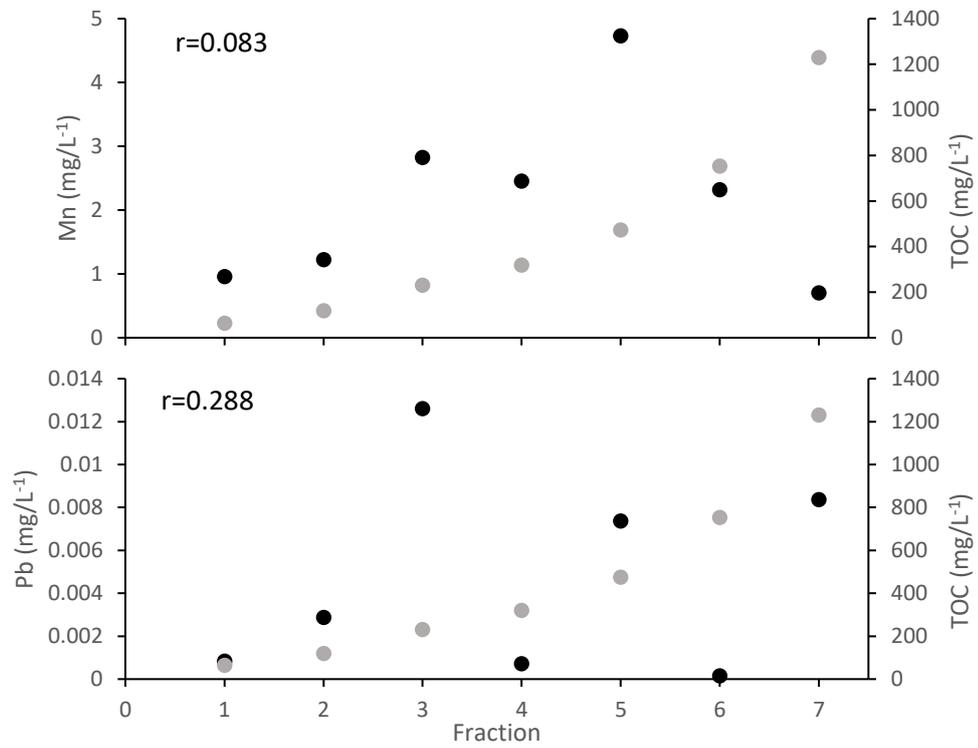
Figure 4.8b. Pearson's correlations of TOC and heavy metals that showed a significant correlation (p>0.05) from the Modified Dutch Leaching columns using soil from Broxton Cheshire.

4.3.2.2 Harper Adams University Farm soils 0 kg ha^{-1} N and 500 kg ha^{-1} N (Figure 4.9)

Compositional differences between Broxton and Harper Adams University soils can be found in Table 2.7.

Iron

No difference was recorded in the total concentration of Fe from each treatment (p=0.957), however a difference was recorded between fractions (p<0.001) with fraction 5 being higher in Fe than all preceding fractions. Fraction 6 was larger still. Fraction 7 was the largest fraction with a concentration of 21.505 mg L^{-1}. No interaction was recorded between treatment and fraction (p=0.095).

Manganese

A difference in the total concentration of Mn was recorded between treatments (p=0.023) with untreated soils leaching higher concentration (9.3 mg L^{-1}) compared to treated soil (2.14 mg L^{-1}). This indicated that the addition of biosolids to soil decreased the overall Mn leached on average over a 45 year period. Fraction was also significant with fractions 1 and 2 being lower in Mn when compared with fractions 6 and 7. Fraction 6 was the largest fraction overall. No interaction was recorded between treatment and fraction (p=0.134).

Arsenic

Concentrations of arsenic were different between treatments (p=0.007), with biosolid treated soils leaching 0.924 mg L^{-1} and untreated soil leaching 0.484 mg L^{-1}. No difference in concentration released over time were recorded (p=0.437). P values indicated no interaction between treatment and fraction (p=0.154).

Copper

Total Cu concentrations were significant between untreated (0.156 mg L^{-1}) and biosolids treated soils (0.492 mg L^{-1}) (p=0.042), indicating that biosolids increased the concentration of Cu leached from the soil overall. No differences between fractions were recorded (p=0.174). No interaction was recorded (p=0.225).

Zinc

No differences were recorded for mean Zn concentrations between treatments (p=0.479), fractions (p=0.306) or an interaction between the two factors (p=0.425). This therefore indicates that the leaching of Zn out of biosolids soil did not increase following one application of biosolids when compared to background soil concentrations.

Lead

Mean concentrations of lead were not different between treatments (p=0.164) with biosolids treated soil leaching 0.0045 mg L^{-1} compared to 0.0025 mg L^{-1} from untreated soils. Concentrations released over time did differ (p=0.014), with the highest concentration being recorded in fraction 5. An interaction was recorded between treatment and fraction, with leachate from biosolids treated soils in fraction 5 having a higher concentration of Pb than untreated soils.

Silver

No differences were recorded for total Ag concentrations between treatments (p=0.866), fraction (p=0.681) or interactions between the two factors (p=0.098). This therefore indicates that the leaching of Ag out of biosolids soil did not increase following one application of biosolids when compared to background soil concentrations.

TOC

Concentrations of TOC leached from the two treatments did not differ (p=0.372) indicating that the concentration of carbon leach was not influenced by the addition of biosolids. The amount of TOC released over time did differ (p<0.001) (Figure 4.10). Fraction 5 was larger than all preceding fractions, which were all statistically similar to each other. Fraction 6 and 7 were larger than 5 but not different from each other. No interactions between treatment and time was recorded (p=0.668)

Correlations were found between TOC and heavy metals for Ag, Fe and Mn (p=0.010, p<0.001 and p=0.015 respectively) (Figure 4.11a). Correlations for Fe and Mn were positive whereas Ag was negative indicating that as TOC increased, Ag concentrations decreased. All other elements showed no significant differences (Figure 4.11b).
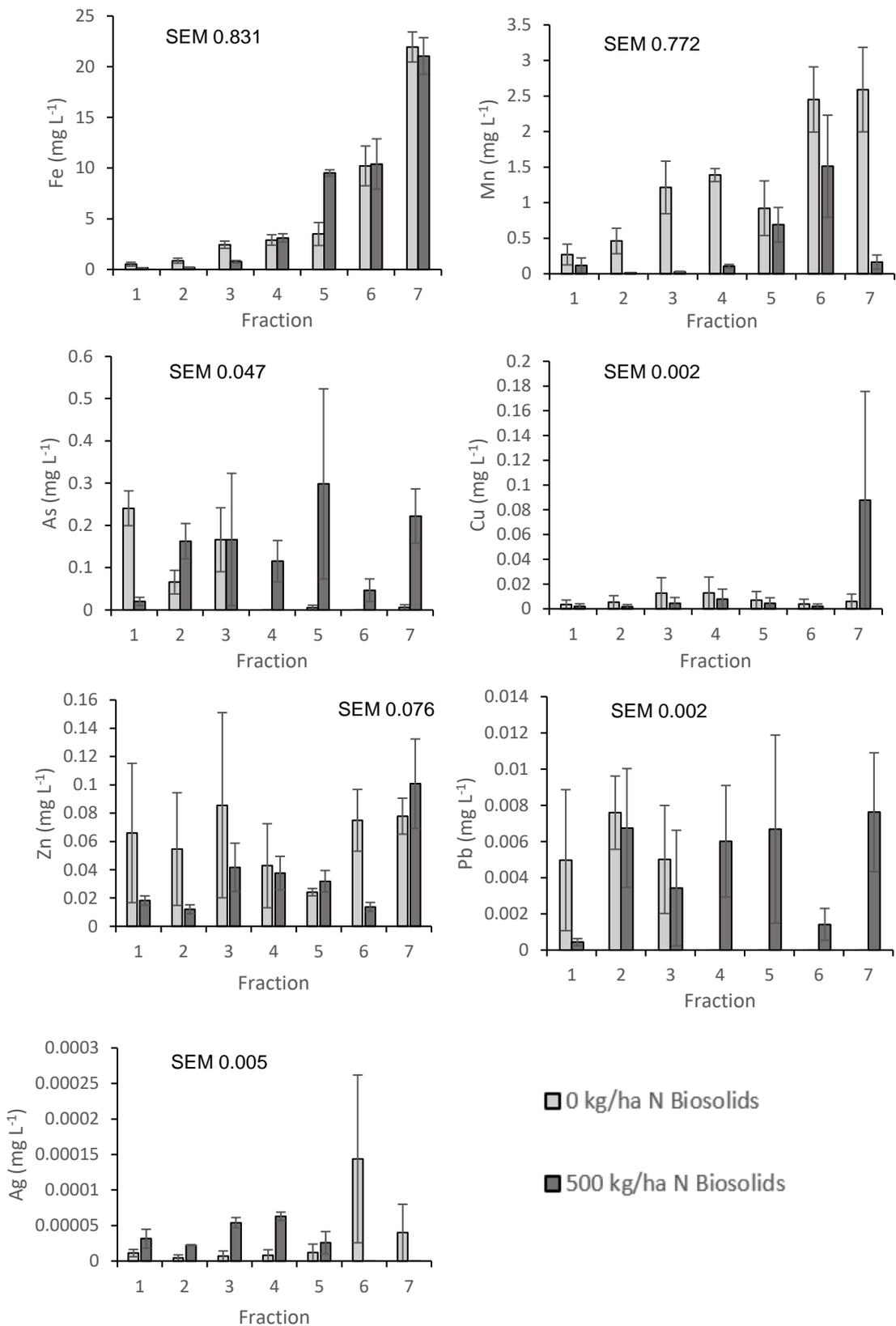
Figure 4.9. Mean elemental leachate concentrations extracted using the modified Dutch leaching investigation on Harper Adams University Farm soil following a single application of biosolids. Note, graphs are presented in decreasing order of concentration due to range of concentrations extracted. Error bars signify ± SE. Residual d.f. 23.
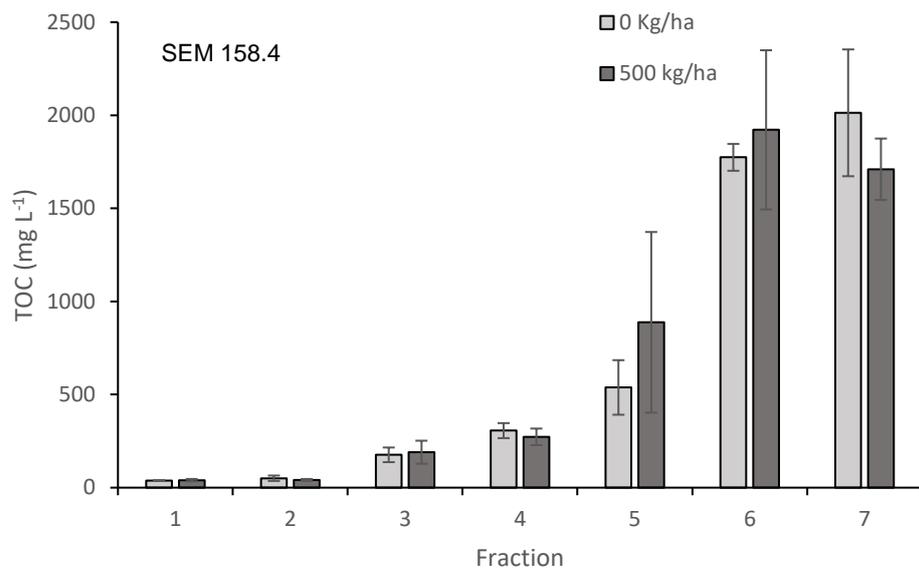
Figure 4.10. Mean TOC of leachate using the Dutch Modified Leaching experiment on Harper Adams university soil following a single application of biosolids (± S.E.). Residual d.f 23.
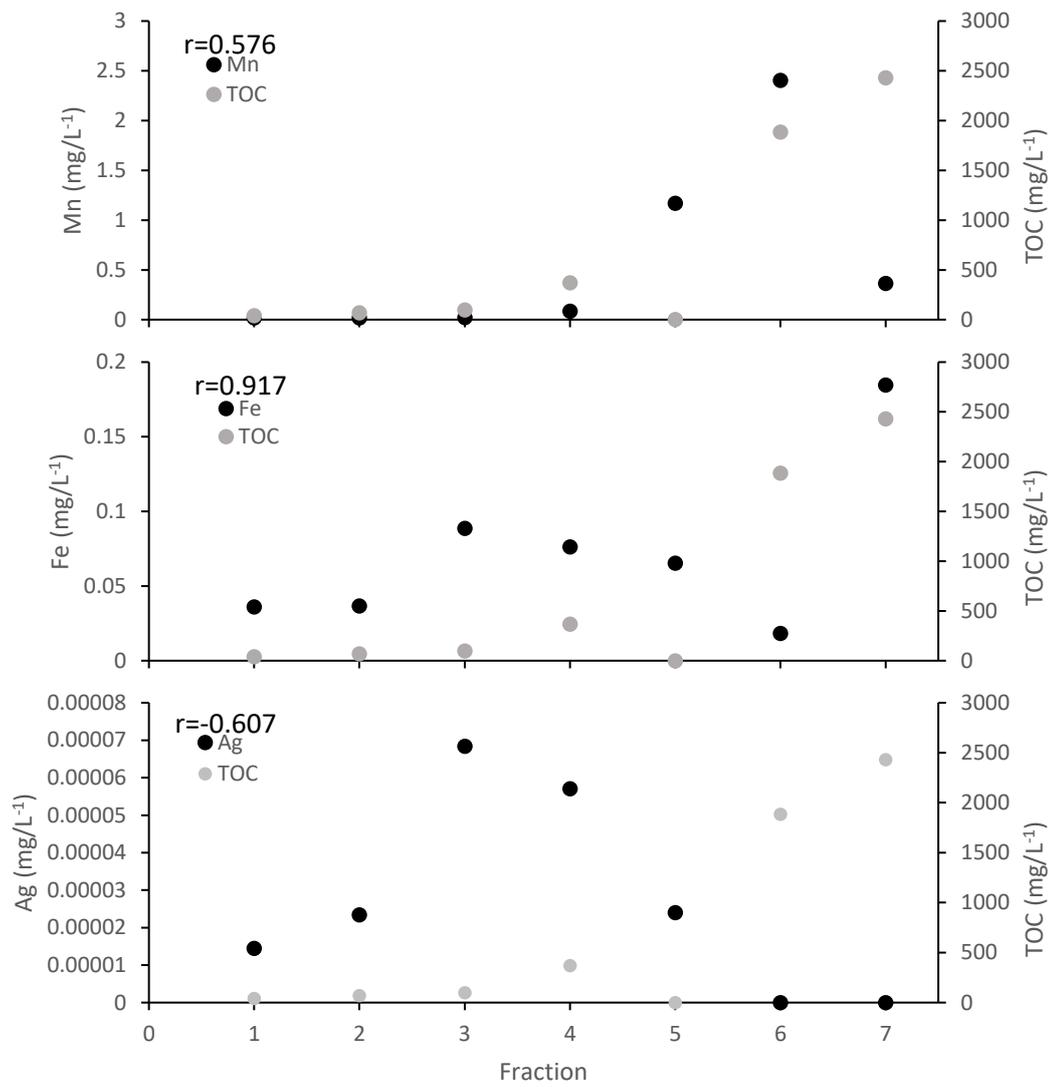
Figure 4.11a. Pearson's correlations of TOC and heavy metals that showed a significant correlation (p<0.005) from the Modified Dutch Leaching columns using soil from Harper Adams University.
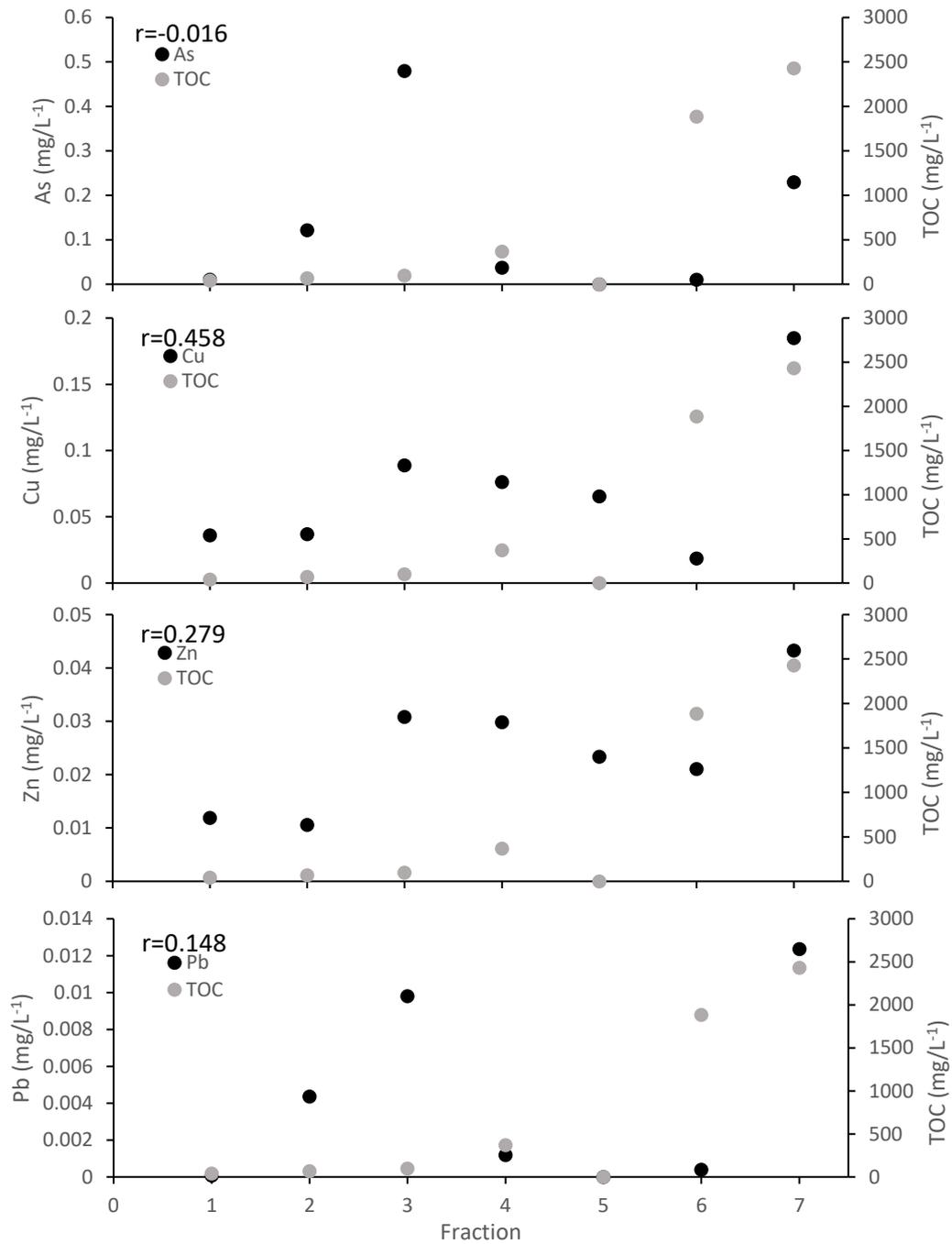
Figure 4.11b. Pearson's correlations of TOC and heavy metals that showed no significant correlation (p>0.005) from the Modified Dutch Leaching columns using soil from Harper Adams University.

4.3.2.3 Broxton seven years of field applications, untreated, conventional and biosolid treated soils (Figure 4.12)

Iron

Iron concentrations leached from soils did not differ (p=0.067) however they were close to significant. Biosolids treated soils leached 84.4 mg L^{-1} compared to 40.0 mg L^{-1} from conventionally treated soils and 47.6 mg L^{-1} from untreated soils. A significant difference was recorded between fractions with the concentration increasing in each fraction. Fraction 7 had the largest mean concentration of Fe in the leachate (31.14 mg L^{-1}). An interaction was found to occur (p=0.034), with leachate from biosolids being recorded at higher concentrations in fraction 6 and 7 than both conventionally treated and untreated soils.

Manganese

Manganese concentrations on average showed no difference between treatments (p=0.714) indicating that biosolid applications over 8 years did not increase the risk of it leaching from soil. A significant difference was recorded between fractions. Fractions showed the following significance in concentrations 1=2=3=4=5<6=7. A significant interaction was recorded (p=0.034) with both conventionally treated and biosolids treated soils leaching more Mn at fraction 7.

Copper

No differences were recorded between the average concertation of Cu leached from each soil (p=0.439) with Cu concentrations in biosolids treated soils leaching 0.038 mg L^{-1} conventionally treated soils leaching 0.117 mg L^{-1} and untreated soils leaching 0.063 mg L^{-1}. No differences were recorded between fractions, with no interaction between treatment and fraction being recorded.

Arsenic

Total As concentrations did not differ between treatments (p=0.085) however, biosolids and conventionally treated soil did leach substantially more As than untreated soils (0.439 mg L^{-1} from biosolid treated soils, 0.558 mg L^{-1} from conventionally treated soils and 0.077 mg L^{-1} from untreated soils). Biosolids are known to contain As but the As in the conventionally treated fertiliser may be the result of contamination in bedrock. Concentrations of As leached did not differ with time (p=0.397). No interaction was recorded between fraction and treatment.

Zinc

Mean concentrations of Zn leached from each soil showed no difference between treatments (p=0.638) indicating that biosolids did not increase the concentration of Zn in leachate overall. A difference was recorded between the fractions with fractions 1, 6 and 7 being statistically similar. All other fractions were similar to each other but lower than fraction 1,6 and 7. No interaction was recorded between exposure time and treatment (p=0.575).

Lead

No differences were recorded in the total amount of Pb leached from different soil treatments (p=0.100). No differences in Pb concentrations leached over time were recorded (p=0.406). No interaction between fraction and treatment was recorded (p=0.124).

Silver

Untreated soil leached more (p=0.003) Ag on average than biosolids and conventionally treated soil (0.00018 mg L^{-1}, 0.00000 mg L^{-1} and 0.00013 mg L^{-1} respectively). A difference was also recorded between fractions with fraction 7 having a higher Ag concentration than all other fractions (p<0.001). An interaction was recorded between treatment and fractions (p<0.001) with the leachate from untreated soil being significantly higher in Ag than both the conventionally and biosolids treated soils in fraction 7 only.

TOC

No differences were recorded in total TOC leached from each treatment (p=0.171). A difference was found for time with fraction 7 containing the highest TOC concentration. Fractions showed the following statistical significance in concentrations 1=2=3<4<5 <6 <7. An interaction (p=0.004) between treatment and fraction was recorded, however no differences were observed between treatments at any one fraction (Figure 4.13). TOC concentration was shown to positively correlate with Ag (p=0.018), Fe (p<0.001), Mn (p<0.001) and Zn (p=0.044) indicating that as TOC increases, as does the concentration of each of these elements (Figure 4.14a). No other elements showed a significant correlation with TOC (Figure 4.14b).
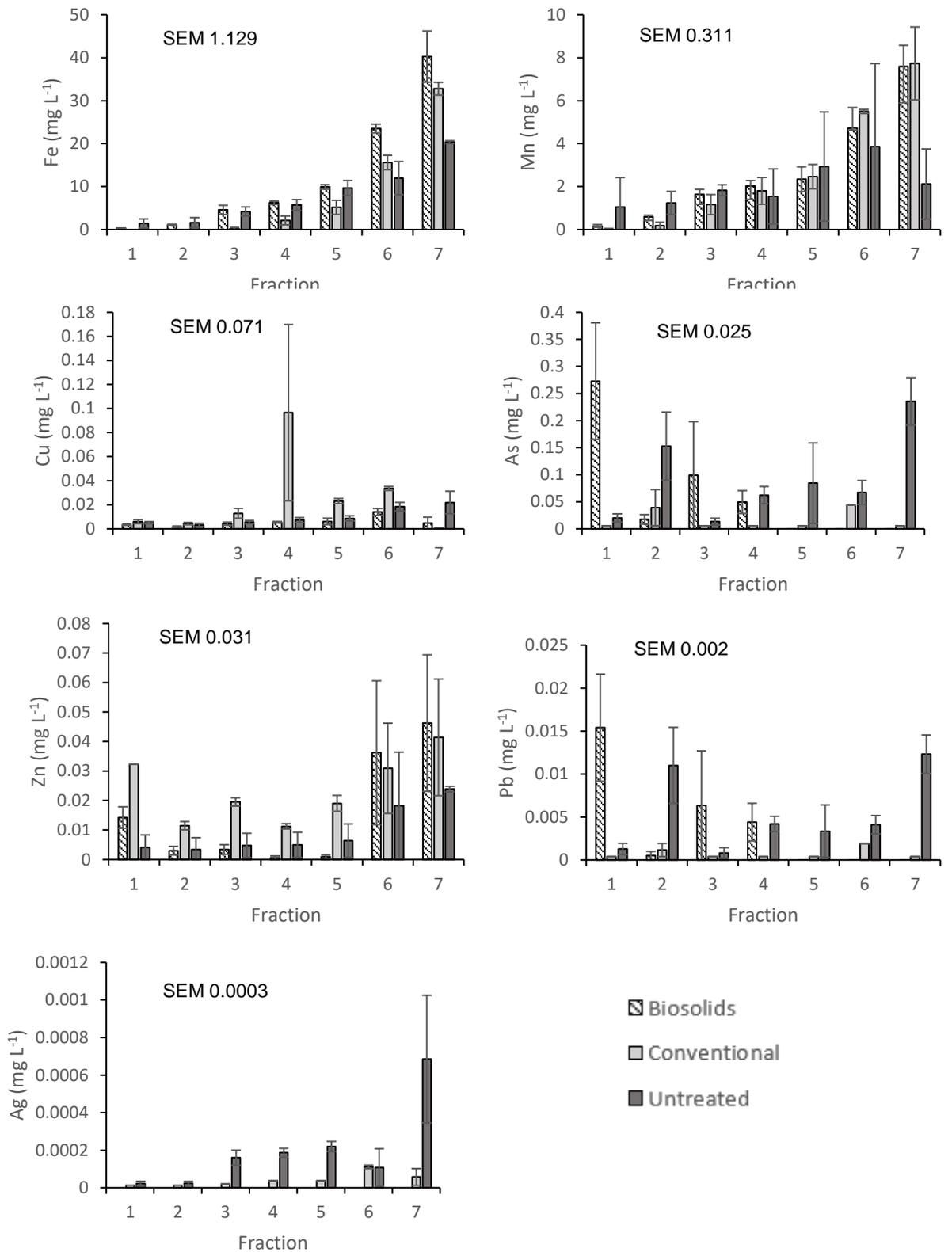
Figure 4.12. Mean elemental leachate concentrations of heavy metals extracted using the Dutch Modified Leaching experiment on Broxton soil following eight years of biosolid applications. Note, graphs are presented in decreasing order of concentration due to range of concentrations extracted (± S.E.). Residual d.f. 23
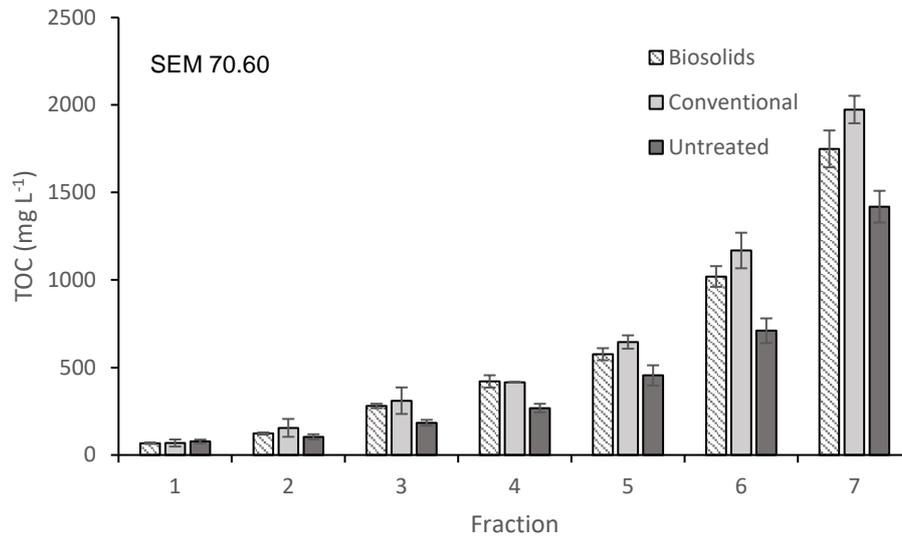
Figure 4.13. Mean TOC of leachate using the Dutch Modified Leaching experiment on Broxton soil following eight years of biosolid applications. Error bars signify +/- SEM. Residual d.f. 23
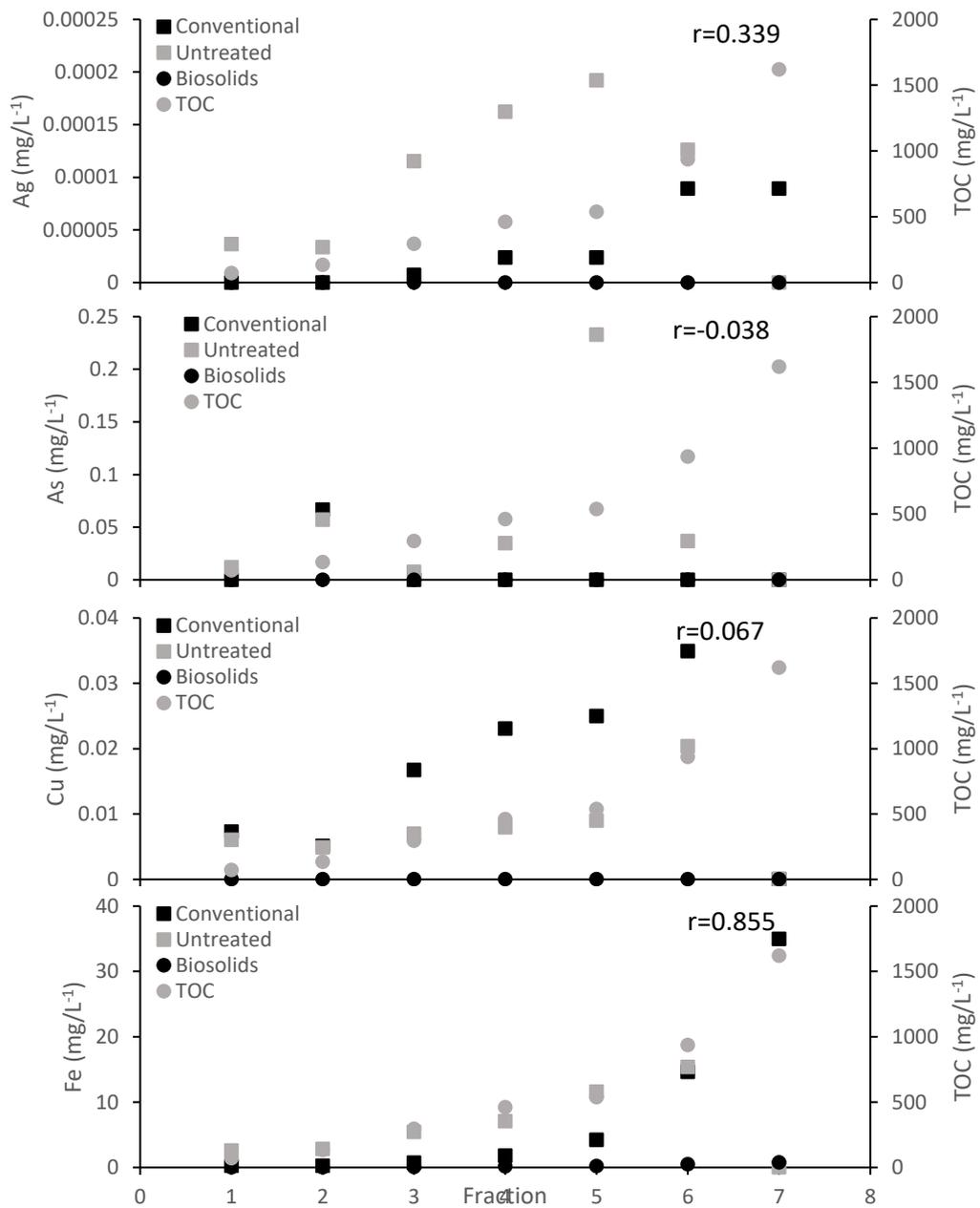
Figure 4.14a. Pearson's correlations between TOC and Ag, As, Cu and Fe for the soil at Broxton, Cheshire, which had received seven years of biosolids applications.
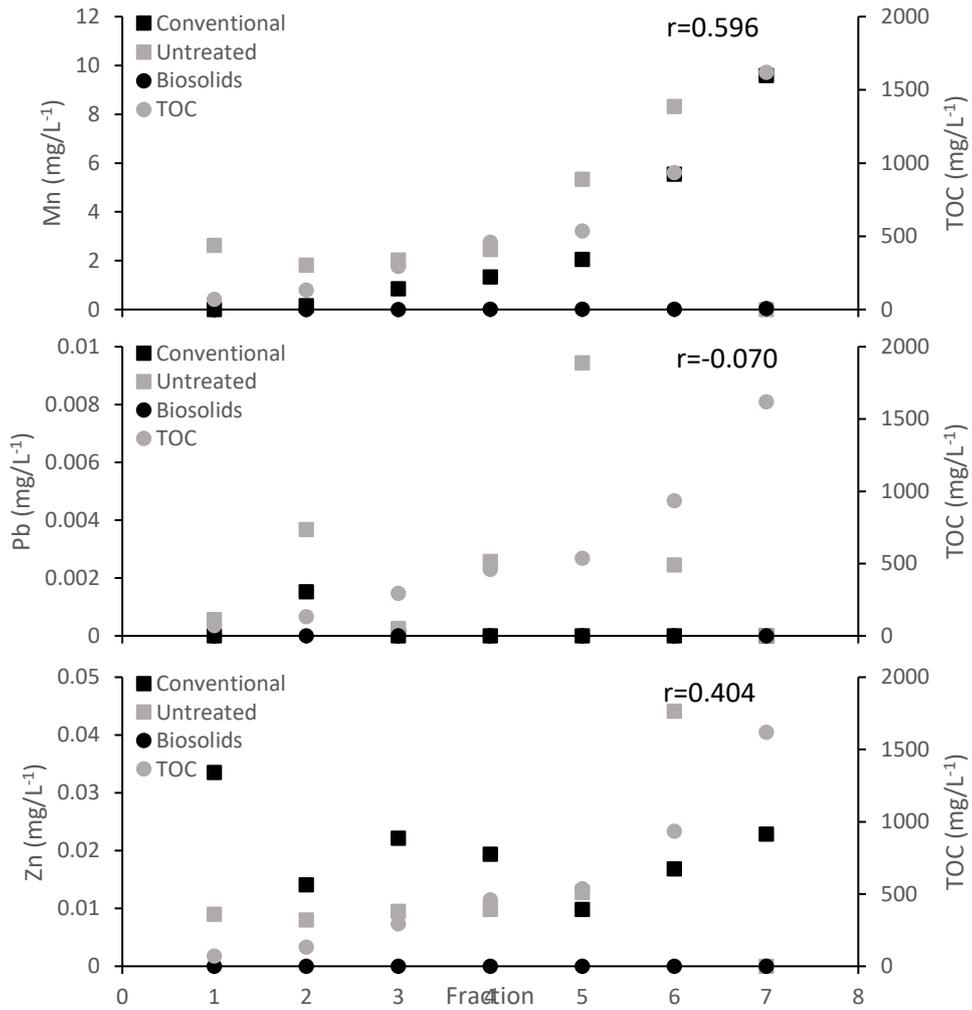
Figure 4.14b. Pearson's correlation for TOC and Mn, Pb and Zn for the soil at Broxton, Cheshire, which had received seven years of biosolids applications.

4.3.2.4 Broxton 500 kg ha^{-1} N and Broxton seven years of applications

The leaching data collected from the two leaching assessments containing Broxton soils were analysed to assess if there was an accumulative increase in heavy metal concentration when comparing a single application of biosolids to 8 years of applications. No statistical differences were recorded between the two leaching sets for Ag, Mn, As, Pb or Zn. (p=0.402, p=0.168, p =0.153, p=0.412 and p=0.367 respectively). Copper concentrations were higher in the leachate from soils receiving a single application of biosolids (0.077 mg L^{-1}) compared with leachate from soils which had received multiple applications (0.038 mg L^{-1}). Conversely, Fe concentrations were found to be statistically higher in leachate from soils that had received seven years of applications indicating that biosolids were increasing the concentration of Fe in leachate (p=0.002). Following a single application of biosolids to soils (500kg ha^{-1} total N), Fe concentrations were 46.8mg L^{-1} but after seven years of applications, concentrations leached were 84.4 mg L^{-1}. No other elements showed statistical differences between the two treatments.

No statistical differences were recorded between the mean TOC values of the two treatments (p=0.501) indicating that multiple applications of biosolids to soil did not increase TOC content in leachate compared to a single application.

4.4. Discussion

Biosolids, like other organic manures, have the potential to increase a soils organic matter content and improve structure resulting in increased crop yields and reducing the impact of modern day farming practices on ecosystems. Whilst their applications have been linked with environmental contamination and bioaccumulation of heavy metals in crop tissues, they also have the potential to supplement diets through biofortification. In order for their applications to made responsibly, their leaching potential must be understood to assess what elements may pose dangers in different soil types and under different soil pH's.

Wet and dry experiments using two contrasting pH levels (4 and 7) established what elements would leach from the biosolids in the absence of soil. Results indicated that only Fe, As, and Pb concentrations were different between the pH's with each of these elements leaching more under pH4 conditions. Concentrations of Pb were found to increase with decreasing pH by Stenhouwer *et al.* (2006), however, in contrast to the results obtained here, Stenhouwer *et al.* (2006) also found that Mn, Cu and Zn concentrations increased with decreasing pH. Reddy *et al.* (1995) suggests that the speciation of an element may be an important factor regarding the release of nutrients at different pH levels. Reddy *et al.* (1995) found that at neutral pH levels, the dissolved organic carbon was the key factor regarding the release of heavy metals into soil solution, yet at lower pH levels, the free ionic

forms of an element were more important. However, it was also found that with decreasing pH, dissolved carbon levels also decreased, which contrasts with the data collected here. No significant differences were recorded for TOC under the two pH treatments.

For all elements, except Pb, the first 24 hours leached the highest concentration, indicating that the biosolids released the largest proportion of readily available heavy metals rapidly. For example, in the first 24 hours the following percentage of the total concentration extracted were leached, Zn 18%, Mn 43%, Fe 32%, Cu 40%, Ag 25%, As 32%. In contract, Pb leached only 5% of the total concentration extracted in the first 24 hours, with the highest concentration being found at 96 and 120 hours. Lead concentrations are often found at very low levels in biosolids and therefore they have not been widely studied for their release rates (Department of Environment and Conservation, 2012 and Brown, no date). Arsenic and Pb were the only elements to show an interaction between pH concentration and time, with As being more concentrated at 24, 48 and 120 hours under the pH4 solution and Pb being more concentrated at 120 hours under pH4 solution. This corresponded with their release patterns and was similar to the two peak timings of TOC release, yet, whilst As was found to positively correlate with TOC at pH 4, Pb was not. The second peak in TOC release was much smaller than the first, and may indicate the water reaching the core of the granules.

Whilst the increase in Fe, As and Pb concentration at pH4 suggests that with decreasing pH mobility increases, few agricultural fields are found with such low pH levels and therefore, further research should study at which pH such differences are recorded for each element to determine the extent of the hazard posed.

Modified Dutch Leaching column experiments showed contrasting result for the two soil types (soil compositional differences are detailed in Table 2.7). Concentrations of elements leached from Broxton soils (0 kg ha^{-1} and 500 kg ha^{-1} N) did not differ when comparing biosolids treated to untreated soils, with the exception of Cu and Zn. Concentrations of Zn leached were on average more than three times higher from soils treated with 500 kg ha^{-1} N as biosolids compared to untreated soil, indicating that there may be a greater chance of Zn uptake into plants, which may help with the issues associated with hidden hunger. However, under Harper Adams University Farm soil, As and Cu concentrations were found to increase under the 500 kg ha^{-1} N as biosolids treatment. Whilst Cu is required in the human diet for metabolic processes and therefore its translocation into plants may not be an issue if below regulatory levels, As is no longer considered beneficial. Daily tolerable intake levels for As are currently under review, but Pb levels are provided due to its lack of use for protective health. The uptake of Cu and Zn into the grain and their concentration in drinking water would therefore need to be studied to determine how much of a risk this poses.

Where differences in the concentrations leached from fractions occurred, they all occurred in the last 3 fractions, indicating that the greatest threat to the environment possibly occurs years after application. Interactions between treatments and time in the Broxton soils were not recorded.

Conversely, in the Harper Adams University Farm soils, Mn leachate concentrations were found to be significantly higher in the soil treated with 0 kg ha^{-1} N as biosolids. This indicates that the application of biosolids to soil at 500 kg ha^{-1} N resulted in Mn becoming more mobile. Arsenic leachate concentrations from the same soil were significantly higher in the biosolids treated soils (500 kg ha^{-1} N as biosolids), indicating that a single application of biosolids to this soil, may result in increased As concentrations, however no interaction between time and treatment was recorded and therefore this risk does not appear to be elevated with time. Again, the hazard this poses is dependent on the levels reaching water courses and being translocated into plants.

Total leached TOC concentrations were not found to differ for either soil type, under each treatment, however both the Harper Adams University Farm and Broxton soils saw the largest concentration recorded in the fractions 6 and 7, indicating that TOC leaching increases with time. No interaction between fraction and treatment was recorded and therefore the application of biosolids does not increase the risk of TOC leaching, even over several decades. However, the work by Wijesekara *et al.* (2017) suggested that biosolid applications increase TOC cannot be supported by the data in this chapter. This may be due to a difference in application rates and the type of biosolids used.

Previous experimental work has suggested that with increasing dissolved organic carbon, the concentration of heavy metals increase (Fang *et al.* 2016), which concurs with some of the correlation data presented here. Total organic carbon was found to increase over time in both the Broxton and Harper Adams University Farm soils. Correlations between elements and TOC indicated positive relationships between for Fe and Mn in the leachate from Harper Adams University Farm soils and Ag, As, Cu, Fe and Zn in Broxton soils. Schaecke *et al.* (2002) found that the Zn, Cd, Cu, Ni and Cd were all correlated with the DOC and could be mobilised up to 50cm down through the soil profile in 11 years. However they did not study any correlation with Fe. Liptzin and Silver (2009) found that Fe reduction (the reduction of Fe (III) to Fe(II)) was correlated with increasing carbon. Ferrous iron (Fe (II)) has high water solubility compared to Fe (III) yet, in oxidising environments Fe(III) is more common. Whilst the leaching columns would not be fully representative of the reducing environment found in soils, it indicated that Fe may become lost from the agricultural system to ground water.

Soils were collected from the field experiments at Broxton following seven years of biosolid applications to assess how multiple applications would impact on the leaching of heavy metals.

Water leached from untreated soils recorded a higher concentration of Ag when compared to soil treated with biosolids and conventional fertilisers. Leached Ag concentrations were very low (0.00018 mg kg^{-1} being the highest mean), but still within the limit of detection of the ICP-MS. Therefore even a small amount of contamination from a source other than the soil, may have impacted on the result. Iron concentrations were close to significant, with biosolids soils having almost double the concentration when compared to the untreated and conventionally treated soils. There was a low number of residual d.f. for this experiment (2) and therefore with more replicates, this may become a significant result. Should that occur, there may be a risk associated with multiple applications of biosolids to soils and the leaching of Fe into ground waters. Silver, Fe, Mn and Zn concentrations released were highest from fraction 7. Arsenic, and Pb did not show any difference in concentrations with fraction. Total organic carbon concentrations showed no significant difference between treatments.

The comparison of total heavy metal contents of the two biosolids treated soils from Broxton (following a single application and seven years of applications), showed only an increase in Fe following seven years of applications. However, Cu concentrations were higher in the leachate from soils receiving a single application of biosolids. This therefore suggests that multiple applications of biosolids, over multiple years only results in the increase of Fe in soil leachate and that the concentration of all other heavy metals studied does not increase. Such increases in Fe concentrations could pollute ground waters. Iron is expensive to remove from waste water but is necessary if levels become excessive as a diet too rich in Fe, can cause cirrhosis of the liver and heart disease. High levels of Fe in water can also result in it becoming darker in colour as well as changing its taste which would need to be rectified before being used for human consumption (Kumar *et al.*, 2017).

Again, TMDA recoveries for Fe, Mn and Zn were all below 95% however they were all above 92%. This may indicate that the concentrations presented here are slightly lower than the true values. The addition of a collision cell to the ICP-MS could improve element recovery.

The data presented in this chapter cannot support the null hypothesis as heavy metal and TOC concentrations have been found to differ between biosolids amended soils and untreated soils.

4.5. Chapter conclusions

Whilst the results from this chapter indicate the concentration of the elements in the leachate are dependent on the soil type, biosolid application only increased the leachate concentration of As and Cu in Harper Adams University Farm soils and Cu and Zn in Broxton soils following a single application of biosolids. Total organic carbon was found to positively correlate with a number of elements in both Broxton and Harper Adams University

soils, indicating that it may be a contributing factor to their mobilisation. Overall, the impact of any increase in leachate concentration cannot be assessed due to a lack of information regarding the dilution of the elements into ground waters and what is likely to be translocated into edible plant tissues. Such factors will dictate the final concentrations for the leachate at these two endpoints and therefore impact what hazard may be associated with them. Therefore it is recommended that the concentration of heavy metals be studied in the grain of plants to see if the concentrations differs and correlates to the results found here.

Chapter 5.

Biological Indicators to Assess Biosolid-Treated Soils

5.1 Introduction

Soil health is an important aspect when considering the sustainability of food production. Linked intrinsically with greenhouse gas emissions and water pollution, soil health impacts on many parts of everyday life. The UK Government has pledged to ensure all soils are managed sustainably by 2030, indicating the level of importance soils have on society (House of Commons, 2016).

Many agricultural procedures for the maintenance of soil can be attributed to cross compliance schemes, whereby farmers receive payments for making soil improving measures. Yet the impact of such practices on soil biology is not considered under these schemes. European legislation regulates biosolids only on its chemical and bacterial content (Artuso *et al.*, 2011), yet they have the potential to support soil populations through their organic matter (OM). Biological indicators of soil health are often focused on three main aspects (Knoepp *et al*, 2000);

1. Soil structure development
2. Nutrient storage
3. Biological activity

Whilst each of these aspects are important, soil biology population dynamics are the unifying factor influencing each.

Currently there is no general consensus regarding assessment of soil quality (Gil-Sotres *et al.*, 2005), and there is a necessity to standardise methods and sampling strategies (House of Commons, 2016; Swift *et al.*, 2004), especially following application of organic amendments to agricultural land.

For agriculture, applications of organic manures are a key input for maintaining or improving the sustainability of soil (Melero *et al.*, 2008; Zhao *et al.*, 2009; Rani *et al.*, 2014), but over time, they will affect soil organism communities and it is therefore essential to monitor these effects (Nannipieri *et al.*, 2003). As the most economical way to dispose of biosolids, land application is viewed to be the most effective strategy (Singh and Agrawal, 2008). This practice has been shown to provide essential nutrients and enhance soil physical and biological properties (Coors *et al.*, 2016). Containing six times the amount of organic matter (OM) found in slurry (Kabirinejad and Hoodaji, 2012), granulated biosolids offer the possibility to add beneficial OM to soil, without the large volumes of water associated with slurries.

5.1.1 Methods of assessing soil biological health

Earthworms have been cited as the single most important group of soil organisms in moist temperate conditions regarding their effects on soil structure (Coleman *et al.*, 2004). As such, they are often used as bio-indicators of soil quality (Crittenden *et al.*, 2014; Bartz *et al.*, 2013; Birkas *et al.*, 2004). They are intimately linked with pedogenesis (Edwards and Bohlen, 1996), improving and maintaining fertility and aggregate stability (Marinissen, 1994). Earthworms predominantly feed on OM and soil fungi (The Earthworm Society of Britain, no date). With biosolids containing OM, they have the potential to support population growth. Kizilkaya and Hepsen (2004) found that biosolid applications increased the nutrient availability of soil following through monitoring earthworm casts. Casts represent what has been digested by the earthworms. During this process of digestion, earthworms break down organic matter, increasing the mobility of elements. Whilst this can be beneficial, Kisilkaya and Hepsen (2004) found that with increasing biosolid application rates, levels of dehydrogenase decreased. Dehydrogenase is an intracellular enzyme, linked to the decomposition of OM. This enzyme's activity can be impacted upon due to heavy metal contamination and therefore it was proposed that this was the reason for the recorded decrease.

Populations of earthworms have also been found to decrease following biosolid applications. Waterhouse *et al.* (2014), found populations of earthworms decreased 100% when biosolids had been applied. This experiment, along with Kizilkaya and Hepsen (2004) were both carried out in either a laboratory or glasshouse environment, which may have introduced an element of stress, causing changes to population dynamics which may not have been seen in a field.

Culliney (2013) emphasised that the use of earthworms as biological indicators of soil health is often over used. Whilst they are easy to collect without specialist equipment, they cannot be fully representative of all soil biota as they represent only one member of the Oligochaeta class, whilst globally, it is estimated that soils contain 25% of all living species (European Commission, 2010).

As an alternative, arthropods are thought to represent approximately 85% of soil biota. More specifically, the microarthropod group, including Collembola (springtails) and Acari (mites) undertake a range of roles in soil food webs, providing an invaluable link for energy transfer between microflora and fauna to macrofauna (Coleman *et al.*, 2004).

Soil dwelling Acari are a large subclass of Arachnida. Oribatids are the characteristic mites of the soil and of the 9000 species documented, most inhabit the soil (Culliney, 2013). Acari have been found in soils ranging in pH and nutrient status, in addition to being found at depths up to 10 m (Kethley, 1990). Species range in feeding types, from dead plant material to nematodes and fungi. Oribatids are the most populous group numerically and

are therefore the most documented. They can loosely be grouped into 4 categories based upon their feeding modes;

1. Macrophytophages – feeding on plant material and some fungi
2. Microphytophages – feeding on fungi, bacteria and microflora
3. Panphytophages – feeding on fungi, bacteria, microflora and plant materials
4. Coprophagous – feeding on faecal material in addition to fungi, bacteria, microflora and plant material

Due to this range of feeding styles, Acari are thought to be essential for decomposition and nutrient cycling within the soil (Moore *et al.*, 1988).

Minor and Norton (2004) reported that biosolids had a beneficial effect on Mesostigmatid mite populations, one year after lime stabilised biosolids were applied. Yet Oribatid populations were found to decrease under the same treatment in a willow planting. This experiment used high application rates (1400 kg ha^{-1} total nitrogen), which would not be permitted under UK regulations.

Collembola are another large group of important microarthropods. Currently, more than 6500 species have been identified (Verma and Paliwal, 2010). Similarly to oribatids, they have a relatively slow reproductive rate of 1-4 generations per annum and prefer a moist soil environment. Collembola as a group can be divided by their feeding methods, masticators and fluid feeders. Whilst they can feed on a greater range of foods than Acari, the majority are fungivorous. Applications of biosolids and the population dynamic of Collembola have detrimental impact on reproduction. Population numbers were unaffected in adults, but statistically lower at 2 Mg ha^{-1} application rates. Yet, this experiment was similar to those where earthworms were the target organism, whereby it was carried out in a laboratory environment.

Microbial activity in soils can be measured using a range of methods, each having its own limitations. Fliessbach *et al.* (1994) studied the effect of heavy metal contaminated sewage sludge application on the soil microbial biomass carbon. They showed that following 10 annual applications a low metal contaminated sludge increased the soil microbial biomass carbon. Whilst the same effect was seen for a high metal contaminated sludge, this was less pronounced. It was also found that high metal contaminated sewage sludge increased respiration substantially over that of low metal contaminated sewage sludge.

The collection of microarthropods from soil poses many challenges and it is generally agreed that no single method exists that is suited to all microarthropods (Akoijam *et al.*, 2013; Kuenen *et al.*, 2009; Robertson *et al*, 1998). Broadly, methods are classified as mechanical or dynamic. Mechanical include those which physically separate microarthropods from the soil through physical force, normally by flotation. In contrast, dynamic methods expel microarthropods from the soil using an external heat and/or light

source. Method selection is normally determined by the general behaviour of the microarthropods (Robertson *et al*, 1998). Whilst the selection of the appropriate collection method is critical to ensure samples are representable to the field, the method of identification is also important. Parisi *et al.*, (2001) developed a method for assessing microarthropods, namely the Qualita Biologica de Suolo (QBS), a biological soil quality assessment using an eco-morphological index (EMI). The index allows for simple evaluation of microarthropod and arthropod communities by assigning scores to the organisms edaphic adaptations. The method does not require identification to species level. Whilst other methods for evaluating soil quality based on microarthropods exist, they are either very simple, using a general classification system, or involve taxonomic identification to species level (Bernini *et al*, 1995). Whilst taxonomic identification is the most accurate, few people possess the level of training required for accurate identification and therefore, it is not a tool available for use for most research. The QBS uses simple taxonomic identification, based on the theory that as soil quality improves, so does the level of eu-edaphic adaptation. The method evaluates microarthropods for their morphological eu-edaphic features. Such features include, reduced or absent eyes, loss of pigmentation and loss of appendages. The system works on the provision of and Eco-Morphological Index (EMI) score being given to microarthropods extracted from the soil. Eu-edaphic receive the highest score of 20, with epi-edaphic microarthropods receiving a score of 1. Hemi-edaphic receive a score depending on the degree of morphological adaptation. Morphological groups may be assigned a single value or values may range depending on the variation between species within the group. Where two EMI scores can be applied to one group, e.g. collembola, only the higher score is counted as part of the QBS. The EMI score is only applied to the sample once, irrespective of the number of microarthropods present in the sample. The QBS is the sum total of the EMI scores.

5.1.2 Risks to soil biology

The Woburn Market experiment is a well-documented example of how organic manure applications can result in decreased microbial activity. The experiment was established to compare the ability of different fertilisers to improve a sandy loam soil. Following 19 years of annual inorganic fertiliser, sewage sludge and Farm Yard Manure (FYM) applications, it was noted that the clover plants receiving sewage sludge appeared yellow and stunted. Following a series of analyses which were not detailed, it was determined that soil cadmium (Cd) and zinc (Zn) levels were affecting the rhizobia, reducing N_2 fixation (Giller *et al.*, 2009). Previously, McGrath (1987) in McGrath *et al.*, (1988) indicated that Cd and lead (Pb) were too insoluble to directly impact upon yield. A further experiment using soil from the original experimental site, showed that the visible symptoms (decreased yield and yellow plants) were due to a lack of nitrogen fixation (McGrath *et al*

1988). Further studies by Chaudri *et al.*, 2000; Chaudri *et al.*, 1992 and Chaudri *et al.*, 1992, also support this theory through demonstrating effects of low level Cd (<3 mg kg^{-1}) contamination in soils. This experimental site has also been used to demonstrate the long-term effects of heavy metal contamination on soil biota, with adenosine triphosphate (ATP) levels in sewage sludge plots being recorded at approximately half that of the other plots in the experiment. Inversely, the sewage sludge treated plots recorded a high respiration rate, indicating that biota within the soil were suffering from stress.

Mossa *et al.*, (2017) demonstrated that high application rates of biosolids (1000 – 2000 mg kg^{-1} Zn) caused stress to soil microbial diversity through the accumulation of Zn concentrations, yet at lower concentrations (700-1000 mg kg^{-1} Zn) microbial and fungal populations were at their most diverse. Whilst this study shows that biosolids have the potential to both increase and decrease the microbial and fungal populations in soil, the application rates were far in excess of those currently permitted under UK legislation. Charlton *et al.*, (2016) revealed that microbial carbon was reduced by 7-12% due to elevated Zn and copper (Cu) concentrations, following eight years of sewage sludge application.

Parisi *et al.* (2005) studied the effect of sewage sludge applications on microarthropod QBS scores. Two out of three sites showed higher QBS scores than untreated soils, however details regarding how many years of applications, what rates of applications and the exact QBS score were unclear. No increase in the concentrations of Cu, Zn, Pb and Hg were recorded in the soil, yet Cd was recorded to have bio-accumulated in earthworm tissues.

Recently Austruy *et al.* (2016) recorded microarthropods in soils with 30,000 mg kg^{-1} Pb, they also recorded an increase in abundance, but a decrease in the diversity of the microarthropods recorded where carbon/nitrogen (C/N) ratio increase. Migliorini *et al.* (2004) studied the effect of shooting grounds on microarthropod numbers. Pellets from shotgun cartridges contain a range of heavy metals (Cu, Zn, Pb etc), and their use can result in an increase in the heavy metal concentration found in the soil. Migliorini *et al.* (2004) found that such increases did not universally decrease microarthropod populations and that Collembola, among other species, increased in number. Symphylan however decreased. Whilst this study shows that microarthropod populations may not be limited by heavy metals, it is a very different environment with less soil disturbance than is experienced under agricultural systems.

Whilst many studies look at the direct effect of heavy metals on microarthropod populations, Caruso *et al.* (2009) suggest that heavy metals may affect other soil organisms (e.g. fungi) which results in the heavy metals indirectly effecting Acari populations. However, each of these studies show exceptional situations where one or more heavy metals are found at particularly high concentrations. Jerome *et al.* (2013) noted that there is a need for further experimental work to investigate the direct effect of biosolid applications on soil

microarthropod populations. Whilst the study looks at "long term applications", a range of biosolid types are applied to plots 4 years out of 10. Results indicated that the application had no beneficial or detrimental effect on soil microarthropods.

Soil biology is key to ensuring sustainable, productive soils. The application of biosolids to soil has been predominantly been completed in a lab/glasshouse environment. Whilst this allows a controlled study and no loss of organisms to the area surrounding experimental site, it may also have introduced stress, impacting on the results. The effect of thermally dried biosolid applications has not been studied in a field environment. Due to the potential chemical changes to the biosolids, their impacts, or benefits, may not be the same as sludge liquids or cakes and therefore should be studied to understand their impact.

Chapter aim: Analyse the response of soil biology to biosolids applications. Samples were taken from the Broxton field site and fresh applications were made to a field site at Harper Adams University to assess both the short and long-term impacts of biosolid applications.

Chapter Objectives;
- Assess microarthropod community response to both long and short-term applications of biosolids
- Assess the response of soil respiration to different application rates of biosolids over a 12-month period.
- Assess how earthworm numbers respond to biosolid applications.

Null Hypothesis: The application of biosolids has no impact on the soil biology, irrespective of the number of applications or rates applied.

N.B. the term microarthropods used in the following experiments also refers to arthropods.

5.2 Methodology

5.2.1 Soil Respiration experiment

A 12-month experiment was established at Fourgates field, Harper Adams University (SJ 70878 19482) in November 2016. A randomised block design consisting of twenty plots, (5 treatments and 4 replicates) measuring 2 m x 2 m, were marked out and surrounded by plywood boarding (Figure 5.1), Boarding was used as a walkway to reduce the risk of treatments transferring between plots. Applications were made by hand to each plot and immediately raked into the top 2 cm of soil to aid incorporation.

Applications were made in accordance with the maximum permitted rates under the Biosolids Nutrient Management Matrix (250 kg ha^{-1} N) (ADAS, 2014) (Table 5.1). Applications greater than these recommendations were made to assess what would happen if inaccurate spreading were to occur.

A respirometer (LCpro+, ADC Bioscientific Ltd, UK) was used to measure Net Carbon Dioxide Exchange Rate (NCER) and soil temperature (°C) each month (Figure 5.2). Metallic collars (16 cm (L), 10 cm (W), 10 cm (H)) were inserted into the soil to a depth of 5 cm, 30 minutes prior to each plot being assessed. This ensured any carbon dioxide losses resulting from the soil disturbance had dissipated. Collars were positioned to avoid any vegetation which may also affect the result. NCER readings were converted using the equation presented in Figure 5.3. Following each assessment, plots were weeded by hand to limit vegetation growth for the following months (Figure 5.4.). Herbicides were not applied to the site as their application interferes with enzyme and microbial activity within the soil (Singh and Ghosal, 2013, Soil Association, no date).

5.2.2 ATP

Three soil cores (10 cm) were taken from each plot of the respiration experiment and bulked for ATP analysis (Section 5.3). From each bulked sample, 1 g was mixed with deionised water (10 ml) in a polypropylene tube (13 ml, Sarstedt, UK) before being shaken manually and vortexed. A Clean Trace Water Plus ATP swab (3M, UK) was then inserted and mixed (5 seconds) within the top 1 cm of the soil solution. The swab was then tapped twice and inserted back into the receptacle. This was then shaken (5 seconds) before being analysed in a V3.0R luminescence meter (Biotrace Ltd., UK). The luminescence is caused by a reaction between luciferine and luciferase in the presence of ATP. The resulting value can then be converted to ng ATP g^{-1} soil. This value represents microbial activity (Dickinson *et al.*, 2006).

5.2.3 Microarthropod assessments

During the 11th month of the respiration experiment, Tullgren samples were taken from the 0% and 400% application rate plots to assess differences in microarthropod numbers and QBS scores. Samples were not taken at the end of the experiment due to fears over declining weather conditions. Metal collars with a 5 mm wire mesh on the upper edge were knocked into the ground, until flush with the surface. Each collar was then extracted using a spade, with any excess soil outside the collar being removed. The collars were placed above funnels, beneath 60 watt bulbs for a period of 7 days. As the soil dried, the microarthropods migrated down the soil column, away from the heat and into a 30ml centrifuge tube containing 70% industrial methylated spirits (IMS). Samples were collected after seven days. These tubes were then replaced after seven days to assess if this was

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sufficient time to capture the microarthropods contained. Following another 7 days samples were placed into petri-dishes and observed under a binocular microscope (MH4 Microscope; TEC Microscopes LTD, UK), with each QBS group being counted and scored according to Parisi *et al.*, (2005).

A further Tullgren experiment was established at Broxton, Cheshire in the field plots (Figure 2.3) to assess the effect of multiple applications of biosolids and conventional fertilisers on the microarthropod populations. Due to the original experimental design of the field, further samples were collected from the surrounds of the experimental area as an untreated control.

Block 4	0%	200%	400%	100%	50%
Block 3	100%	50%	200%	0%	400%
Block 2	200%	100%	400%	0%	50%
Block 1	400%	200%	0%	50%	100%

Figure 5.1. Respiration experiment design.

Table 5.1. Weight of biosolids (in kilograms per hectare of total nitrogen) for each treatment. All applications were made in December 2016, with first assessment being taken a week after the applications were made.

Application rate	Months Biosolid application rate (kg ha^{-1} total N)
0%	0
50%	125
100%	250
200%	500
400%	1000

Figure 5.2. ADC respirometer with metal collars in situ. Wooden boards were used to prevent compaction. The collar was positioned to avoid any visible weeds which may respire, impacting on the result.

NCER (μmol m2 sec^{-1}) x 0.01201(μmol weight of carbon in grams) = NCER (g C m2 sec^{-1})
NCER (g C m2 sec^{-1}) x 60 = g C m2 min^{-1}
NCER (g C m2 min^{-1}) x 60 = g C m2 hr^{-1}
NCER (g C m2 hr^{-1}) x 24 = g C m2 day^{-1}
NCER (g C m2 day^{-1}) x 365 = g C m2 yr^{-1}
NCER (g C m2 yr^{-1})/1000 = kg C m2 yr^{-1}

Figure 5.3 The NCER conversion calculations used for the respirometer.

Figure 5.4. The respiration plots, pre and post-weeding.

5.2.4 Earthworm assessments

Earthworms were quantified at the experimental site at Broxton, Cheshire in 2012 and 2013 following crop harvest. These counts were taken in the same plots (conventionally treated and biosolids treated plots) as the yield and Tullgren experiments (Figure 2.3). An additional data set was collected from a grass silage experiment in the same field (Figure 2.4).

Following the crop harvest each year a 1 m x 1 m wooden quadrate was placed in the centre of each plot (grass plots *n=6*; arable plots, *n=12*). A 10 ltr solution containing 90 g of mustard powder (Coleman's, Unilever, UK) was then poured evenly over the area outlined by the quadrat. Earthworms that surfaced inside the quadrat were then collected and stored in 70% IMS for counting and weighing. After 10 minutes, another solution of mustard powder (90 g in 10 ltr water) was applied to the soil. Following the 20 minutes

sampling period, a 20 cm (W) x 40 cm (L) x 20 cm (H) core was dug using a spade and placed on a plastic sheet. This core was then manually sorted through to collect any remaining earthworms (Cunningham, 2003).

5.2.5 Statistical analysis

All analyses were completed using Genstat (VSN International, 18th edition). Respiration data was analysed using a linear mixed model fit by restricted maximum likelihood (REML) to account for the interaction between month and temperature. Data was transformed using a fourth root transformation due to a left skew.

Tullgren counts were assessed using generalised linear model (GLM analysis), with ATP and earthworms being analysed using analysis of variance (ANOVA).

Due to the variability of soils and biosolids data producing a p value less than 0.05 was deemed to be statistically significant, whilst data with p values between 0.05 and 0.1 was deemed to be of interest.

5.3 Results

5.3.1 Respiration experiment

An ANOVA analysis comparing months, showed that overall, November had significantly lower NCER values compared to all other months, with May having significantly higher NCER compared to all other months ($p < 0.001$) (Figure 5.5). NCER showed a difference overall between the 0% and 100% application rates ($p = 0.032$) and 0 and 200% application rate ($p < 0.001$), when values were averaged over the 12 months of the experiment (Figure 5.6). This analysis accounted for month and temperature as covariates.
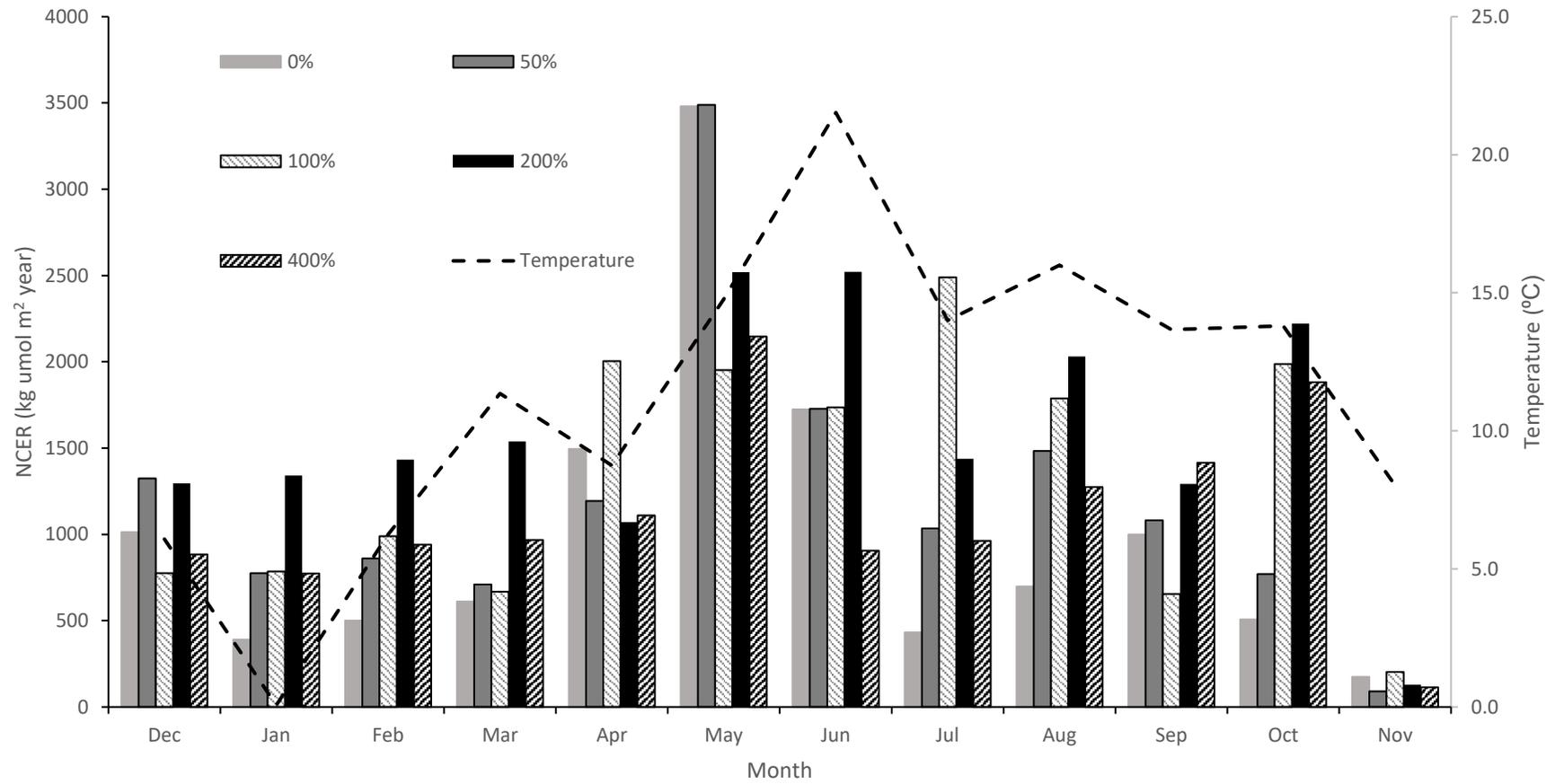
Figure 5.5. Mean NCER and temperature recorded over a twelve-month period ($n = 4$, ± S.E.)
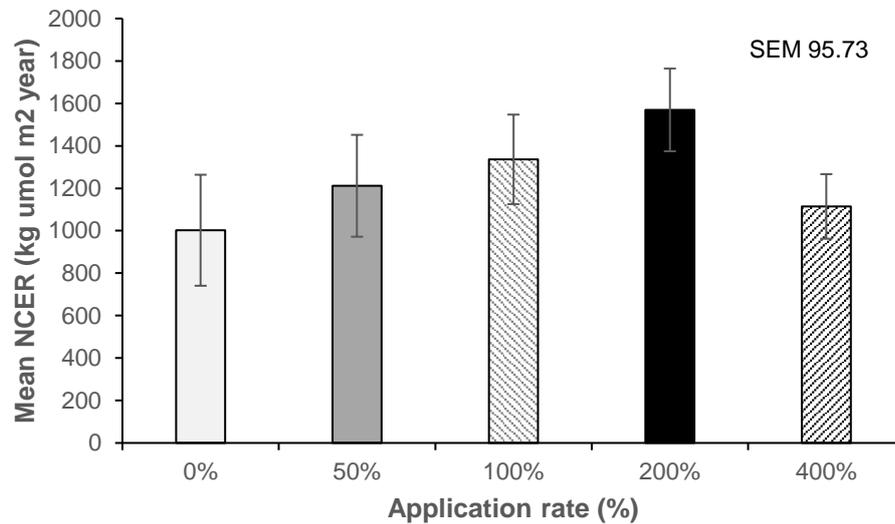
Figure 5.6. Mean values of the NCER readings taken across 12 months under each treatment (±S.E.). Residual d.f. 23

5.3.2 ATP

The data showed a significant difference for the variables month and temperature, as would be expected. ATP showed no difference ($p < 0.323$) across any treatment or any month, indicating no change in microbial biomass (Figure 5.7). When compared with NCER, for each unit increase in ATP, a 0.2 decrease was seen in NCER. This was attributed to the large variability of the ATP data collected (cv% 114.3).

November ATP data was higher than other months, however, the error bars in Figure 5.7 indicate a large amount of variation.
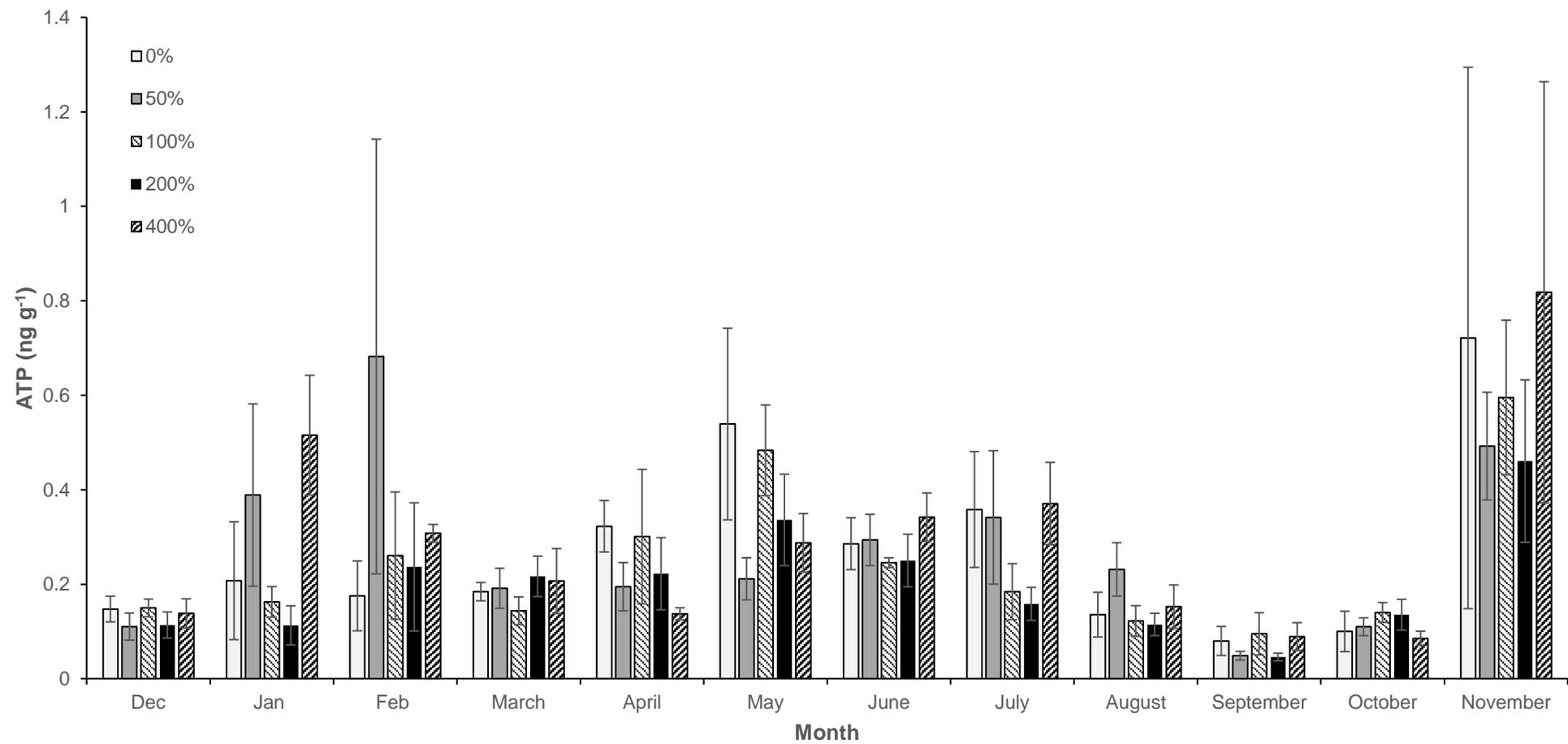
Figure 5.7. Mean ATP concentrations for each treatment over 12 months ($n=5$). Error bars represent ± SE.

A mean of the total year ATP data under each treatment was calculated (Figure 5.8) to simplify and clarify the data shown in Figure 5.5.
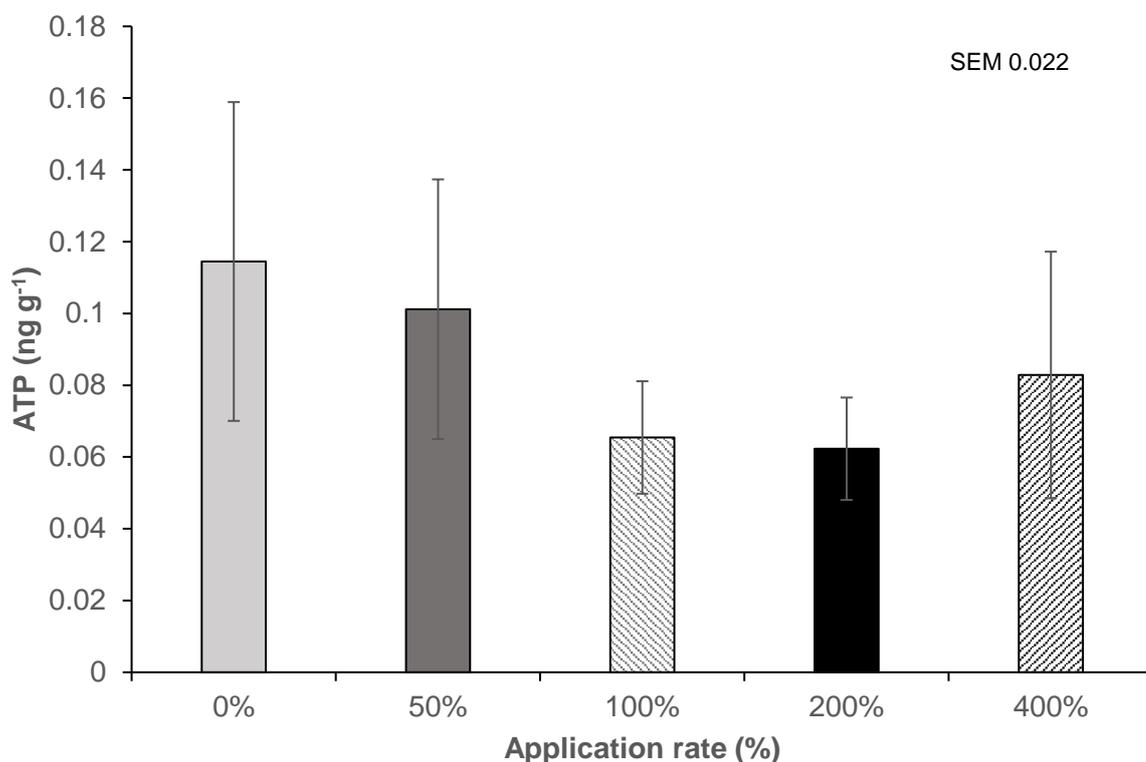
Figure 5.8. Mean ATP for each application rate over 12 months. Error bars signify ± SE. Residual d.f. 237.

5.3.3 Microarthropod Counts at Harper Adams University Farm

Six out of the 12 microarthropod groups collected after seven days showed significant differences, with all significant groups, except the Acari and Diplopodia, recording a higher count in the 400% application group compared to the 0% application group (Table 5.2a). This may be attributed to the additional carbon applied through the higher biosolids applications. Acari, may have been a different feeding type and therefore not utilising the additional carbon found under the higher application rate. Following another seven days (Table 5.2b), Acari, Collembola (8) Diptera larvae and Arachnida were the only microarthropods to be found in the samples. Of these, Acari and Collembola showed significant difference ($p < 0.001$), with the 0% application rate being the highest. Yet, it should be noted that these significances can be attributed to a large number of Acari and Collembola (8) being found in on sample alone (57 of each group were found in one sample). This therefore, may not be representative of the true result.

Overall, when both data sets are combined, the data indicates that seven of the 12 groups showed significant differences overall. Again, all groups showed a significantly higher count for the 400% application rate, with the exception of the diplopodia and the Acari (Figure 5.2c).

The QBS scores for the microarthropods collected at seven and 14 days showed no significant differences, nor was there a significant difference when the two data sets were combined (Table 5.3).

Following the statistical analysis of the 0 and 400% application plots, it was decided to assess the 100% application rate to see if any further information could be gained from the experiment (Table 5.4).

Table 5.2. Tullgren microarthropod counts for 0% and 400% application rates at a)7 days post-collection, b) 7-14 days post-collection and c) mean totals.

a)

Application rate	Acari	True insect	Coleoptera	Collembola (1)	Collembola (8)	Collembola (20)	Diptera larvae	Diplopodia	Cullicidae	Paurpoda	Arachnida	Elateroidea
0%	121	0.33	0.58	2.25	12.5	8	0.42	0.5	1.58	1.4	0.58	0.92
400%	104.2	0.58	0.33	4.16	22.5	14.73	0.5	0.17	2.48	5.43	1.65	3
p vale	<0.001	0.3631	0.363	0.008	<0.001	<0.001	0.763	0.148	0.115	<0.001	0.011	<0.001
Residual df	22	22	22	22	22	22	22	22	22	22	22	22

b)

Application rate	Acari	True insect	Coleoptera	Collembola (1)	Collembola (8)	Collembola (20)	Diptera larvae	Diplopodia	Cullicidae	Paurpoda	Arachnida	Elateroidea
0%	6.75	0	0	0	6.17	0.25	0.25	0	0	0	0	N/A
400%	3.71	0	0	0	0	0	0.33	0	0	0	0.08	N/A
p vale	<0.001	N/A	N/A	N/A	<0.001	0.041	0.705	N/A	N/A	N/A	0.239	N/A
Residual df	22	22	22	22	22	22	22	22	22	22	22	22

c)

Application rate	Acari	True insect	Coleoptera	Collembola (1)	Collembola (8)	Collembola (20)	Diptera larvae	Diplopodia	Cullicidae	Paurpoda	Arachnida	Elateroidea
0%	127.75	0.33	0.58	2.25 a	18.67	8.25	0.67	0.5	1.58	1.4	0.58	0.92
400%	107.91	0.58	0.33	4.16 b	22.5	14.73	0.83	0.17	2.48	5.43	1.73	3
p vale	<0.001	0.902	0.443	0.05	0.004	<0.001	0.928	0.06	0.181	<0.001	0.035	0.413
Residual df	22	22	22	22	22	22	22	22	22	22	22	22

*Numbers in brackets denote the QBS score allocated depending on euedaphic features.

Table 5.3. The mean QBS scores for 7 and 14 days post-collection and the overall total

Application rate	7 days	14 days	Total
0%	65.7	12.8	67.0
400%	70.9	8.5	71.0
p vale	0.333	0.403	0.501
cv%	17.5	107.5	19.5
SEM	11.34	10.89	12.77
Residual d.f.	10	10	10

Table 5.4. Mean microarthropod counts for 0, 100 and 400% application rates.

Application rate	Acari	True insect	Coleoptera	Collembola (1)	Collembola (8)	Collembola (20)	Diptera larvae	Diplopodia	Cullicidae	Paurpoda	Arachnida	Elateroidea
0%	127.80c	0.33	0.58a	2.25a	18.67a	8.25b	0.67	0.50	1.58a	1.42a	0.00	0.58a
100%	34.40 a	0.99	1.75b	26.5b	39.60c	5.20a	0.63	0.17	9.91b	1.08a	0.00	0.25a
400%	109.80 b	0.36	0.36 a	3.64a	24.20b	15.90c	0.00	0.09	2.36a	6.01b	0.16	1.45 b
p vale	<0.001	0.064	0.001	<0.001	<0.001	<0.001	0.200	0.125	<0.001	<0.001	0.118	0.003
Residual df	32	32	32	32	32	32	32	32	32	32	32	32

The inclusion of the additional application rate resulted in significant differences for six microarthropod groups. Significantly fewer Acari were recorded in the 100% application plots (p<0.001) when compared to the 0% and 400% application rates. Conversely, significantly more Cullicidae and Elateroidea were recorded in the 100% application rate plots compared with the other two treatments (p<0.001). Significantly great number of Coleoptera were recorded in the 100% application rate compared to the plots receiving the 0 and 400% application rate. Collembola (1) counts were significantly higher in the 100% plots compared to the 0 and 400% plots.

QBS scores showed no significant differences between the treatments, however the results were trending towards significance between 0 and 400% application rates (Table 5.5)

Table 5.5. Mean QBS scored for the three applications rates, 12 months after the first biosolid application.

	Mean
0%	67.1
100%	84.2
400%	70.8
p value	0.065
cv%	24.5
SE	5.24
Residual d.f.	33

5.3.4 Tullgren Counts at Broxton, Cheshire

The number of microarthropod groups recorded at the Broxton site (16) were greater than those at Harper (12). This may be attributed to either the difference in soil type and geographic location.

Significant differences in microarthropod counts were recorded in Acari (p<0.001), Arachnida (p=0.007), Tipulidae larvae (p<0.001), Symphyta (p<0.001) and Elateroidea lavae (<0.001), along with all classifications of Collembola (p<0.001). Acari counts were significantly higher under the biosolids treatment when compared with the untreated and conventional treatments. Symphyta counts were significantly higher in biosolids and untreated Tullgrens, when compared with conventional fertiliser Tullgrens. Tipulidae larvae were significantly higher in biosolids Tullgrens when compared to the conventional treatment, but there was no significant difference between biosolids and untreated or conventional and untreated. Arachnida were also significantly higher in the biosolids treated Tullgrens compared to the untreated control but were not significantly different to conventional fertilisers (Table 5.6.). Treatments effected the different groups of Collembola differently, with Collembola (1) having the highest counts under biosolid treated and

untreated soils, Collembola (8) highest under biosolid treated soils and Collembola (20) highest under untreated soils. Elateroidea populations were highest in the biosolids treated soils, with untreated soils having the lowest population counts statistically ($p < 0.001$).

QBS scores allocated for the eu-edaphic features of each microarthropod group also showed a significant difference ($p = 0.003$), with microarthropods collected from biosolid treated Tullgrens scoring significantly higher than both the control and the conventional fertiliser Tullgrens (Table 5.7).

Table 5.6. The Tullgren counts for different microarthropod groups under different fertilisers at Broxton, Cheshire.

Fertiliser	Formicidae	True Insect	Arachnida	Coleoptera larvae dark	Coleoptera larvae light	Collembola (1)	Collembola (8)	Collembola (20)	Diplopodia	Diptera	Tipulidae larvae	Coleoptera mature	Acari	Symphyta	Elateroidea larvae
Biosolids	0.00	0.41	0.65 b	0.94	0.76	16.47 b	3.70 b	32.12 b	0.06	1.765	2.88 b	0.53	86.47 c	13.65 b	6.12 c
Conventional	0.06	0.06	0.05 a	0.08	1.06	11.88 a	1.47a	20.59 a	0.06	0.878	1.06 a	0.29	31.70 b	1.94 a	2.65 b
Untreated	0.00	0.12	0.30 a	1.00	1.18	18.28 b	2.06 a	57.37 c	0.00	1.050	1.29 a	0.24	26.17 a	13.00 b	1.24 a
p value	0.333	0.05	0.007	0.86	0.453	<0.001	<0.001	<0.001	0.445	0.056	<0.001	0.328	<0.001a	<0.001	<0.001
residual d.f.	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48

Table 5.7. The QBS scores for the Tullgren counts from the Broxton, Cheshire field site.

Fertiliser type	QBS
Biosolids	79.9 b
Conventional	69.3 a
Untreated	70.4 a
p value	0.003
CV%	12.5
SEM	2.21
residual df	32

To further explain the differences in the microarthropod counts, soil carbon was measured for each treatment (Figure 5.9). Results indicated that no significant differences were recorded between treatments (p=0.066) however, they were close to significance between plots receiving the 0% application rate and the 400% application rate, with untreated soil containing 1.7% carbon, conventionally treated soil containing 1.76% carbon and biosolids treated soil containing 1.87% carbon. The precision for this equipment is 0.00025% and thus should be capable of identifying small differences in C between the treatments.
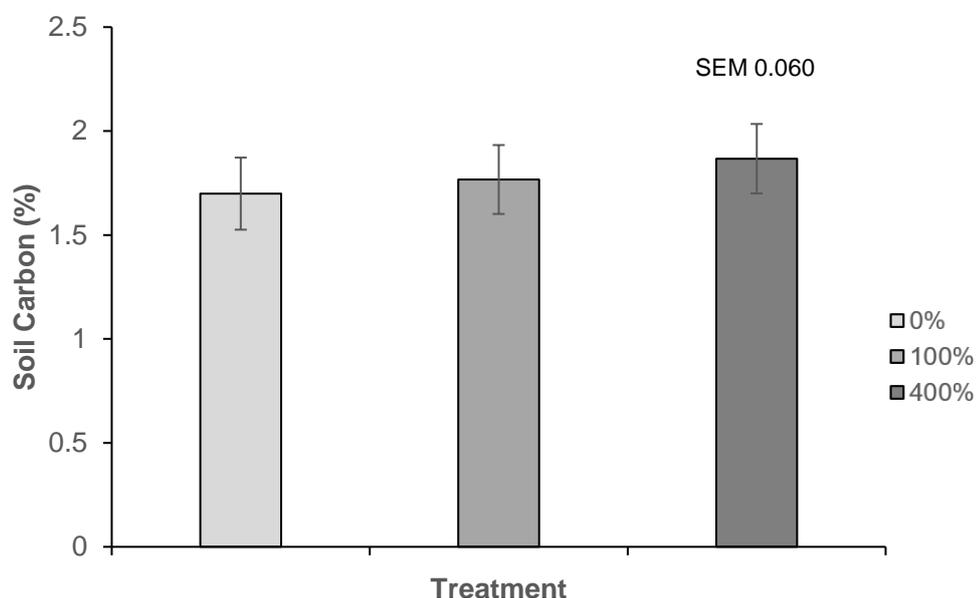
Figure 5.9. Mean soil carbon under 0%, 100% and 400% application rates, 12 months after the first biosolid application was made. Residual d.f. 15.

5.3.5 Earthworm Counts, Broxton Cheshire

2012

No significant difference was recorded between the number (p=0.389) or weight (p=0.402) of earthworms collected under biosolids or conventionally treated soil in the arable rotation (Figure 5.10). Similarly, no significance was recorded between treatments for either the number (p=0.860) or weight (p=0.508) in the glass land (Figure 5.11
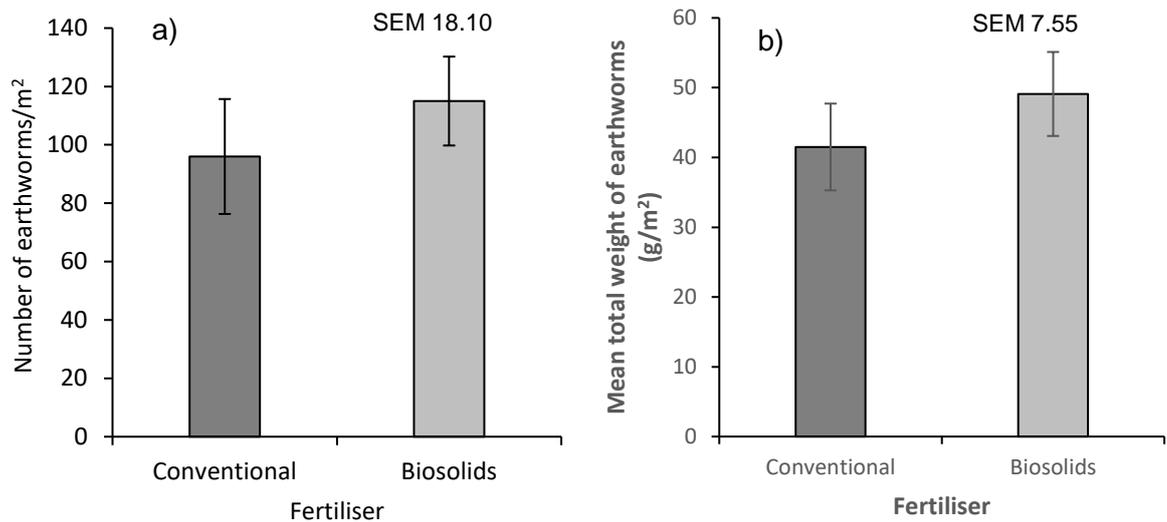
Figure 5.10 Mean number (a) and total weight (b) of earthworms collected in 2012 from the arable plots. Error bars signify ± S.E. Residual d.f. 11.
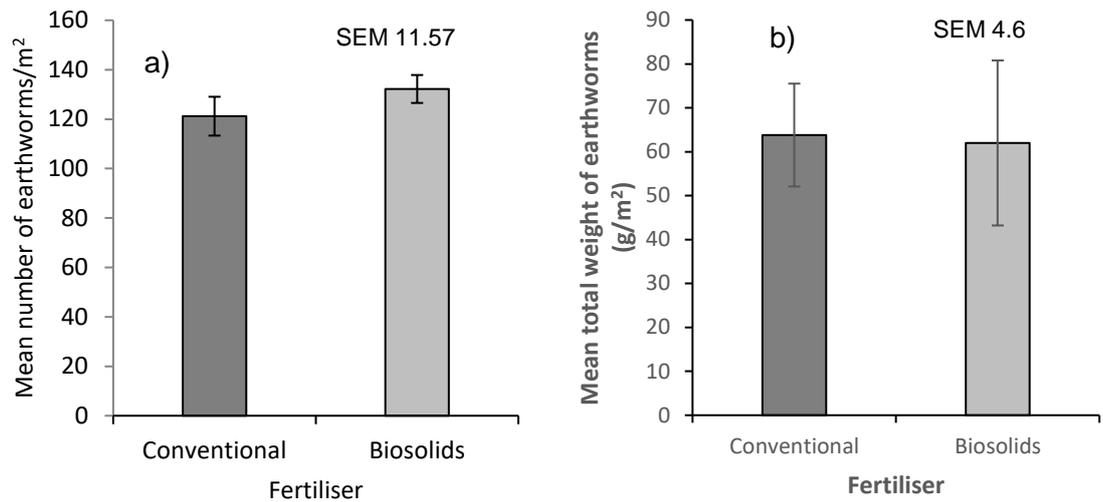
Figure 5.11. Mean number (a) and total weight (b) of earthworms collected in 2012 from the grassland plots. Error bars signify ± S.E. Residual d.f. 5.

2013

Whilst the mean number of earthworms collect in 2013 was substantially higher, there was no significant difference between treatments, however this was close to significance (p=0.072). There was also no significant difference between the weight of the earthworms collected (p=0.120) (Figure 5.12). No significant differences were also seen between treatments for the grassland plots for either number (p=0.676) or weight (p=0.388) (Figure 5.13).
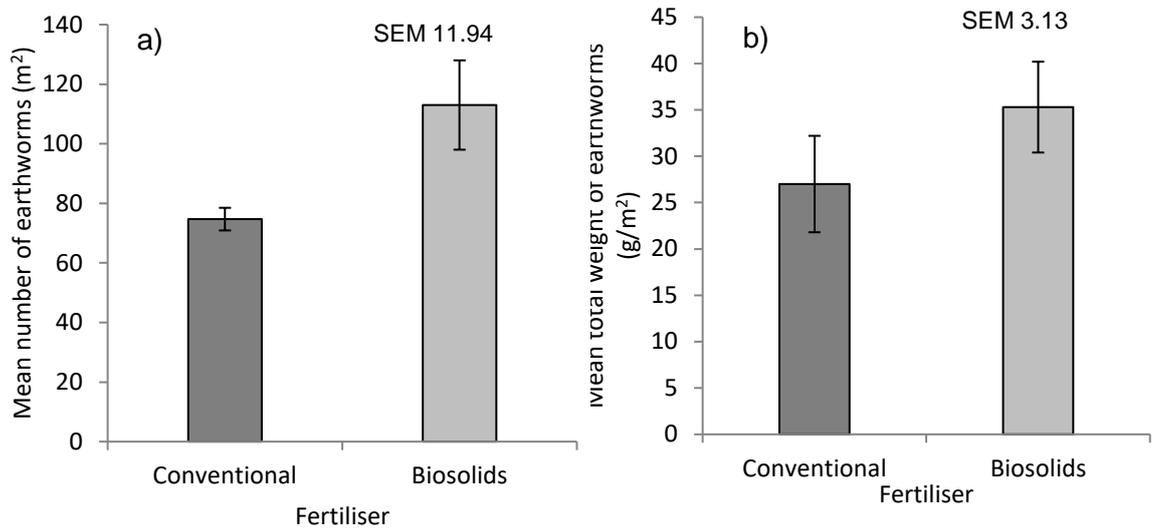
Figure 5.12. Mean number (a) and total weight (b) of earthworms collected in 2013 from the arable plots. Error bars signify ± S.E. Residual d.f. 5.
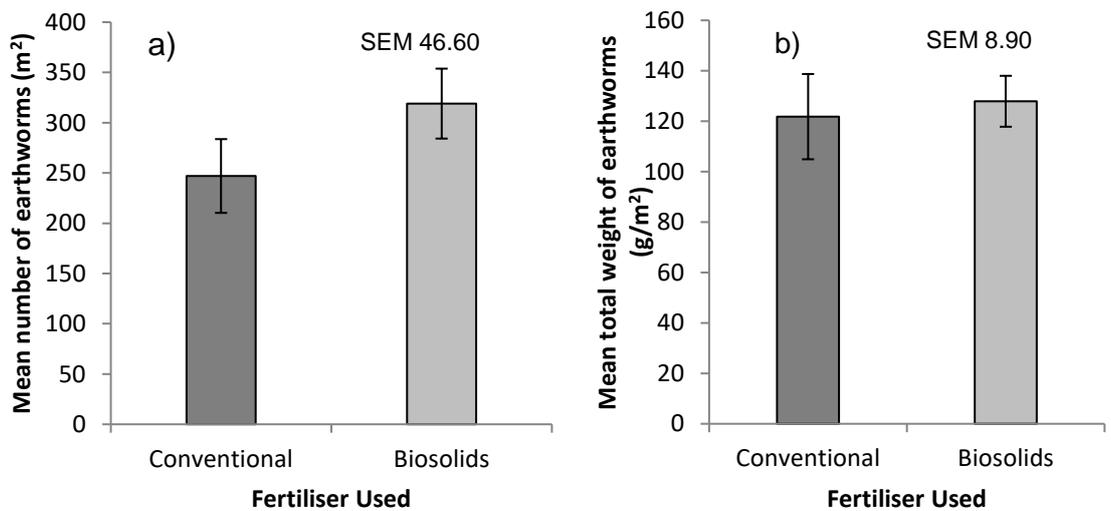
Figure 5.13 Mean number (a) and total weight (b) of earthworms collected in 2013 from the grassland plots. Error bars signify ± S.E. Residual d.f. 5.

Due to the lack of significance in the two datasets, an F test was used to see if the data could be combined to make the analysis statistically stronger. Each F test produced a p value > 0.001 indicating that the two years differed too significantly to combine data sets.

5.4 Discussion.

Soil microorganisms are fundamental for the maintenance of a healthy, productive, sustainable soil. They assist with the recycling of nutrients and the breakdown of SOM to SOC, improving soil drainage and water holding capacity, whilst reducing erosion. However modern practices such as cultivations and the application of pesticides have seen a decrease in microbial activity (Lo, 2010). Often organic manures, such as biosolids, are cited as beneficial to the soil as they help replenish SOM, feeding these organisms (Zhen *et al*, 2014; Fageria, 2012). However, biosolids contain heavy metals which may detrimentally impact on such microorganisms. The work presented in this chapter looked to explain the impact of both long and short-term biosolids applications on soil biology using a range of assessment methods.

Soil respiration data indicated increased respiration from plots receiving 100% and 200% application rates when compared to the untreated plots. This would suggest that when biosolids are applied at sufficient quantities, microbial respiration increases, however, over applications (400% application rate) decreases respiration back to the a level seen when no application is made. A similar response to higher biosolid application rates was seen by Artuso *et al*. (2011), whereby, application rate at 2 Mg ha^{-1} increased Collembola populations but applications at 5 Mg ha^{-1} and above decreased populations. This was not attributed to heavy metal accumulation in the soil, but instead, ammonia production, resulting in a lack of oxygen. The biosolids used in this work contain low concentrations of available nitrogen and therefore the amount of ammonia released is unlikely to have caused such a response. It is however, unclear as to whether the increase at 100% and 200% application rates is due to microbial stress or an increase in the microbial populations present. Tan *et al*. (2011), suggests that soil carbon dioxide (CO_2) levels peak in May and decrease to their lowest in October. Whilst the data set represented here indicate that the highest levels of CO_2 were obtained in May, the lowest were reached in November. During the collection of the NECR data in November, the ground was found to be very wet which may explain the low CO_2 recordings. Weather details for the year can be found in Appendix 1.Stres *et al*. (2008) suggest that microbial activity may decrease in wet environments due to low oxygen supplies, which would concur with results detailed by Artuso *et al*. (2011) but would have occurred via a different mechanism. They also suggest that changes in microbial community structure may result from environments experiencing extreme soil moisture contents.

ATP analysis of the soil showed no significant differences between the treatments or months, indicating that the application rate of biosolids did not affect the level of ATP found in the soil. It should, however be noted that this data was very variable (cv 114.3%) indicating that the methodology and/or technique may not have been robust enough. A study by Whitley *et al*. (2015) investigated the variability and precision of such swabs across

a range of manufacturers. It was concluded that the precision of this technique was too poor to be valuable, yet Dickinson *et al.* (2006) used the same technique and did not question their reliability. An alternative method for assessing soil ATP analysis is one outlined by Jenkinson and Oades (1979). This involved the ultra-sonification of soil with trichloroacetic acid (TCA) amongst other reagents. However, in order to prevent TCA from binding ATP strongly, preventing its accurate extraction, bipyridinium dichloride (also known as Paraquat) must be added. As Paraquat is now a banned pesticide in the UK this methodology is also not without problems. Jenkinson and Oades (1979) methodology can be completed without paraquat, however its extraction if less efficient resulting in data only comparable to itself.

Tullgrens at the Harper Adams University site saw a significant difference in the counts of six groups identified using the QBS score categories to compare the 0 and 400% application rates. Whilst an additional count was carried out on the 100% application rate plots, this occured three weeks later and therefore these values may not be comparable to the 0 and 400% application rate due to changes in soil conditions. Both Acari and diplopodia showed decreased counts under the 400% application rate, with six groups, (Collembola (1, 8 and 20), Paurpoda, Arachnida and Elateroidea) showing increased counts compared to the 0% application rate. Whilst the QBS method of identifying microarthropods is suitable for scientists to classify microarthropods into categories without having a detailed knowledge of species identification, it makes interpretation difficult. Often identification to species level is required to determine their role ecologically and thus importance to soil habitats in detail.

Data collected here may suggest that Acari are not being supported by the addition of biosolids, indicating that they may not be coprophilous Acari and instead may rely on fungi and plant material as a food source. Further research would look to identify what species the Acari are and thus why there was a significant decrease in their numbers. Diplopodia also showed a significant decrease in their number, yet it should be noted that only seven were recorded across the 0 and 400% treatments, therefore, there were few data points supporting this significance. An increase in the number of Collembola were seen in the 400% application rate plots when compared to the control. This would indicate that biosolids were helping to support their survival. Rusek *et al.* (1998) suggests that the larger euedaphic Collembola can only occupy the surface horizons whilst the smaller euedaphic Collembola can occupy both the surface and deep horizons. Their feeding is considered to be highly specialised and thus control much of their population dynamic (Parkinso, 1983). When comparing the 0 and 400% application rates, data would suggest that the Collembola were actively feeding on the biosolids (or a by-product of) at a range of levels, indicating that this supporting food may have been reaching beyond the soil's surface horizon. Whilst Collembola are an important microarthropod with regards to the breakdown of SOM to SOC,

they also serve as a food source for larger organisms such as Arachnida. This may explain the increase in Arachnida in the 400% plots.

Low counts recorded from the second week of the Tullgren samples indicates that the additional time did not impact on the QBS score for each treatment, and therefore it would be recommended that the Tullgren samples be left under lights for one week rather than two. It is believed that although there may have been other microarthropods within the samples, that the soil become very dry and hard before these organisms could move down and out of the soil column, entrapping them inside. When the soil was disposed of, it was noted that the samples split into planes. The number of microarthropods in the sample at the end of the two weeks was not quantified, however, this may be useful to quantify in future to add further clarity as to which methodology is best.

Overall, QBS scores did not show a significant increase between 0% and 400% treatments at the Harper Adams University site, however when the 100% application rate treatment was added this was close to significance. This data should be treated with caution and instead should be repeated, as detailed above.

The Broxton site noticeably supported different microarthropods than the 12 Harper Adams site (16 in total). This may be attributed to either the length of the biosolid applications or most probably the soil type and geographic location. Six of the microarthropod groups showed significant differences. In contrast to the Harper Adams University site, Acari showed a significant increase in number under the biosolids treatment when compare to the untreated and conventional treatments. Again, in contrast to the Harper Adams University site, each Collembola group showed no significant difference between treatments. Without identifying the species in each sample, further specific explanation as to why these differences occurred is not possible as the differences in the counts between the two sites may be attributable to reproductive strategies, their respective lengths or their species. Arachnida, Tipulidae larvae and Elateroidea all had significantly higher counts under the biosolids treatments compared with the untreated. Whilst Collembola are a beneficial microarthropod within the soil, Elateroidea and Tipulidae larvae are a pest in agricultural systems. Elateroidea only feed on plant roots but Tipulidae larvae may feed on organic matter, of which the biosolids applied here contained 61.4% and therefore may be acting as a food source to aid their survival. Such increases could result in farmers increasing pesticide applications to control the problem, causing the death of non-target organisms. Collembola (20) numbers were highest in the untreated and lowest in the conventionally treated plots. Both the biosolids and conventional plots received inorganic fertilisers to meet plant requirements and as such, this may have resulted in changes in population dynamics through ammonification. Another factor these two treatments had in common was the cultivations. The use of farm machinery has been shown to detrimentally impact earthworms. For example. populations of earthworms have been

shown to decrease from 319 individuals per square meter to almost undetectable following the cultivations associated with a single crop of potatoes (Curry *et al.*, 2002).

In contrast to the QBS scores obtained at the Harper Adams University Farm site, the Broxton mesocosms showed a higher QBS score in plots receiving biosolids than untreated and conventionally treated plots. This increase would suggest that on the whole, biosolids are supporting a wider range of microarthropods than conventional treatments, yet it cannot be said if this increase, which was not seen at the Harper Adams University Farm site, was due to the number of applications, or the experimental site location. Further applications would be needed at the Harper Adams University Farm site to determine this.

Earthworms are often used as a biological indicator of soil health (Crittenden *et al.*, 2014; Bartz *et al.*, 2013; Birkas *et al.*, 2004). The method of mechanical extraction used in this work has allowed the assessment of earthworms from biosolid and conventionally treated plots. Results indicated that there were no significant differences between the treatments in the 2012 and 2013 data sets, however the 2013 data had a p value 0.072, with the biosolids treated soils having a higher mean count. When this method is compared with that of the Parisi (2005) QBS assessments, it is suggested that whilst earthworms are easier to extract and quantify, they do not fully represent the effect on the broader microorganism spectrum and therefore should only be used in conjunction with other assessment methods. Sandor *et al.* (2015) indicate that mustard extraction for earthworms may not be the most consistent method. The mustard is applied to the soil as it acts as an irritant to the earthworms, causing them to surface. However, Sandor *et al.*, (2015) suggest that the effect of mustard may not be equal to all species and that some may be more susceptible than others. Another issue with this method is the lateral movement of the mustard solution outside of the frame. Whilst this would not be a problem on flat ground, any slope results in some loss and therefore unequal distribution and/or application to the plot, leaving the potential for anomalous results.
The data presented in this chapter would suggest that the application of biosolids to soil, does impact on soil biology, therefore the null hypothesis cannot be accepted.

5.5 Chapter conclusions

The impact biosolids have on soil biology is not currently considered when applications to land are made, yet, they have the potential to support populations through increased OM content but also cause toxicity to the same organisms. The application of biosolids to soil has been assessed using a range of techniques to understand the impacts they may have on different organisms. Respiration data showed an increase in NCER at 100% and 200% application rates, but a decrease at 400%, indicating that biosolids may increase soil respiration at certain levels, but also could decrease respiration at excessive

application rates. It is unclear if this increase in respiration is a result of increased populations or increased stress on the same population numbers.

Tullgren counts showed contrasting results between the two soil types, with the Harper Adams University Farm site showing no difference in QBS scores, and Broxton showing an increase under biosolids treated plots. This may be attributed to the higher number of applications made to the Broxton site.

Earthworm counts found no statistical differences in the two years assessments were made at the Broxton field site. When comparing this data to the data collected for QBS scores, it is evident that earthworm assessments only represent a small proportion of soil organisms, and may not be wholly representative.

Chapter 6

The yield response of crops to biosolid applications

6.1 Introduction

The ever-increasing global population (currently 7.6 billion but forecast to grow to 11.2 billion by 2100) (United Nations, 2017) will inevitably produce more sewage, requiring responsible disposal routes. The UK currently produces 1.05 million tonnes of dry solids each year, with 85 % already being utilised by agricultural and energy industries. However, the potential increases in population also mean that food production must increase by between 60 and 110 % to meet consumer demands (Pradhan *et al.*, 2015; Alexandratos and Bruinsma, 2012; Tilman *et al.*, 2011). Global malnutrition problems associated with low nutrients in grains can only be exacerbated by such demands. Biosolids are often cited as a useful source of plant nutrients (Sharma *et al.*, 2017; Adair *et al.*, 2014; Binder 2002) and therefore, may be a suitable organic amendment to meet such pressing demands.

Plants nutrients can be split into two categories, macro and micronutrients. Macro nutrients are those which are required in large quantities such as nitrogen (N), phosphorus (P), potassium (K) and sulfur (S). Micronutrients are those that are still essential for plant growth but are required in smaller quantities. These nutrients include magnesium (Mg), manganese (Mn), sodium (Na), copper (Cu), zinc (Zn), boron (B), molybdenum (Mo). Macro and micronutrients requirements vary depending on the crop and the geographic location in which they are grown, amongst others. Whilst excessive applications of nutrients such as nitrogen (N) will result in the crop lodging and failure to meeting the specification of processors, the impact of excessive applications go beyond this and have the potential to affect the surrounding environment.

Biosolids generally contain the macronutrients N and P amongst a range of micronutrients (Chapter 2). The ratio of N and P in biosolids, however, is not in the same proportions that are required by plants. Biosolids have a greater proportion of P than total N. This can result in soil receiving a higher rate of P than required if biosolids are applied as the sole fertiliser. This could result in environmental pollution and eutrophication of water systems. The Biosolids Nutrient Management Matrix was introduced in 2014, in hope to limit biosolid applications to areas requiring P (ADAS, 2014). Improper disposal of biosolids could potentially cause contamination of the food chain and ground waters, in addition to the degradation of land (Shober *et al.*, 2007. Whilst other disposal options have been explored (for example incineration), they often result in a waste that still needs disposing of, for example ash. Biosolid applications to land have many benefits over that of commercially available, inorganic fertilisers. Whilst they increase organic matter and provide

micronutrients in addition to macronutrients through one fertiliser, they also act as a slow release fertiliser (AHDB, 2017).

6.1.1 Biosolids and crop yield

Penarete *et al.* (2013) carried out a yield experiment comparing 3 different types of biosolids (dehydrated, thermally dried and lime stabilised) at two application rates (100% and 200% required N rate), with inorganic fertiliser and untreated plots of sugarcane. Results indicated no significant differences between treatments, suggesting that biosolids had the potential to produce similar yields to that of inorganic fertilisers, regardless of their physical form, or application rate.

Following 17 years of biosolid applications, Miah *et al.* (2012) completed pot experiments assessing the effects of biosolids on barley yields. Results suggested that barley receiving inorganic fertilisers were significantly higher yielding than those receiving biosolids. This was attributed to a potassium (K) deficiency, as K levels are low in biosolids generally (AHDB, 2017). During the 17-year period of application the treated ground had been cropped with barley (*Hordeum vulgare* L.) maize *(Zea mays* L.) and rye (*Secale cereale* L.). Their biosolid treatments contained 0.07 – 0.5% K and therefore it was likely that the lack of K input over several years impacted on yield. Fernandez *et al.* (2009) also reported a decrease in barley yields from biosolid applications. Composted and thermally dried biosolids were applied to the soil at two applications rates (20 and 80t/ha) for three years. Barley grown in composted biosolids yielded better than the thermally dried biosolids, with this being attributed to the more stabilised organic matter. Overall, better yields were achieved at the lower biosolids application rate. The release of nutrients from thermally dried biosolids is known to be slower and therefore may explain the difference in the crop yields.

Binder *et al.* (2002) found that the optimum application rate of biosolids to maize crops (441 kg ha^{-1} N) had a similar use efficiency to that of inorganic fertilisers. However, this application rate is very high compared to an inorganic fertiliser, where closer to 150 kg ha^{-1} N would be used. Whilst the organic N can be utilised by plants, it must first be converted to inorganic forms (ammonium or nitrate) to be utilised. Such high rates could increase the risk of leaching into ground water. Kuotroubas *et al.* (2014) also studied the effect of a range of biosolid applications on crop growth. Wheat (*Triticum aestivum* L.) was grown over two years using the application rates 20, 40 and 60 Mg ha^{-1} and compared to inorganic fertilisers and an untreated control. In year one, differences between treatments were recorded with the 60 Mg ha^{-1} application rate producing a similar yield to the inorganic fertiliser. Both the 20 Mg ha^{-1} and 40 Mg ha^{-1} application rates were lower the 60 Mg ha^{-1}, however they were still statistically higher than the control. In year two the 60 Mg ha^{-1} biosolids treatments was the highest yielding treatment, with all other treatments being

similar. This application rate experiment poses conflicting results to those found by Fernandez *et al.* (2009)

Previous experimental work on the Broxton field site (Chapter 2) (Deeks *et al.*, 2013) found only one crop (out of 11 grown) with a significant difference in yield (conventionally treated wheat yielded higher than biosolids treated wheat), however, no data was collected on the heavy metal content of the grain produced under any of the crops.

6.1.2 Heavy metals in biosolid treated crops

Concentrations of heavy metals in biosolids vary depending on the input and the treatment wastewater receives at waste water treatment works (WWTW), therefore the concentration found in crops must be expected to vary (Table 6.1). Daily intake recommendations have been provided by World Health Organisation (WHO, 2018) (Table 6.2)

Table 6.1. Normal concentration range of heavy metals in cereal grain (Gramss and Voigt, 2013).

Element	mg kg^{-1}
Cu	2-20
Fe	26-90
Mn	14-30
Zn	10-100
As	0.2
Pb	0.2

Table 6.2. Recommended maximum daily intakes of heavy metals for the average adult.

Element	Limit	Human health impacts
As	<0.05 mg L^{-1}	Limit is under review
Ag	No data	No benefit to health
Cu	0.5 mg kg^{-1} bw/day	Excess can cause liver cirrhosis
Fe	0.8 mg kg^{-1}/day	Excess can cause liver failure
Mn	0.16 mg kg^{-1}/day	Excess can cause Manganism
Pb	No level	No benefit to health
Zn	0.3^{-1} mg kg^{-1} bw/day	Excess can cause Cu deficiency

*milligrams per kilogramme of body weight per day (mg kg^{-1} bw/day)

Mengel and Kirkby (2010) suggested that elements considered essential for plant growth should be classified into 1 of 4 groups. Of the elements discussed in this thesis, P is classified as group 2 (nutrients that are important in energy storage or structural integrity), Mn is group 3 (nutrients that remain in ionic form) and Fe, Mn and Cu in group 4 (nutrients involved in redox reactions). However, such classification is difficult to apply as some elements should fall into multiple categories (e.g. Mn could be group 3 and 4).

Mantovi *et al.* (2005) completed field experiments in Italy over a period of 12 years. Three fields underwent a rotation on an annual basis between wheat, maize and sugar beet (*Beta vulgaris*), with each crop having three replicates in each field. Each crop received a low and a high rate of biosolids with additional N in the form of urea. Biosolids were applied as liquid, dewatered (cake) and compost material individually. The annual applications of biosolids were found to increased soil fertility. This was evidenced by increases in soil organic matter, with the higher rate of biosolids application showing the greatest impact. The greatest effect was observed in the biosolids compost. The soils showed increased alkalinity over the 12 year period which is also thought to have resulted in an increase in nutrient availability. Nitrogen increases were thought to have occurred due to organically bound N in the biosolids. Increases in P content are thought to be due to both phosphate in the biosolids but also a change in the pH, making the P more available (pH change was from 7.8 to 8.0).

A significant increase of Zn and Cu was recorded in the topsoil of the highest biosolids treatment. Cadmium (Cd) increases was recorded in the plots treated with inorganic fertilisers. This was accounted for through applications of superphosphate, which is increasingly showing high Cd levels. Importantly, after 12 years of biosolid applications, the levels of all heavy metals remained below the EU regulatory limits. Wheat grain from biosolid treated plots showed an increase in N, P, Zn and Cu compared to inorganic fertiliser. N and Cu showed increases in sugar beet and Cu in maize grain. Lead was not detectable in the wheat grain or sugar beet, whilst Cd was not detectable in maize. This is attributed to the increase in soil organic matter limiting their availability. Zinc and Cu showed increases in the plant tissue analysis compared with inorganically treated grain. This is thought to have been caused by changes in pH.

The effects of biosolids on grain heavy metal concentrations have been documented to last beyond the duration of the applications. Granato *et al.* (2004) studied the effects of biosolid applications on heavy metal contamination of grain and soils following 10 years of applications. Whilst soil organic carbon was found to decrease rapidly, heavy metal contamination differed. Zinc and Ni concentrations were not statistically lower in 1995-1997 compared with 1985-1987. However, Cd and Cu concentrations did decrease. In maize, both grain Cu and Zn tissue concentrations remained similar when comparing maize grown

in 1985-1987 to grain grown 1995-1997, but grain concentrations of Cd and Ni, along with leaf Cd Cu, Ni and Zn concentrations decreased.

Conflicting results from the literature do not provide a clear message regarding the most likely yield impact biosolids may have on crops. Generally, it is thought that biosolids increase heavy metal concentration in grain, yet this cannot be certain due to the different types of biosolids available and their differing elemental compositions. Work by Deeks *et al.* (2013) on the Broxton field site indicated that when used in conjunction with inorganic fertilisers, thermally dried biosolids have the potential to produce similar yields to conventional fertilisers, with the exception of one wheat crop.

Following on from the sequential extraction and leaching data collected in Chapters 3 and 4, experiments were established to assess how crops were affected by biosolids applications. Yield studies were undertaken at Broxton, Cheshire as a continuation of previous field experiments, in addition to two mesocosm glasshouse studies. Grain was analysed from selected field studies and mesocosm experiments to assess if biosolid applications impacted on the grain elemental composition.

Chapter aim: To analyse yield response of grain crops to biosolids applications in both field and glasshouse conditions.

Chapter Objectives;
- Assess the response of crop yield to biosolid applications over a three-year period in a field situation
- Assess the effect of biosolid application on soil pore water, grain yield and elemental composition under two soil types in mesocosms.

Null Hypothesis: The application of biosolids has no impact on the yield of any crop, or its grains elemental composition.

6.2 Methodology

6.2.1 Broxton Yield Experiments

Three years of field experiments were established in accordance with the experimental plan in Figures 2.3 and 2.4. These experiments (occurring between 2012 - 2014) were a continuation of this work, with all treatments remaining in the same location (Figure 6.1). The arable plot area was cultivated by the resident farmer in an East to West direction and drilled North to South, with all fertiliser applications made using a pneumatic Kuhn Aerospreader to ensure uniformity for the applications, in conjunction with the work detailed in Chapter 2. A range of crops were grown, deemed suitable to the geographical location (Table 6.3) at suitable seed rates for the time of drilling (Table 6.4). All plots were maintained in accordance with commercial practice, following recommendations from BASIS qualified advisers. Fertiliser application rates were given by a FACTS qualified advisor (Table 6.5). Biosolid applications were made to meet crop P requirement, with additional inorganic fertilisers being used to ensure the crop had sufficient N and K. Nitrogen was applied in the form of urea with biosolids, whilst the conventional treatment N source was ammonium nitrate. Such decisions were made to follow commercial practice where possible (i.e. ammonium nitrate being the most common form of N used in the UK), whilst not causing a hazard through the use of an oxidising agent with a carbon source. All treatments received the same quantities of nutrients to limit the effect of difference sources of nutrients on the crop. Muriate of Potassium (MOP) was used for all K applications and conventional P applications were made using Triple Superphosphate (TSP). Cereal and oilseed rape (OSR) (*Brassica napus* L.) plots were harvested using a Wintersteiger Nursery Master plot combine (Wintersteiger, Germany), with plot weights and grain quality assessments being completed in the laboratory. All cereal and OSR yields were adjusted for moisture content (8% OSR and 15% cereals), with the moisture being determined using a Sinar GrainPro 6070 (Sinar Technology, UK). Thousand Grain Weights (TGW) were established using a FarmTec CountAmatic Console and feeder (FarmTec, UK) and a Kern KB2000 – 2N (Kern and SOHN GmbH, Germany) balance. Specific weights were taken using a Chondrometer. Due to poor establishment, the spring oilseed rape (OSR) was not harvested in 2012/2013.

Table 6.3. Cropping history of the Broxton field experiment, harvest years 2012- 2014.

Row	2011-2012	2012-2013	2013-2014
4	*Avena sativa* L.(winter oats var. Gerald	*Brassica napus* L. (Spring OSR var. Ability)	*Hordeum vulgare* L. (spring barley var. Shuffle)
3	*Triticosecale* (triticale var. Grenado)	*Zea mays* L. (maize var. Ballade)	*Hordeum vulgare* L. (spring barley var. Shuffle)
2	*Triticum aestivum* L. (winter wheat var. Solstice)	*Hordeum vulgare* L. (spring barley var. Shuffle)	*Hordeum vulgare* L. (spring barley var. Shuffle)
1	*Triticum aestivum* L. (winter wheat var. Solstice)	*Hordeum vulgare* L. (spring barley var. Shuffle)	*Hordeum vulgare* L. (spring barley var. Shuffle)

Table 6.4. Seed rates for each crop of the Broxton field experiment.

Harvest Year	Crop	Seed rate (m2)
2012	Winter oats	350
2012	Triticale	300
2012	Winter wheat	250
2013	Spring oilseed rape	120
2013	Maize	10
2013	Spring barley	250
2014	Spring Barley	250

Table 6.5. Macronutrient applications made to the Broxton field experiment over 3 years. Applications were made to meet P requirements in each instance.

Year	Crop	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
2012	Winter oats	130	45*	60
2012	Triticale	190	45*	60
2012	Winter Wheat	190	65*	60
2013	Spring Oilseed Rape	70	60*	20
2013	Maize	60	55*	20
2013	Spring Barley	110	45*	60
2014	Spring Barley	110	45*	60
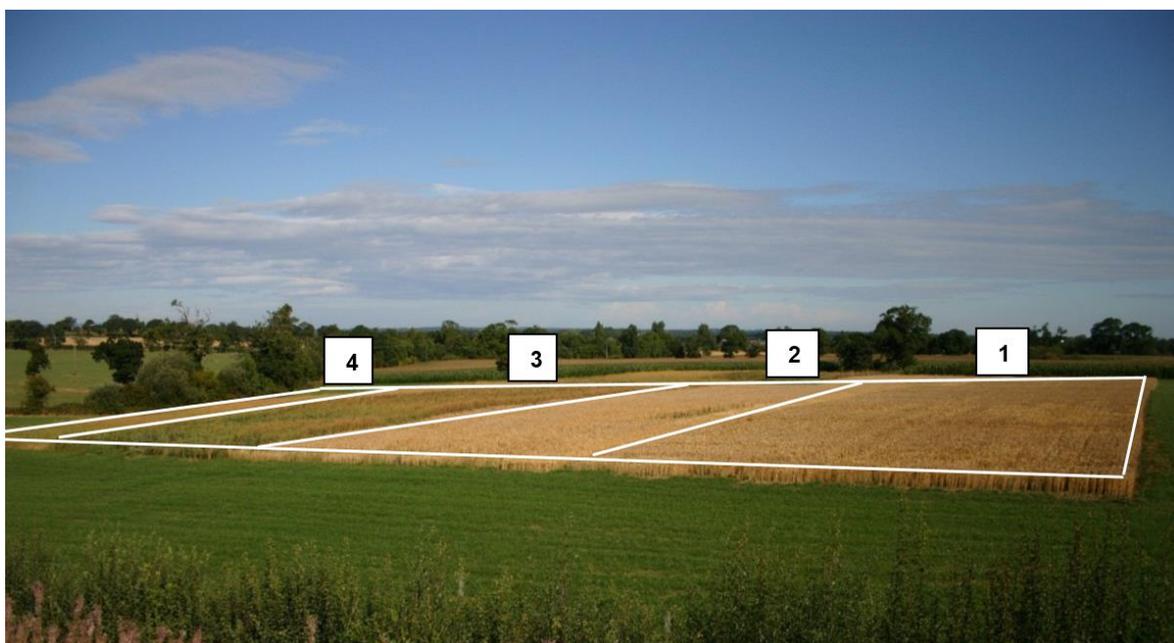
Figure 6.1. Broxton, Cheshire field site with its four cropping rows (1-4). Each block contained 2 treatments (biosolids and conventional fertilisers) and 6 replicates.

6.2.2 Mesocosm Studies (Broxton and Harper Adams University Farm soils)

Mesocosms studies were undertaken to enable an assessment of fertiliser treatments in controlled conditions. This also allowed an increase in the residual degrees of freedom in comparison to the field experiments. A low residual degree of freedom means each data point has a larger influence on a result, thus it is likely to incur error.

Two Mesocosm experiments were established to study the effect of biosolid applications on the contrasting soil types (Figure 6.2 and Figure 6.3). Bulk soil samples (taken to a depth of 20cm) were collected from the Broxton field experiment and transported to Harper for experimentation. For the Harper Adams mesocosms, bulk soil was collected from the top 20cm of Fourgates field (SJ 70878 19482). Following the results from the Broxton Mesocosm experiment (investigated outdoors at Harper Adams University), additional treatments were added to the Harper Adams University Farm soil experiment under glasshouse conditions. Treatment lists for both experiments are shown in Tables 6.6 and 6.7. Inorganic fertilisers can contain ranging heavy metal concentrations which may cause additions to the soil inadvertently (Table 6.8).

Different conditions were used due to time restrictions on the experiments and therefore the plants grown under glass would have had accelerated growth and therefore may have utilised the treatments applied differently when compare with outdoor studies.

A 16 hour, 15 ºC day, 8 hour, 5 ºC night cycle was implemented with a minimum natural light threshold set at 30 Klux. Any additional light requirements were met by 400W sodium lights. Mesocosms were watered every two days for both experiments.

Plot	22	23	24
Block	8	8	8
Entry	Biosolids	Untreated	Conventional
Plot	19	20	21
Block	7	7	7
Entry	Conventional	Biosolids	Untreated
Plot	16	17	18
Block	6	6	6
Entry	Untreated	3	Biosolids
Plot	13	14	15
Block	5	5	5
Entry	Biosolids	Untreated	Conventional
Plot	10	11	12
Block	4	4	4
Entry	Conventional	Biosolids	Untreated
Plot	7	8	9
Block	3	3	3
Entry	Untreated	Conventional	Biosolids
Plot	4	5	6
Block	2	2	2
Entry	3	Biosolids	Untreated
Plot	1	2	3
Block	1	1	1
Entry	Biosolids	Untreated	Conventional

Figure 6.2. Experimental design for the Broxton field soil mesocosms. n=8

Plot	22	23	24
Block	4	4	4
Entry	2	6	5
Plot	19	20	21
Block	4	4	4
Entry	1	3	4
Plot	16	17	18
Block	3	3	3
Entry	3	6	2
Plot	13	14	15
Block	3	3	3
Entry	5	4	1
Plot	10	11	12
Block	2	2	2
Entry	4	5	6
Plot	7	8	8
Block	2	2	2
Entry	2	1	3
Plot	4	5	6
Block	1	1	1
Entry	1	5	2
Plot	1	2	3
Block	1	1	1
Entry	4	6	3

	Treatment
1	Untreated
2	Conventional
3	Biosolids + Conventional
4	Biosolids (250kg/kg N)
5	Biosolids (500kg/h N)
6	Biosolids at conventional rate

Figure 6.3. Experimental design for the Harper Adams University Farm field soil mesocosms. *n=4.*

Table 6.6 Treatment list and application rate equivalent for the spring wheat crop grown in Broxton field soil. All treatments were applied at GS 28. Numbers in brackets indicate the amount of nutrients supplied from the biosolids alone.

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Untreated	0	0	0
Conventional	160	55	45
Biosolids + Conventional	160 (3)	55 (55)	45 (0)

Table 6.7. Treatment list and application rate equivalent for the spring wheat crop grown in Harper Adams University Farm soil. All treatments were applied at GS 28.

Treatment	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Untreated	0	0	0
Conventional	160	55	45
Biosolids + Conventional	160	55	45
Biosolids (250kg ha^{-1} total N)	250*	(485)	45
Biosolids (500kg ha^{-1} total N)	500*	(970)	45
Biosolids at conventional rate (using total N)	160*	(310)	45

N.B. * signifies the application rate met through biosolids alone.

Table 6.8. Average heavy metal concentrations of inorganic fertilisers used as the conventional treatment.

Element	mg kg^{-1}
Cu	12.9
Fe	ND
Mn	ND
Zn	12.5
As	ND
Ag	ND
Pb	ND

For both experiments (Figure 6.4 and 6.5) plastic containers (n=*24*, 30 litre, 40 cm diameter, 33 cm high) were filled with soil (8 replicates for each treatment) and planted with 15 spring wheat seeds (*Triticum aestivum*.L. var. Belvoir) sown at approximately 2 cm deep in each mesocosm. Upon germination, seedlings were thinned to 12 plants to ensure equal populations in each mesocosm. Plants removed through thinning were selected due to poor establishment, close proximity to other plants and poor health. Plants were grown until grain maturity (growth stage 92), when they were hand harvested.
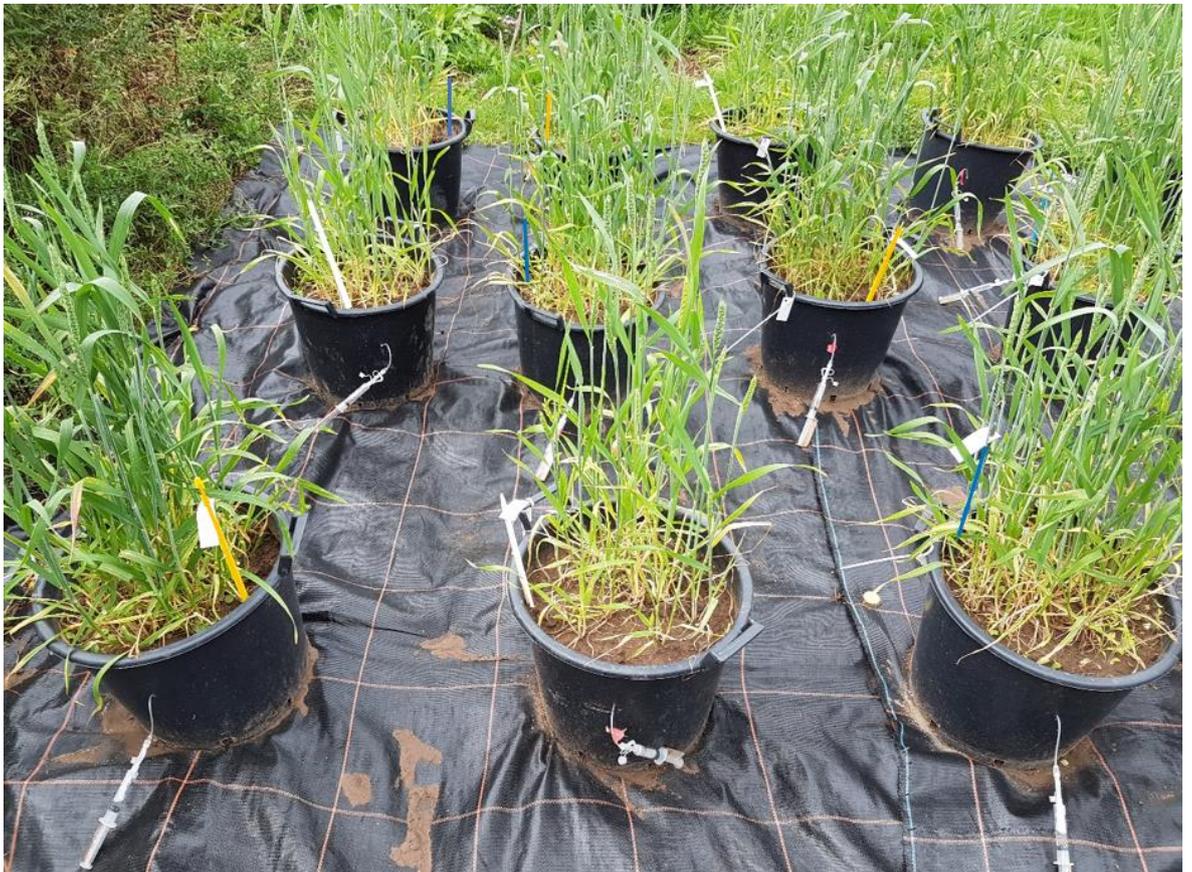
Figure 6.4. First three replicates from the experiment at growth stage 61, with the white peg indicating untreated, blue for biosolids and yellow for conventionally treated mesocosms.
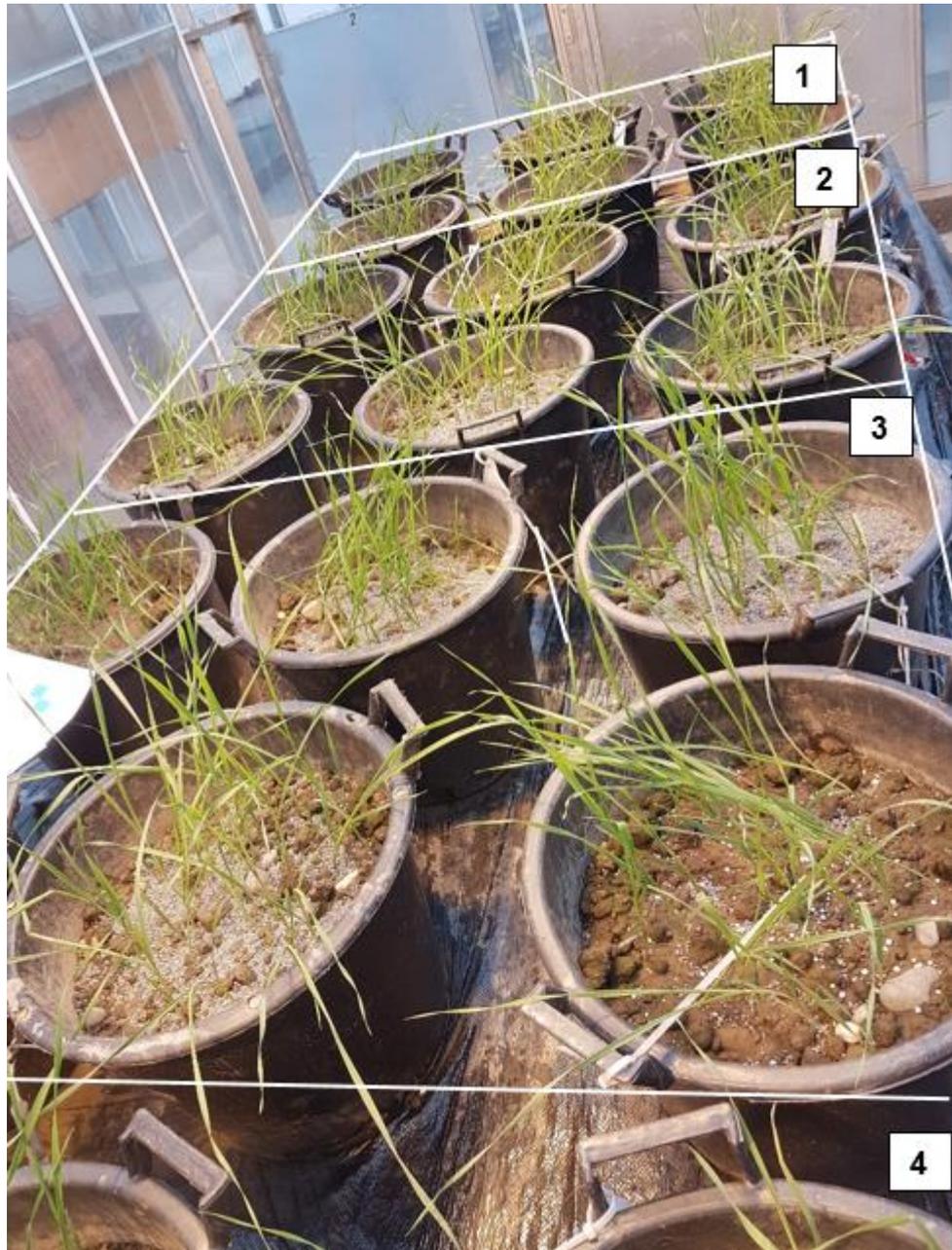
Figure 6.5. Harper Adams Mesocosm experiment post-fertilisation with grid lines demonstrating the experimentation blocking.

6.2.2.1 Soil Pore water sampling and collection

Two rhizon soil moisture samplers (consisting of a 10 cm porous PVS tube) were inserted 10 cm from the base of each mesocosm, at a 45-degree angle. A Leur-Lock connector was then used to connect a syringe to establish a vacuum, allowing soil pore water samples to be extracted (Hartley *et al.*, 2010). Soil pore waters were collected fortnightly until the crop began to senesce when watering was reduced to once weekly.

Following pore water collection, samples were stored in a fridge at 4ºC until processing for pH and heavy metal. Heavy metal analysis for Fe, Cu, As, Ag, Pb, Mn and Zn was completed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (X-series 2 ICP-MS, Thermo Scientific MA, USA). Each pore water sample was analysed in accordance with the procedure outlined in section 2.7. Samples were analysed in triplicate.

6.2.2.2 Wheat yield

Each mesocosm was harvested once the crop had reached Growth Stage (GS) 92, with the ears being separated from the straw. All samples were oven dried (60 ˚C) for 48 hours to remove any water, ensuring differences in weight were due to grain fill the grain and not maturity. The number of tillers and ears were counted and weighed before the ears were threshed (Walter-Winterstiger 1 GD 180 STA, Austria) with the grain, counted and weighed.

6.2.2.3 Grain analysis

Grain from each plot was passed through a Perten 100 mill producing a flour. Subsamples (0.20 g) were digested using a Mars 6 microwave digester using the methodology outlined in section 3.2.4.

Grain samples were quantified using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (X-series 2 ICP-MS, Thermo Scientific MA, USA).
Wet and dry and all leaching samples were analysed without dilution, using the following volumes;

- 4.85 ml sample solution
- 100 µl HNO_3 (14M)
- 50 µl internal standard solution (1 ppm gallium (Ga)).

Samples were vortex mixed for 5 seconds to ensure they were mixed uniformly.

Lake Ontario Water (CRM TMDA 64.2) was used as a water reference material (Table.6.9). This was to ensure that the values being produced by the ICP-MS were within the accepted range of the reference sample.

Table 6.9.TMDA recoveries using ICP-MS.

Element	Recovery	TMDA 64.2 reference value	Tolerance	Recovery %
Mn	0.276	0.295	0.0223	93.559
Fe	0.283	0.306	0.0271	92.484
Cu	0.266	0.274	0.0241	97.080
Zn	0.284	0.310	0.0265	91.613

6.2.3 Statistical analysis

All data was analysed using GenStat (VSN International, 18th edition). One-way analysis of variances (ANOVA's) was used to analyse yield and grain heavy metal data from the Broxton field site and mesocosm experiments. A repeated measures ANOVA was completed on all pore water data. Significant differences were recorded at $p<0.050$. Where statistical differences were found, a post hoc test (Tukey) at a 5% probability was used to establish significance between treatments and times.

6.3 Results

6.3.1 Broxton Field Yield Experiment

2011/2012 season

No differences were recorded between treatments for yield (p=0.112), Specific Weight (SW) (p=0.157) or Thousand Grain Weight (TGW) (p=0.095) indicating that fertiliser type did not impact on oat yield. Similarly, no significant differences were recorded for wheat yields with p values of 0.183, 0.065 and 0.069 for yield, SW and TGW respectively. Results were close to significant for SW and TGW with biosolids having the higher mean yield for both. Triticale did show significant differences for yield however (p=0.033) with biosolids producing 3.26 Mg ha^{-1} and conventional fertiliser producing 2.6 Mg ha^{-1}. Both SW and TGW showed no significant difference between treatments (p=0.560 and 0.725 respectively) (Figure 6.6).
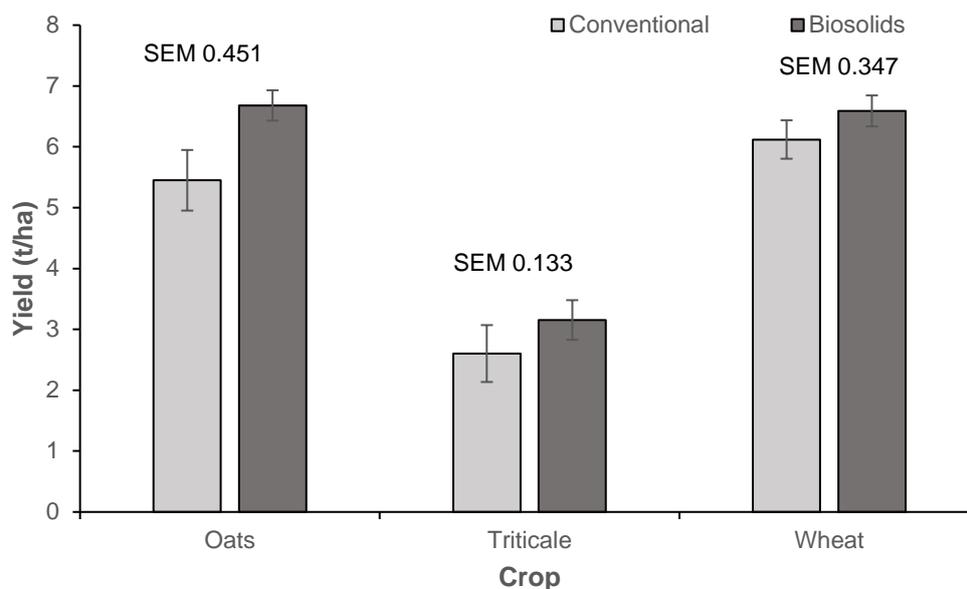
Figure 6.6. Yield for 2011/2012 cropping season. Residual d.f. oats 5; triticale 5, wheat 11. Error bars signify ± SE.

2012/2013 season

The spring OSR crop was not taken to harvest due to extensive pigeon damage during its establishment. The maize crop showed no differences in fresh yield (p=0.184), dry yield (p=0.332) or dry matter content (p=0.148). However, a difference was recorded between treatments for barley, with conventional fertiliser producing a higher yield (4.46 Mg ha^{-1} compared to 3.61 Mg ha^{-1}) (p=0.002) (Figure 6.7). Whilst no significant differences were recorded for the SW (p=0.252), conventional fertiliser produced a higher TGW (55.43 g compared to 51.45 g) indicating the conventional fertilisers produce heavier grain (p<0.001).
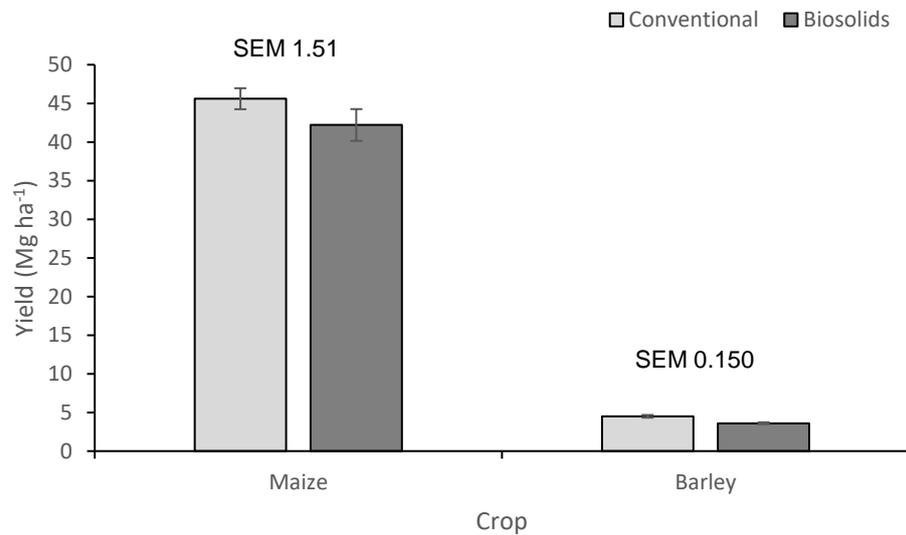
Figure 6.7. Yields for the 2012/2013 cropping season. Residual d.f. maize 5; barley 11, SOSR not harvested. Error bars signify ± SE.

2013/2014 season

For the final year of the field yield experiment, the cropping area was uniformly drilled with barley. No difference was recorded between the treatments for yield (p=0.130) (Figure 6.8), however SW was higher for biosolids treated grain (p=0.005). No difference was recorded for the TGW.
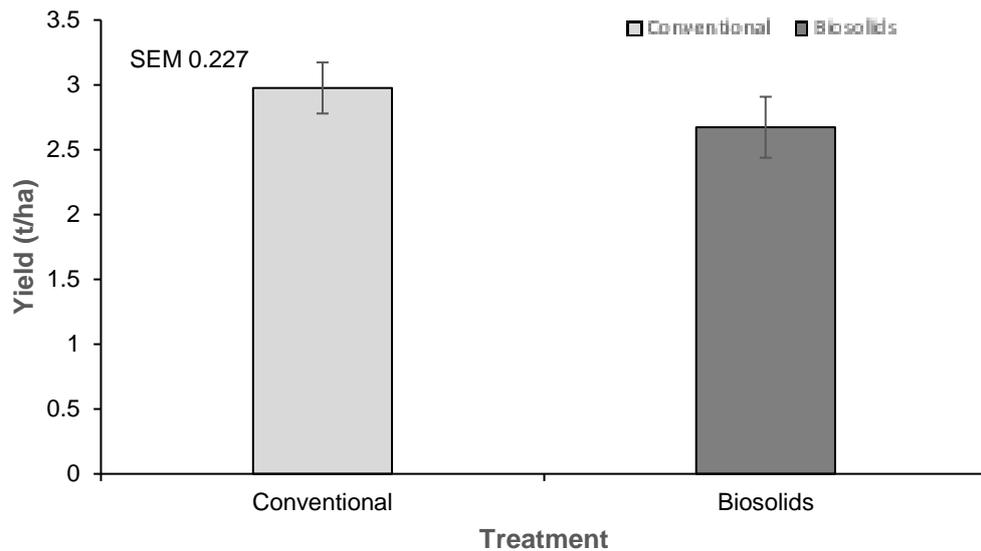
Figure 6.8. Yield for the 2013/2014 cropping season. Residual d.f. 11. Error bars signify ± SE.

Selected crops from the three-year rotation were digested and analysed for heavy metal content based on their yield results. Triticale showed differences for Ag, As, Fe, Pb and Zn; conventionally treated grain had a greater concentration of each element. Copper and Mn were close to showing a statistical difference between treatments (p=0.066 and p=0.059 respectively, with the highest concentration being found in the conventionally treated grain. Spring barley (harvested 2013) showed no significant difference between any of the elements, whilst a significant difference in 2013/2014 was only recorded between treatments for Mn concentration with again, conventionally treated grain being significantly higher (Table 6.10).

Table 6.10. Grain digest results from selected crops at Broxton Cheshire; a) 2011/2012 triticale, b) 2012/2013 spring barley, c) 2013/2014 spring barley.

a)

Treatment	Ag (mg kg^{-1})	As (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Biosolids	0.06	0.18	53.70	77.80	23.80	6.22	185.10
Conventional	0.10	0.22	60.20	100.80	38.50	6.78	202.20
p value	0.021	0.019	0.066	0.044	0.059	0.010	0.033
cv%	20.5	8.0	7.0	13.3	27.5	2.5	4.1
SEM	0.007	0.006	1.620	4.860	3.500	0.066	3.230
Residual d.f	3	3	3	3	3	3	3

b)

Treatment	Ag (mg kg^{-1})	As (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Biosolids	0.05	0.14	16.60	87.20	14.86	0.91	50.20
Conventional	0.06	0.16	17.370	76.90	17.14	1.15	54.20
p value	0.549	0.407	0.433	0.488	0.187	0.337	0.388
cv%	71.1	26.4	13.5	42.8	24.6	56.7	21
SEM	0.011	0.011	0.664	10.140	1.134	0.169	3.160
Residual d.f	10	10	10	10	10	10	10

c)

Treatment	Ag (mg kg^{-1})	As (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Biosolids	0.09	0.16	20.01	88.00	13.26	0.55	45.80
Conventional	0.08	0.18	17.58	141.00	16.17	0.59	38.50
p value	0.609	0.309	0.207	0.142	0.001	0.889	0.125
cv%	46.1	35.2	23.1	70.4	10.0	130.1	25.2
SEM	0.011	0.017	1.252	23.200	0.426	0.213	3.070
Residual d.f	11	11	11	11	11	11	11

6.3.2 Broxton Mesocosm Experiment

6.3.2.1 Pore water analysis

Concentrations of Ag and Mn were below the LOD and LOQ and so no results are presented for these elements.

Differences between treatments were recorded for Cu and Fe only with all other elements recording p values greater than 0.05. Pore waters from biosolids treated soils recorded higher concentrations of Cu overall, compared to both untreated and conventionally treated soils. Pore water samples taken from untreated soils yielded less Fe than biosolid and conventionally treated soil pore water. The majority of the data was within

the limits of Detection (LOD) and limits of quantification, however Pb concentrations were very low and therefore these results should be treated with caution.

Differences between timings were recorded for all elements except Pb, however, there was no single timing in which each element was found at its highest concentration. Arsenic levels peaked at sample timing 4 but remained similar for the remaining sample times. Copper concentrations increased at sample timing 5 and remained similar until the final sample timing when concentrations decreased back to the concentration of the first 4 sample times. The first two sample timings were the highest for iron, with sample times 2 and 3 being statistically similar to each other. The remaining sample times did not statistically differ from each other. Zinc concentrations in pore water samples were statistically similar for the first three sample times, decreasing at sample 4. Concentrations then increased and remained statistically similar at sample timings 5, 6, 7 and 8 before decreasing again at sample time 9. An interaction between time and element concentration was only recorded for Fe. At sample times 1, 2 and 3, the pore waters extracted from untreated soils recorded lower concentrations than both the pore waters taken from biosolids and conventionally treated soils. No differences were recorded between treatments and any other time points (Figure 6.9).

No significant difference was recorded between treatments with regards to pH of the pore water (p=0.455), with no difference being recorded over time (p=0.190). No interaction between treatment and time was recorded.

6.3.2.2 Wheat yield

Grain weight and number of grains produced were all significantly higher where biosolids and conventional fertiliser had been applied (p<0.001) (Figure 6.10, 6.11 and 6.12) compared with untreated pots. However, there was no significant difference between biosolids and conventionally treated mesocosms. Biosolid and conventionally treated grain had a higher TGW average than the untreated control (p=0.049).

6.3.2.3 Grain analysis

Grain elemental concentrations were statistically similar between treatments for Ag, As, Cu, Fe, Pb and Zn. Grain harvested from untreated mesocosms had a lower Mn concentration than both biosolids and conventionally treated grain (p=0.007) (Figure 6.13), however, this was still within what is considered to be the normal limits (Table 6.1).
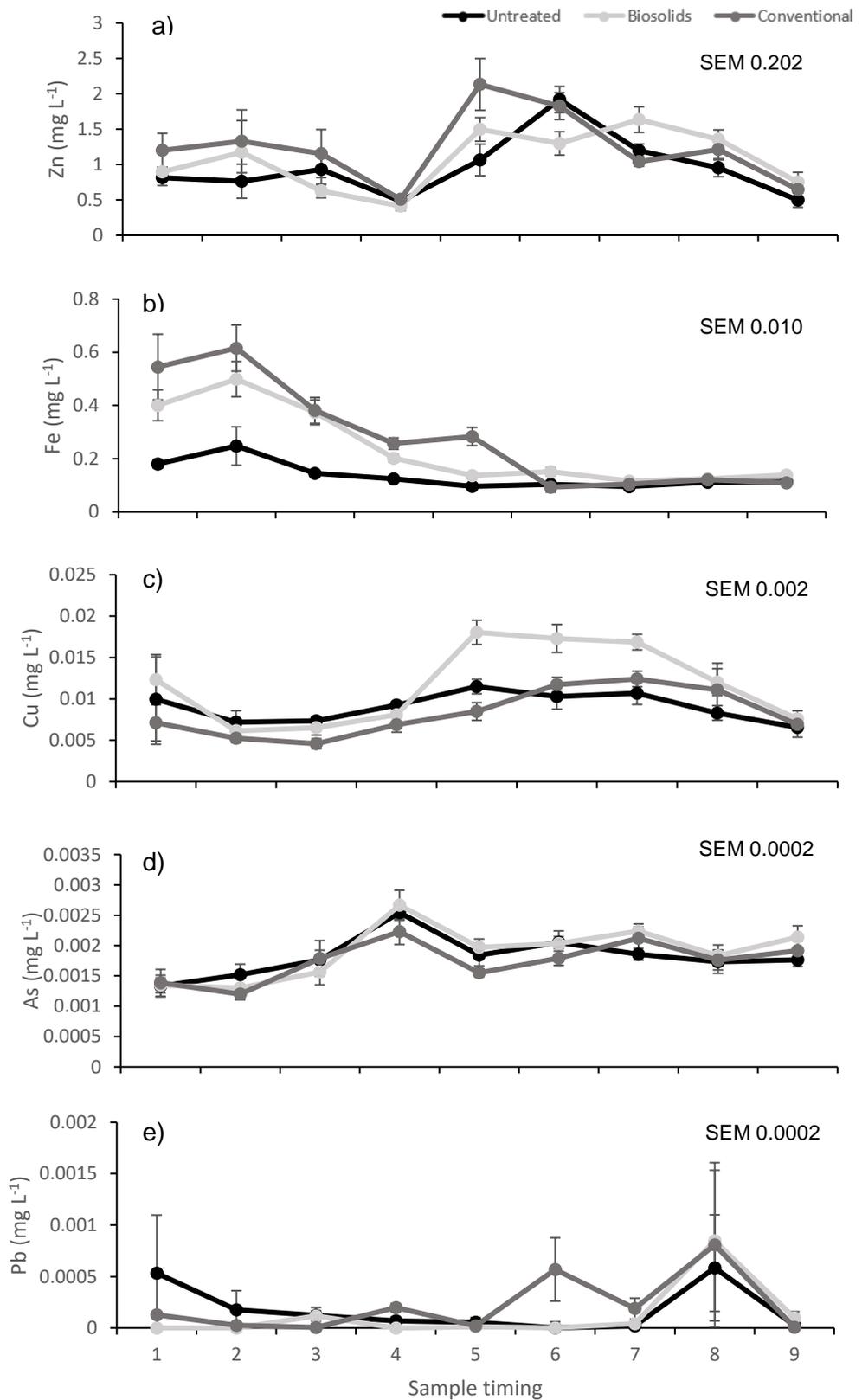
Figure 6.9. Elemental concentrations a) Zn, b), Fe, c) Cu, d) As, e) Pb of pore water over time in the Broxton mesocosm experiment. Elements are presented in order of decreasing concentration due to the large variability in concentrations recorded. Error bars represent ± SE. Residual d.f. 148.
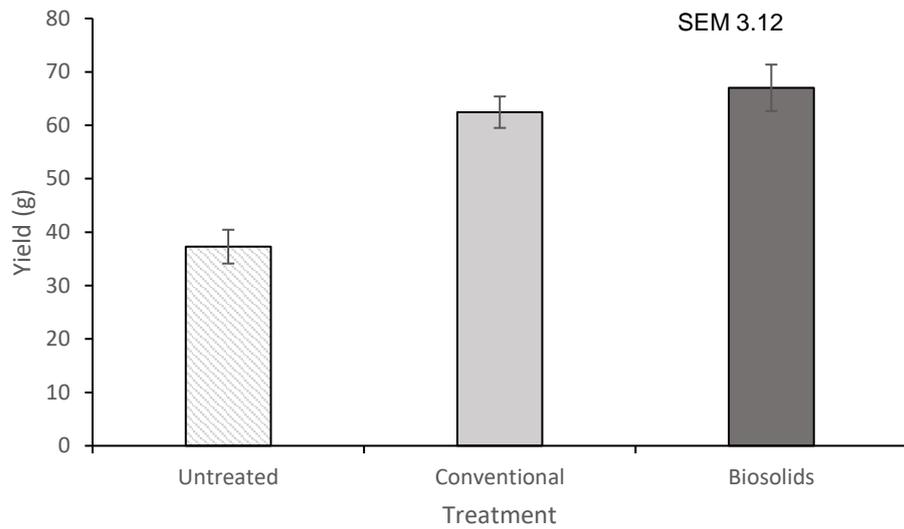
Figure 6.10. Grain yield of the Broxton mesocosm study comparing different fertiliser treatments. Error bars indicate ±SE. Residual d.f. 14.
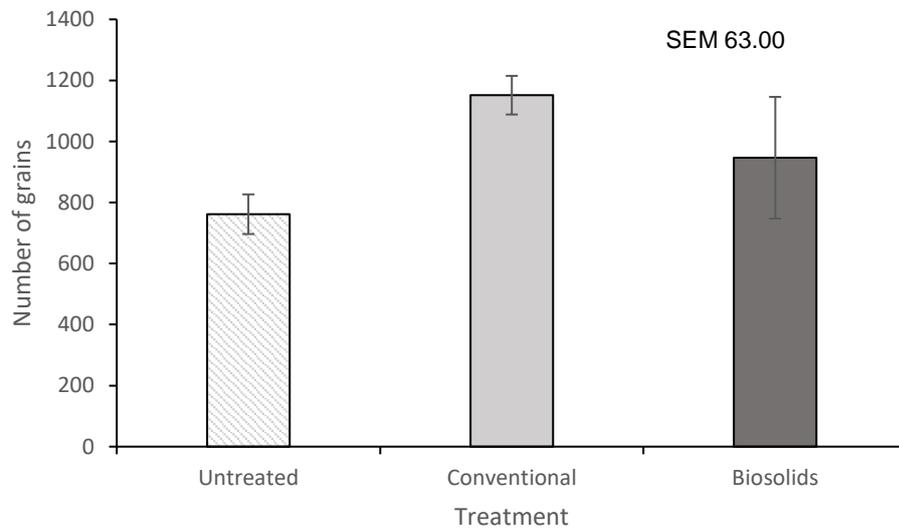
Figure 6.11. Number of grains produced by the respective treatments from the Broxton mesocosm study. Error bars indicate ± SE. Residual d.f. 14.
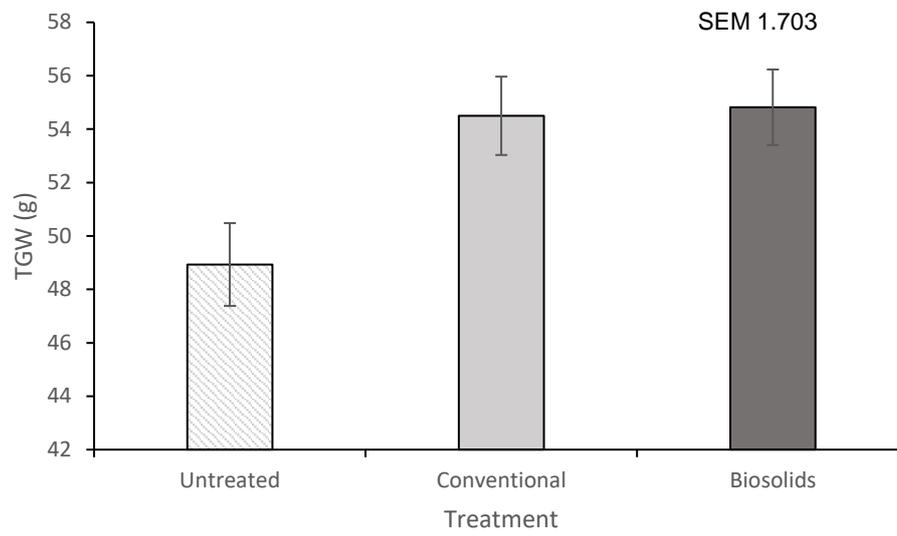
Figure 6.12. TGW produced by the respective treatments from the Harper Adams mesocosm study. ± SE. Residual d.f. 14.
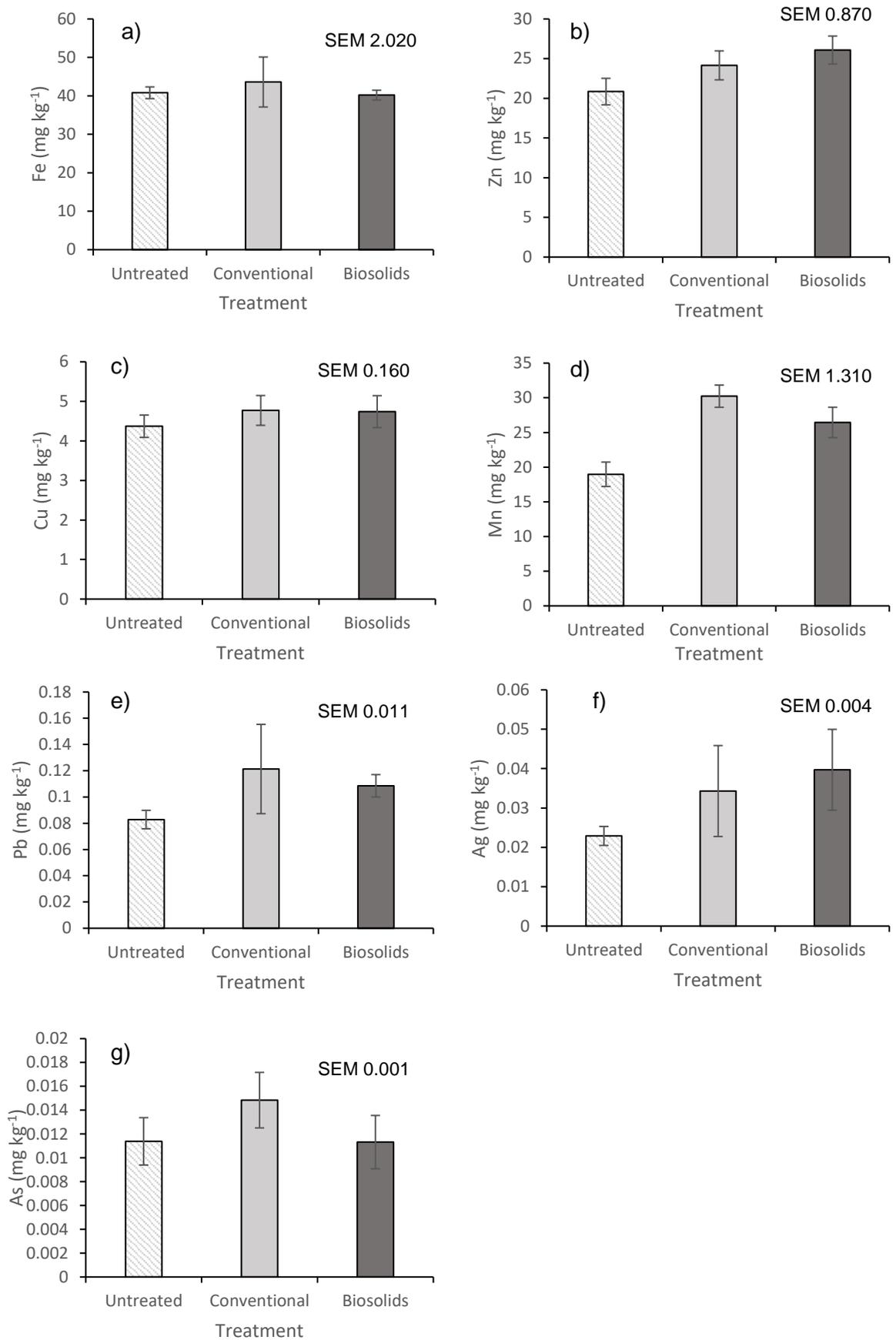
Figure 6.13. Grain elemental concentrations, a) Fe, b) Zn, c) Cu, d) Mn, e) Pb, f) Ag, g) As in the Broxton mesocosm experiment. Elements are presented in order of decreasing concentration due to the large variability in concentrations recorded. Residual d.f. 14

6.3.3 Harper Adams University Farm mesocosm study

6.3.3.1 Soil Pore water

Concentrations of Pb were not high enough (below the LOD and limits of Quantification (LOQ) to record on the ICP-MS and therefore no data was collected.

No differences in the concentration of Ag, Cu, Fe, Mn or Zn were recorded overall in the pore waters ($p > 0.05$). Copper was close to significance ($p = 0.062$) with pore waters collected from conventionally treated soils having a higher concentration than all other treatments, however this cannot be explained through any historical applications of manure such as pig slurry. Higher concentrations of As were recorded in the untreated soil pore waters compared to the biosolids applied at 250 kg ha^{-1} total N pots.

Differences in concentration of elements at different timings was recorded for Ag, Fe, Mn and Zn. The final sample was significantly higher in Ag and Zn in comparison to all other samples, whilst the Cu was higher at the second sample timing and Mn was higher at the third.

No interactions were recorded between time and element concentration for any of the elements (Figure 6.14).

Pore waters showed no difference in pH regarding treatment, but the pH did decrease significantly at the last sample timing (sample set 4) just before the crop was harvested. No interaction was recorded between treatment and time.

6.3.3.2 Wheat yield

No differences were recorded between treatments for the weight of grain ($p = 0.585$) or the number of grains ($p = 0.543$), indicating that fertiliser did not increase the yield above the untreated control (Figure 6.15, 6.16 and 6.17). No differences were recorded between treatments for TGW ($p = 0.533$). This may indicate that applications of fertiliser to the field preceding the experiment were influential on the results.

6.3.3.3 Grain analysis

Grain digests indicated no significant differences between any treatments for any element analysed. This suggests that the application of biosolids to the mesocosm, even at twice the maximum rate, did not have a detrimental effect on the grain produced (Figure 6.18) and therefore under these circumstances, did not pose a health risk to any consumers above what was seen the other treatments. This would also indicate that the use of inorganic fertiliser did not affect the uptake of heavy metals into the grain.
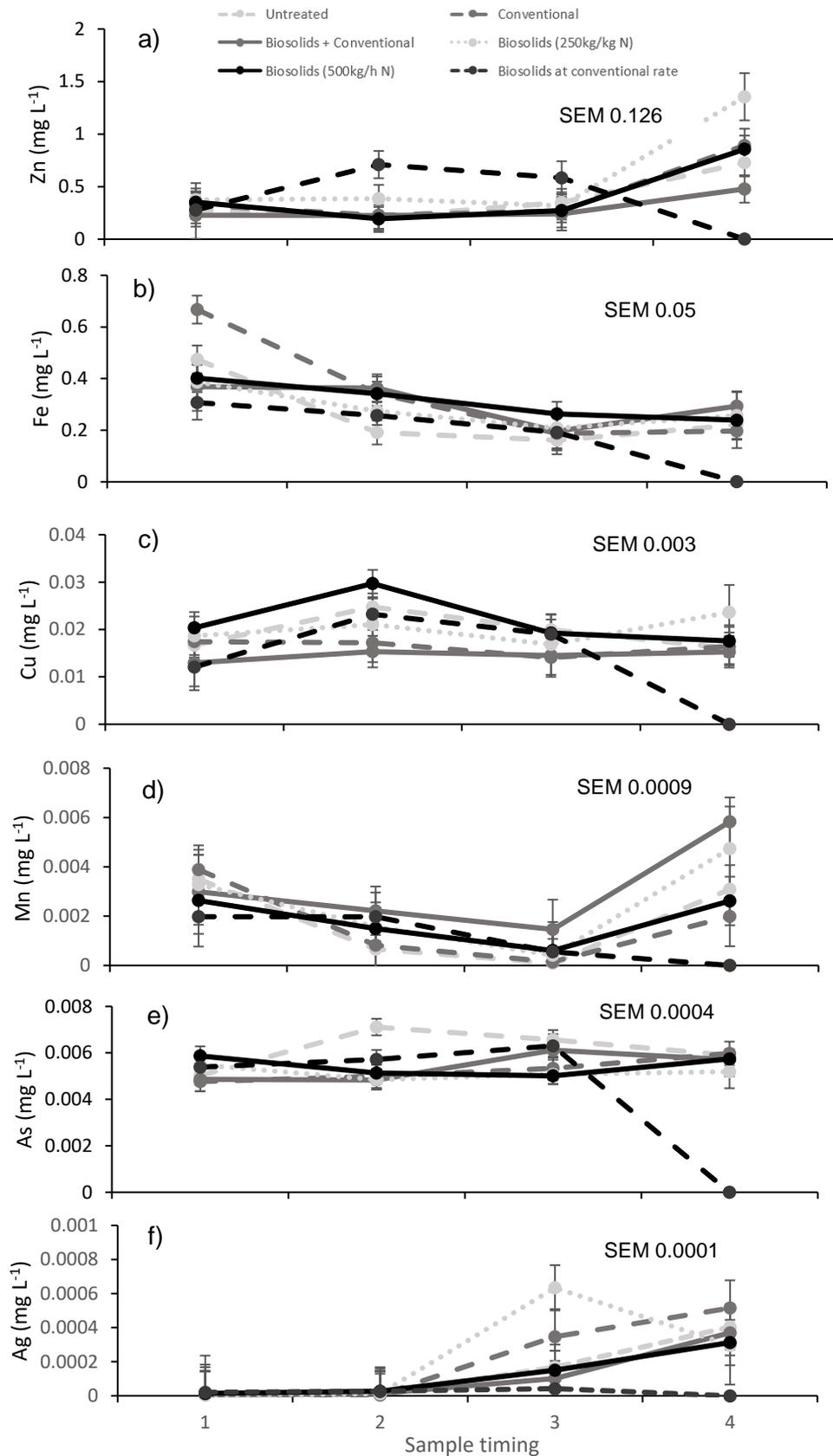
Figure 6.14. Elemental pore water concentrations, a) Zn, b) Fe, c) Cu, d) Mn, e) As, f) Ag over time in the Harper Adams University Farm mesocosm experiment. Elements are presented in order of decreasing concentration due to the large variability in concentrations recorded. Residual d.f. 148.
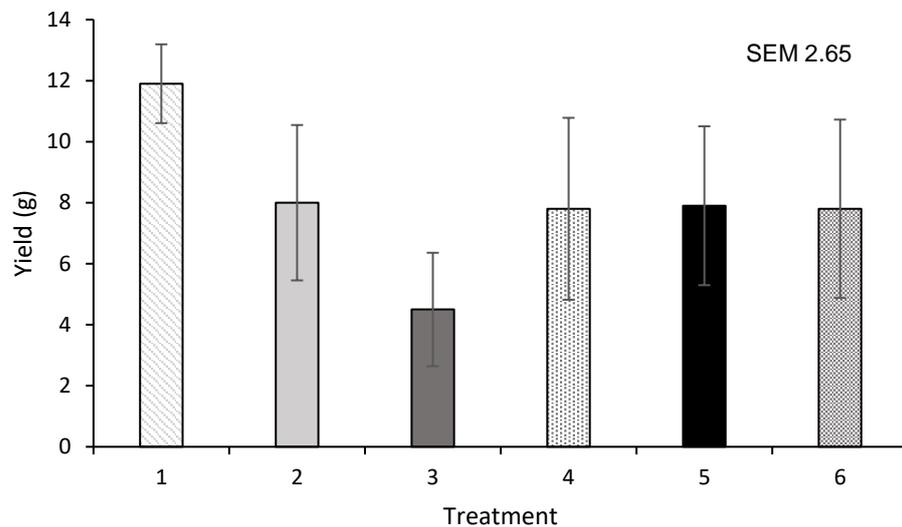
Figure 6.15. Grain yield of spring wheat grown in the Harper Adams University Farm mesocosm experiment under each treatment. Error bars indicate +/- SE. Residual d.f 15.
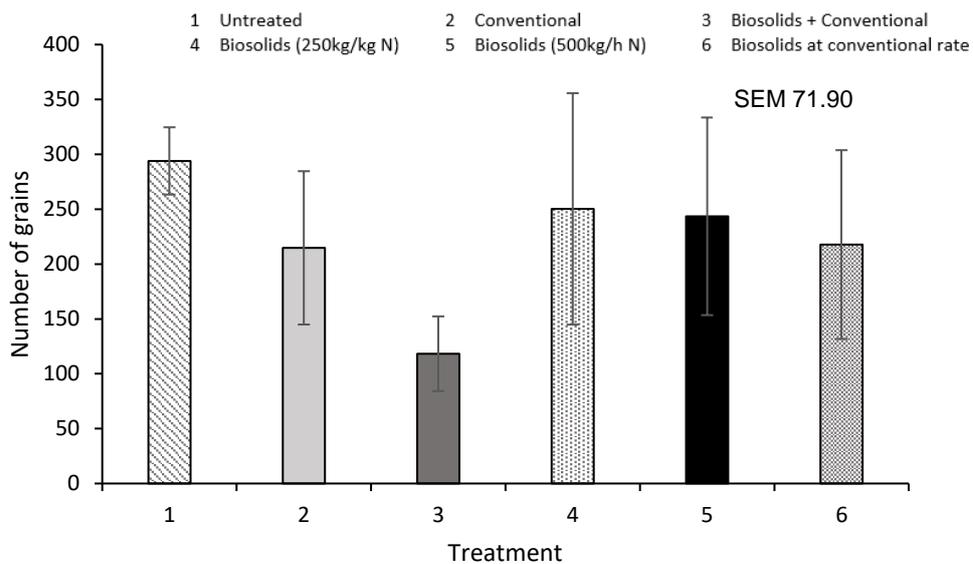
Figure 6.16. Number of grains produced by the respective treatments from the Harper Adams mesocosm study. Error bars indicate +/- SE. Residual d.f 15.
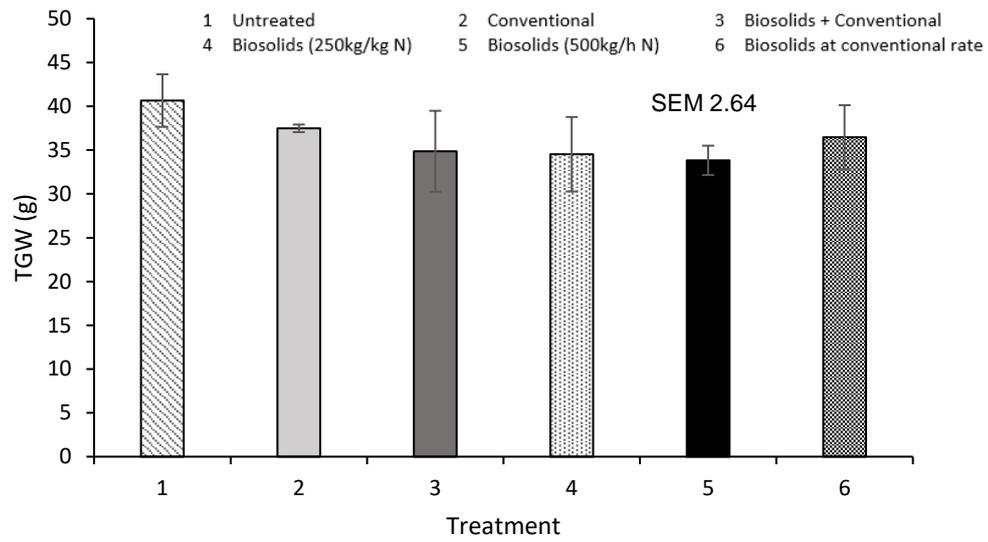
Figure 6.17. TGW produced by the respective treatments from the Harper Adams mesocosm study. Error bars indicate +/- SE. Residual d.f 15
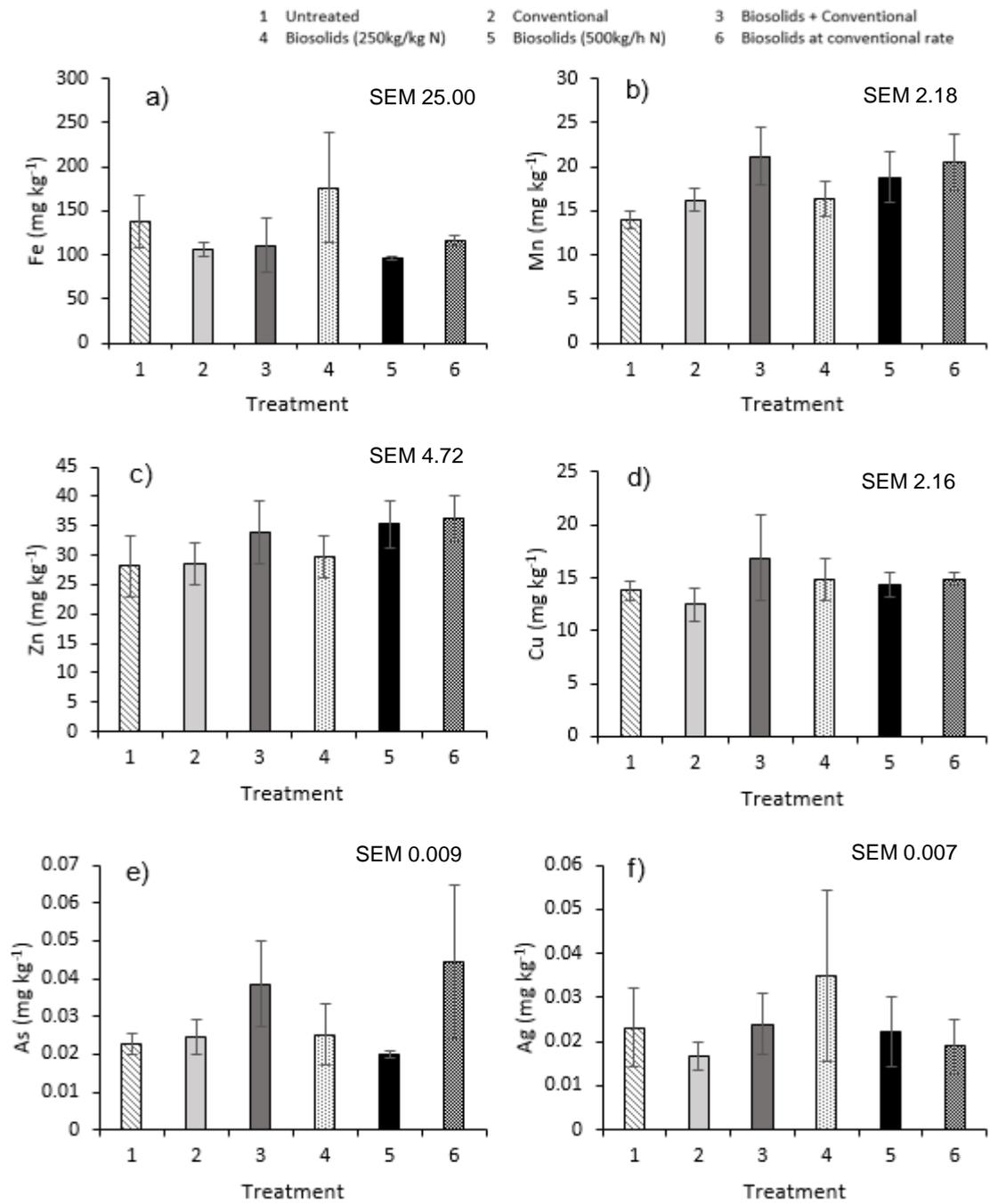
Figure 6.18. Grain elemental concentrations, a) Fe, b) Mn, c) Zn, d) Cu, e) As, f) Ag in the Harper Adams University Farm mesocosm experiment. Elements are presented in order of decreasing concentration due to the large variability in concentrations recorded. Residual d.f. 15

6.4 Discussion

Sustainable crop production is required to meet the demands of an increasing global population, whilst limiting the impact of food production practices on the environment. Foley *et al*. (2011) suggested that crop yields are rising more slowly than in previous decades, which may pose serious problems should population forecasts be correct. Studies have demonstrated that fertiliser use generally, increases crop yield, with Mueller *et al.*, (2012) suggesting that 45-70% increases can be expected across a range of crops when fertilisers are utilised. Biosolids pose a potential source of nutrients for crop and and their use in crop production could help alleviate a waste disposal problem, whilst contributing towards sustainable food production.

A series of experiments investigated the impact of biosolids on crop yield and quality in comparison to commercial fertilisers. Grain was analysed to establish, what effect such applications may have on the use of biosolids.

Recovery values for the ICP-MS were lower than the desired 95% for Fe, Mn and Zn, indicating that the values presented, may be lower than the true concentrations.

A large field experiment (a continuation of KTP and European Commission funded projects) was undertaken over three years. Both oat and wheat crops showed no differences in yield in the 2011/2012 cropping season. Specific weight and TGW were close to significance in the wheat crop, with grain produced using biosolids having higher values for both. Both SW and TGW are measures of crop quality, with SW measuring how dense the grain is and TGW measuring how heavy grain is. Higher values indicate a better grain, which if used for seed, is more likely to germinate. The specific weight for this grain was low compared to normal market requirements (normally 72 kg hl^{-1}) (Yara, no date). This may have been a result of poor ground and a poor growing season. Triticale yielded higher under plots receiving biosolids, with the grain showing significant differences in in Ag, As, Fe, Pb and Zn concentrations. In each instance conventionally treated grain had a greater concentration of each element. These concentrations were found to be high in accordance with levels detailed by grain Gramss and Voigt (2013). Nevertheless, where a difference in concentration was recorded between the treatments, conventionally treated grain had higher concentrations. The values provided by Gramss and Voigt (2013) were for cereal crops in general and not specifically triticale and therefore may not be applicable to this crop. The differences between concentration and treatment may be accounted for by a dilution effect. This may be advantageous for elements such as As and Pb which are not beneficial to human diet.

In 2012/2013 maize showed no yield differences between treatment, however, spring barley yields and TGW's were higher under plots fertiliser with conventional fertiliser, yet there were no differences in the grain element concentrations. The concentrations of heavy metals in this grain were within guideline limits details by Gramss and Voigt (2013).

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With the exception of Pb, which was elevated for both treatments. All plots were drilled with spring barley in the 2013/2014 season. No yield differences between treatments were recorded, although grain Mn concentrations were higher in conventionally treated plots. Concentrations in grain treated with biosolids were still within the normal limits and therefore, whilst they concentration was significantly lower, this would not be detrimental to consumers.

Whilst oats, wheat, triticale, and maize were only grown for one season, the barley grown in 2012/2013 and 2013/2014 showed contrasting results, possibly indicating that environmental factors such as rainfall and temperature may have influenced the crops nutrient demands. The experimental site had a very low number of residual degrees of freedom (5 residual d.f where only a single row of a crop was grown), which may have impacted on the results also. Ideally, a residual degrees of freedom should be greater than 12. In such instances, each data point has a greater influence on the result, when compared with experiments containing a higher number of residual degrees of freedom. For this experimental site, it may also explain why, treatment differences were not observed.

Mesocosm yield experiments were carried out on the two soil types detailed in Chapters 2 and 3. Containing a higher number of residual degrees of freedom, they allowed a more controlled assessment of the effect of fertiliser on yield and grain heavy metal content. Broxton mesocosms treated with biosolids and conventional fertiliser yielded more than untreated mesocosms. The TGW from the mesocosms receiving fertiliser was also higher. Elemental grain analysis showed that Mn was significantly lower in grain from untreated mesocosm. This showed that the fertiliser applied was influential on crop yield. However, each treatment produced grain within the normal limits (Gramss and Voigt, 2013). Pore water Cu was higher in the biosolids treated mesocosms, whilst Fe was higher in the biosolids and conventionally treated mesocosms. These elements were not at elevated levels in the grain, indicating that if an increased amount was translocated into the plant, this was stored elsewhere (e.g. leaves) or that the increase in concentration was not utilised by the plant. A study by Garnett and Graham (2005) showed that 77 % of the Fe in the grain was translocated when the grain reached maturity and that the Fe was redistributed from the leaves, therefore possibly explaining why there was no increase in grain Fe concentrations in biosolids treated mesocosms. Elemental grain concentrations were all within the expected range (Table 6.1), with the exception of Fe in each case which was higher. This would suggest that no element was deficient. Cereals crops have the ability to alter the rhizosphere should Fe or Zn concentration be below their requirements. The release of phytosiderophores increases the concentration of Zn and Fe by mobilising the elements, however whilst the untreated grain was lower in Fe than the biosolids and conventionally treated mesocosms, concentrations were still within the normal range, indicating that Fe and Zn concentrations were sufficient for growth.

Biosolids in both the Broxton field and mesocosm experiments were supplemented with inorganic fertilisers due to the unsuitable ratio of N:P and their lack of K. This would be consistent with commercial practice, although it did not provide evidence of their potential as a product to be used on its own. Harper Adams University Farm mesocosms contained additional treatments to assess whether the application of biosolids on their own impacted on yield and grain content. There was no difference between treatments with yield or elemental content, indicating that the field soil had sufficient supply of nutrients to sustain growth. Pore water concentrations did differ between treatments with overall As concentrations being higher in untreated mesocosms compared to biosolids applied at 250kg N/ha. As these mesocosms were receiving no fertiliser, this may be due to natural variation in concentration. The trend towards increased levels of Cu in conventionally treated mesocosms may be explained by contamination in the TSP used as the phosphate fertiliser. Whilst the concentration shown in Table 6.8 are not excessively high, Chibueze *et al.* (2012) presented data indicating how variable fertiliser contamination can be. Silver, Fe, Mn and Zn all showed significant changes in concentration over time, with Cu concentrations peaking at sample timing 2, Mn at timing 3 and Ag and Zn at timing 4. Fertiliser applications were made between 1 and 2 sample times and therefore the increase in these elements may be attributed to the addition of the fertilisers. All grain concentrations fell within normal limits with the exception of Fe, where concentrations were elevated under each treatment, indicating that this was not likely due to a treatment effect.

6.5 Chapter conclusions

The null hypothesis of this chapter cannot be fully accepted without further experimentation. Grain yield and element concentrations show conflicting results across the field and mesocosm experiments. The field experiments provided conflicting data regarding crop yields, with similar yields being achieved for the majority of crops, yet both higher and lower yields were also recorded. Mesocosm experiments showed that biosolids could produce similar yields to conventional fertilisers in the Broxton soils, but no difference in yield was recorded between any of the treatments for the Harper Adams University Farm mesocosms. This was attributed to sufficient levels of nutrients already being present in the soil prior to the experiment commencing. Differences in grain concentrations were recorded in both the Broxton field and mesocosm experiments, however, these concentrations were within normal grain limits. In each case of statistical significance, grain concentrations were higher under conventional fertilisers, with the exception of Mn in the Broxton mesocosm, where Zn was significantly lower in the grain from untreated pots. Heavy metal concentrations were not elevated in any grain from biosolids treatments compared to the commercially treated crops suggesting that biosolids did not increase heavy metal

concentrations above conventional fertilisers. Whilst this indicates that heavy metal bioaccumulation is not a problem, it also would suggest that biosolids are not suitable for biofortification. Further work should look to vary environmental conditions to analyses how this may impact on crop yields and grain heavy metal concentrations.

Chapter 7.

Conclusions and Suggestions for Further Work.

7.1 Conclusions

The work detailed in this thesis has profiled the Bestways Fertiliser, produced by Southern Water, using and developing a range of techniques, to assess their effects on soil chemistry and biology. This work provides a detailed account of how their application affects the soil environment over both the short and long term, in addition to their effect on crop production. As a consequence of different treatment methods, no batch of biosolids is ever identical and therefore they have the potential to impact the soil differently unless more is understood about them and their potential to cause harm. This could be achieved through analysing the soil and biosolids themselves, especially over time. Research often looks at singular scientific topics (e.g. soil chemistry) for associating a cause and effect, however, this thesis combined chemistry, biology and crop response to produce a more detailed analysis.

Thermally dried biosolids are advantageous in comparison to other biosolid products such as cake due to their lower water content, resulting in them being less dense. This allows them to be transported further at lower costs. The UK is subject to an East-West split regarding organic manures, with the dairy industry supplying sufficient organic amendments to meet farmer demands in the West, whilst the east is mainly reliant on anaerobic digesters in order to add organic matter back into soil. Thermally dried biosolids offer the possibility of redistributing surplus biosolids in the West to the East in a more economical manor. Yet the drying process involved in the production of these biosolids has the potential to alter the chemistry of the nutrients and contaminants, in addition to concentrating them. As of March 2018, no waste water treatment works (WWTW) produces thermally dried biosolids due to the expense of drying the material. Yet, some water companies are investing money into their production in the hope that enhanced treated biosolids will become the mainstream form that are applied to land. Thermally dried biosolids are classified as enhanced treated, therefore allowing them to be applied to a wider range of agricultural and horticultural crops. However, their use is still closely monitored over fears of contamination to both the environment and food chain.

A series of experiments were undertaken to understand the short and long term mobility of heavy metals in soils amended with biosolids. Firstly, a series of sequential extractions were undertaken to partition the heavy metals silver (Ag), arsenic (As), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn), into their chemical binding groups in two soil types. Results showed no significant increases in heavy metals in any binding group as a result of biosolid applications. Whilst this studied the implications associated

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with a single application of biosolids, it suggested that biosolids were not increasing any chemical binding fraction in the soil. This can most likely be attributed to the low biosolid:soil ratio when considering their volumes.

Grobelek and Napra (2015) suggested that the application of inorganic phosphate in the form of triple superphosphate (TSP) may help decrease the mobility of heavy metals from biosolids. However, such applications could only be made if there was no accumulation of phosphorus (P) in soils where biosolids were applied. A further sequential extraction was undertaken to establish if the applications of P, through biosolids, had increased P concentration in any fraction of the soil at the Broxton field site. The data provided here, would suggest that the application of biosolids to soils would increase the concentration of P above that of conventional fertilisers, (the majority being bound to Fe/Mn). Whilst this might not all be immediately available to the plant, there is an increased risk of elevated concentrations of P entering surface waters through erosion, which may result in eutrophication. The lack of increase in heavy metals found following a single application of biosolids to the two soil types, would also suggest that the application of TSP alongside biosolids would not be necessary.

Leaching experiments were carried out to assess the long-term mobility of elements in biosolid treated soils. In contrast to the sequential extraction experiments, leaching assessments show what heavy metals become available over time in water, which would have the potential for plants to translocate. Results from repeated wetting and drying investigations revealed that Fe, As and Pb concentrations all increased in water altered to pH 4. Stenhouwer *et al.* (2006) similarly showed an increase in heavy metal mobility at pH4 for Pb, Mn, Cu and Zn, however not with the elements studied in this thesis. Modified Dutch leaching column experiments showed contrasting results between soil types indicating that this was an influential factor. Broxton soils treated with 500 kg ha^{-1} total N recorded Zn concentrations more than three times higher than leachate from untreated soils. This would suggest that the potential for plants to translocate Zn into tissues would also be higher. Yield experiments in chapter 6 found no differences in Zn grain concentrations, suggesting that the plant may not be utilising this additional Zn, or that it was being translocated to a non-edible part of the plant. Tavarez et al. (2015) found that although some Zn was remobilised and translocated to the grain from other parts of the durum wheat plant during senescence, the majority of the Zn found in grain is directly soured from plant uptake.

Copper concentrations were also higher in leachate from Broxton and Harper Adams University Farm soils amended with biosolids compared with untreated soil. Copper deficiency is a prevalent issue for cereals in Europe (Alloway, 2008), and so biosolid applications may help rectify such issues. The cereals grown as part of this thesis did not show elevated concentrations of Cu in the grain however concentrations fell within normal

limits (Gramss and Voigt, 2013) for all treatments, indicating that the soil was not deficient of Cu or Zn.

Arsenic concentrations were also found to be elevated in leachate from the Harper Adams University Farm soils amended with biosolids. Whilst Cu is required in both human and animal diets for metabolic processes, As has no current recognised, beneficial effect on plants, animals or humans. However, historically As has been used as a growth promotor in animals. Again, no increase in As was recorded in the grain of plants grown using biosolids, indicating that the application of biosolids did not increase As concentrations in the edible parts of the plant. Concentrations recorded were within LOD and LOQ and so this difference is not attributable to the equipment used. However, it may be attributable to changes in bedrock in the field causing localised concentration changes. Whilst this increase in As was recorded in the leaching columns, pore water samples from As were only found to be higher in untreated soils of the Harper mesocosms. There was no evidence to suggest that this may result from problems with detection and therefore this indicates that further work would be needed to understand why this occurred.

Overall, whilst significant differences were recorded between treatments and leachate elemental concentrations, there appeared to be no accumulation of heavy metals in the grain above those recorded by untreated and conventionally treated crops.

Total organic carbon (TOC) from the wet and dry experiment showed significant positive correlations for Ag, As, Cu, Fe, Mn and Zn. Harper Adams University Farm soils showed a positive correlation between TOC and Fe and TOC and Mn. No other significant correlations were recorded, however, Cu was close to having a significant result. In contrast, the Broxton soils receiving a single application were found to have a positive correlation between TOC and Cu and TOC and Fe. Total organic carbon can act as a chelating agent, mobilising heavy metals into solution, causing them to leach from the soil. Whilst this is beneficial to the plant, as it removes toxic heavy metals away from the rhizosphere, it can result in grain nutrient deficiency and cause groundwater contamination (Sherene, 2009). Dissolved organic carbon (DOC) is a constituent of TOC and is used as a water quality indicator. Elevated levels of DOC can interfere with the disinfection processes for water treatment as well as reducing the aesthetic quality of the water being produced. The data presented here found no increase in total TOC leached when comparing the biosolids treated soils with untreated soils, suggesting that this may not be a concern associated with these biosolids.

Whilst the elements in soil solution pose the greatest risk to the food chain through plant translocation, this fraction has also been linked with microarthropod toxicity. Poulson *et al.* (1996) and Radha and Seenayya (1992) suggest that the elements held in soil solution pose the greatest danger to soil microorganisms. However, many microarthropods are detritivores and therefore would be aiding the breakdown of SOM to SOC. Chapter 3

showed the functional chemical groups of heavy metals and their concentrations. The organic matter/sulphide bound fraction is substantial in size when compared to the fractions that might be considered in soil solution (water extractable and exchangeable fractions). Therefore, it may be that microarthropods are mobilising this fraction into solution, indirectly, through decomposition of SOM. Whilst this does not appear to be detrimental at Broxton, as most microarthropods were recorded to have higher population numbers under biosolids treated soils, it may explain why the 400% treatment at HAU Farm often produced lower counts to that of the 100% application rate.

Earthworms and soil microarthropods are a critical part of an agricultural production system. By recycling plant material, they release nutrients into the soil for plants to use, whilst also improving soil structure and drainage. Agricultural cultivations often damage populations of organisms such as earthworms. Therefore, it is important to ensure adequate carbon is available to support such populations. The biosolids used in these experiments contained 61.4% organic matter, potentially providing a good food source for soil organisms. Whilst earthworm populations showed no difference when extracted from plots with differing fertiliser regimes (Chapter 5), their heavy metal content was not studied. The information collected from chapter 4 would suggest that the earthworms may experience a greater exposure to elements such as Zn in solution over time and therefore it may be possible that the heavy metals could bioaccumulate in their tissues. By digesting the earthworms upon collection, tissues could be analysed using ICP-MS to clarify if such differences exist. Kizilkaya and Hepsen (2004) found a decrease in dehydrogenase enzymes following the application of biosolids to soil. This was attributed to an accumulation of heavy metals in the tissue of the earthworms but was not studied. Therefore, further research should assess if population growth is being prevented through such accumulations.

Data collected from the Harper Adams University Farm respiration experiment suggested no increases in Adenosine triphosphate (ATP) content of the soil in the 12 months following applications. The problems associated with the technique used to analyse ATP was outlined in Chapter 5, however, if each sample was analysed in triplicate, the variation in results, may decrease providing a different result. Respiration was shown to increase under 100% and 200% application rates when compared to the control. Whilst this may suggest that there was an increase in the number of microorganisms respiring, it may also be a sign of stress, where fewer microorganisms are respiring harder.

Microarthropod counts for Broxton and Harper Adams University Farm sites allowed the impact of biosolids applications on soil biology to be quantified. Harper Adams University Farm QBS scores showed no difference between application rates of biosolids, indicating that the increased respiration rates recorded under the 100% and 200% application rates were not only due to microarthropods. Broxton soils saw a higher QBS score under biosolids amended soils, indicating that biosolids may be supporting

microarthropods better than conventional or inorganic fertilisers. The contrasting results between the two sites may be a result of differing numbers of applications and reproductive strategies. K reproductive strategists may not have had sufficient time to benefit from the additional OM applied to the soil at Harper Adams University Farm site and therefore population increases were not observed.

Soil microarthropods and microorganisms have the potential to increase nutrient availability and thus may impact on crop production (Trivedi *et al.*, 2017). A series of field experiments were carried out, studying the effect on crop yield and the associated heavy metal contamination levels in grain. Following commercial practices as closely as possible, the experiments were a continuation of previous work (Deeks *et al.,* 2013), which suggested that biosolids could produce similar yields to that of commercial inorganic fertilisers. The results from this experimental series overall, agreed with the previous investigations. Of the 6 crops harvested, 4 produced statistically similar yields to that of commercial inorganic fertiliser, whilst one (triticale (*Triticosecale*)) produced a greater yield and another (spring barely (*Hordeum vulgare* L.)) produced a lower yield. Bestways biosolids were structurally very hard, with some granules requiring up to 10 kg of force to disintegrate them. This may have resulted in only the outer surfaces releasing nutrients, especially in dry years, and may explain why reduced yields were experienced during the field investigations. Biosolids are known as a slow release fertiliser, and applications in previous years may have impacted on spring crops (AHDB, 2017). This may suggest that the wet and dry leaching tests show a worst-case scenario of what can be released by biosolids over time.

Grain heavy metal concentrations were found to statistically differ between treatments at the Broxton field site, with Ag, As, Fe, Pb and Zn concentrations being higher in the conventionally treated grain of triticale. Copper and Mn concentrations were near significance in the conventionally treated grain also. No heavy metal concentration differences were recorded in the spring barley 2012/2013 grain, but a higher concentration of Mn was recorded in the conventionally treated grain from 2013/24014. Whilst no heavy metals were applied intentionally through the inorganic fertiliser applications themselves, contamination of inorganic fertilisers is being increasingly recorded, monitored and regulated. For example, Cd concentration in phosphate fertilisers is already restricted in many countries. This, in addition to natural variation in soil heavy metal concentrations, may have resulted in the results recorded. This data would suggest that the increased microarthropod QBS scores recorded at Broxton, did not impact on the crop yield as has been found by other research (Trivedi *et al,* 2017 and Lloyd, 2017).

In Broxton mesocosm investigations, reduced concentrations of Mn were recorded in the grain grown in untreated soil. There were no untreated plots established at Broxton and therefore a direct comparison between the two could not be made, but it would support

the hypothesis that the conventional treatments were supplementing some heavy metal concentrations. Harper mesocosms showed no significant differences between treatments.

Overall, results indicate that application of biosolids does not enhance grain metal contents and suggests that repeated applications may not be a suitable method for trace metal biofortification of grain. Biosolids contain metals, some of which have a protective role for health (e.g. Fe and Mn).

Overall, the work suggests that the risk to the food chain via accumulation would be limited and therefore should not be a factor when considering their application. However, the data would suggest that application of these biosolids, would not alleviate the hidden hunger issue as detailed in Chapter 1. Elemental concentrations were correlated with TOC, with increased heavy metal mobility found to correlate with increasing TOC. The lack of uptake into the grain, would suggest that the elements may be leached out of the rooting zone of plants, possibly leading to the contamination of ground waters.

7.2 Future work

Biosolids, along with other organic manures, are very variable. Due to input changes and treatment differences at WWTW, nutrients and heavy metal contents for each batch will inevitably vary. Therefore, further work should consider profiling other granulated products, comparing results to establish if the biosolids used here differ in any way different to others. Analysis of their differing treatment techniques may also indicate if any one method has additional benefits or disadvantages over any other. It is believed that similar products to Bestways Fertiliser are in development, but no direct comparisons are currently available. One biosolid based product is available. Milorganite is a granulated product derived from biosolids. In contrast to the Bestways fertiliser biosolids, Milorganite is not a true biosolid product as it is formed from additional bacteria added to wastewater during the treatment process. These microbes die once there is no longer any nutrients in the wastewater and they are then harvested and granulated in a similar way to the biosolids discussed here (Milorganite, 2018). This poses the possibility of decreasing the heavy metal load, whilst using the nutrients from the biosolids. However, other additional benefits, such as addition of OM would not be seen from such practices. With other thermally dried biosolids currently in development, a comparison between the three would help understand how the biosolids tested here would relate on a larger scale.

Earthworms were quantified at the Broxton field experimental site, whereby earthworms were collected and weighed from the conventional and biosolids treated plots. Whilst this provides valuable biomass data, it does not indicate the impact of the

applications on earthworms at a species level. Different species are important for different ecological roles. For example, *L. terrestris* has been linked with environmental remediation of contaminated soils (Sizmur *et al.,* 2011). No differences were recorded between the earthworm numbers of biomass in the experiments detailed in this thesis, but further work could have assessed the heavy metal concentrations in the earthworms and whether this affected species dynamics.

Whilst the data suggests a low risk to the food chain through the application of biosolids, their full environmental impact has not been quantified. Several elements have shown greater mobility which has been attributed to TOC concentrations. However, the abundance of an element in soil does not necessarily indicate the level of risk it poses to the environment. An elements oxidation state indicates its potential to form complex chemical species through processes such as acid-base reactions. Further work to establish the oxidation state of the elements would further help profile the full risk posed through applying these biosolids. Donner *et al.* (2011) found differences between the Cu and Zn oxidation states depending on the age of the biosolids, with Cu (I) and Cu (II) sulphides being most dominant in fresh biosolids. Organic matter bound Cu (II) was found to be more dominant in the dried biosolids. This would expand on the results provided in Chapter 3 where the humic and sulphide bound fractions would have likely been extracted to provide one value. Vodyanitskii (2016) details two contrasting standards used to allocate a hazard level to a heavy metal contaminant. Whilst the Russian and Dutch standards differ substantially with regards to what elements are considered the most and least hazardous when found in soils, no further details as to the oxidation state of elements has been used.

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Appendix 1.

Table 1. Soil temperature data recorded at 10 cm depth during the respiration experiment.

Days	December 2016	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	August 2017	September 2017	October 2017	November 2017
1	1.4	5.7	6.9	3.9	9.6	11.0	16.3	15.7	15.8	14.7	14.1	9.3
2	4.4	2.0	8.0	5.4	8.5	10.5	16.7	16.6	15.9	14.5	13.1	9.1
3	4.7	1.7	5.6	5.6	7.9	10.2	15.5	17.6	16.4	14.6	11.1	10.1
4	2.3	4.8	3.4	6.1	10.2	11.5	14.7	17.9	16.4	17	11.2	10.6
5	2.0	1.5	3.0	5.6	8.1	10.9	14.5	17.5	16.7	17	12.3	7.6
6	3.5	1.3	2.3	3.9	8.0	11.1	13.7	19.4	15.0	14.3	10.8	5.5
7	6.4	5.1	4.1	5.5	9.1	10.8	13.4	19.5	15.7	14.6	12.8	9.2
8	9.1	5.4	4.3	8.1	8.4	11.1	15.0	18.2	16.1	15.1	12.0	5.7
9	8.4	6.3	3.4	7.0	10.0	10.7	14.4	19.7	15.2	13.9	13.1	8.7
10	8.6	5.1	2.8	7.8	10.3	10.1	15.3	18.0	15.3	13	13.4	8.4
11	5.7	6.0	2.8	8.5	9.2	11.5	15.3	17.2	15.6	14	13.7	8.5
12	4.7	4.0	3.5	9.4	10.1	12.7	14.7	16.5	16.3	12.7	10.4	7.8
13	6.1	2.3	3.7	6.0	9.3	12.8	15.0	17.2	15.2	12.7	13.3	4.6
14	8.0	2.1	2.8	8.1	9.8	12.6	15.4	16.9	15.9	12.9	15.0	8.1
15	7.6	4.6	5.9	6.8	9.9	12.6	16.5	17.3	15.7	12.3	14.0	8.7
16	7.3	7.0	4.1	6.7	9.6	14.0	16.4	18.4	15.1	13.2	14.2	8.7
17	7.2	6.3	4.9	5.9	9.9	13.9	18.2	15.8	16.5	12.2	12.5	5.6
18	4.8	6.2	6.7	9.4	8.2	11.6	19.0	18.1	16.0	13.1	10.9	6.4
19	5.5	6.3	6.4	9.9	9.1	13.0	20.2	19.5	15.5	12	12.0	5.9
20	6.2	4.1	8.1	9.8	10.7	12.2	20.9	17.9	15.3	13.7	12.3	8.2
21	5.6	2.1	8.5	5.8	11.3	12.2	20.5	16.8	16.1	14.9	11.7	10.2
22	2.8	2.3	9.0	6.8	10.5	13.2	20.3	15.5	17.0	10.6	10.7	10.9
23	4.8	2.5	8.1	6.1	10.2	13.8	17.8	15.9	17.7	14.2	11.3	8.9
24	4.9	1.9	4.5	5.6	10.9	14.7	18.1	17.3	15.9	13.5	12.6	5.6
25	8.6	3.3	7.0	5.0	8.8	16.3	18.2	17.1	15.7	14.7	12.3	4.5
26	5.5	2.6	7.5	5.1	8.4	16.7	16.6	17.9	17.1	15.4	11.6	4.8
27	2.6	1.1	6.1	6.7	7.8	16.9	16.9	15.5	15.5	14.3	9.7	7.0
28	1.9	4.2	4.0	7.3	9.9	16.2	16.3	15.8	17.1	14	8.6	4.9
29	1.9	2.2	-	9.6	10.6	16.6	15.0	15.4	17.9	13.9	11.8	3.5
30	1.6	5.0	-	10.6	10.1	15.8	15.6	16.1	15.8	11.5	7.3	2.6
31	3.9	5.6	-	10.7	-	15.4	-	15.3	13.8	-	9.1	-
Average	5.1	3.9	5.3	7.0	9.5	13.0	16.5	17.2	16.0	13.8	11.9	7.3

Table 2. Precipitation data recorded during the respiration experiment

Days	December 2016	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	August 2017	September 2017	October 2017	November 2017
1	0.0	3.5	1.0	4.5	0.8	0.6	0.0	0.6	3.8	0	0.0	0.0
2	0.0	0.0	0.0	0.2	0.0	0.0	3.2	0.0	0.6	0	0.0	0.2
3	0.0	0.0	4.7	5.1	0.0	0.0	0.2	0.0	0.6	1.2	0.0	10.4
4	0.0	0.0	0.8	3.7	0.0	0.0	0.4	0.0	0.0	0	1.6	2.6
5	0.0	0.0	0.0	2.0	0.0	0.0	19.5	0.0	9.2	3.8	0.2	1.6
6	0.2	4.1	4.5	3.9	0.0	0.0	2.8	0.0	11.8	0	0.4	0.0
7	5.5	0.0	1.2	2.8	0.0	0.0	2.4	0.0	3.2	1.8	0.8	8.2
8	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	11.6	0	0.2	0.0
9	3.9	4.3	0.0	0.0	0.0	0.0	1.6	2.2	0.0	1.8	0.0	0.4
10	5.9	0.0	0.0	0.0	0.0	0.0	0.6	2.4	0.0	4	0.0	5.6
11	0.4	0.0	0.8	0.0	0.0	0.2	0.4	13.6	0.0	0.6	1.6	3.0
12	1.2	4.9	0.0	0.2	0.8	4.5	0.0	0.0	0.0	2.2	0.0	0.0
13	1.4	0.6	0.0	0.0	0.0	3.5	0.0	2.0	1.8	0.8	0.0	0.8
14	0.0	9.5	0.0	0.0	2.4	0.0	0.0	1.4	2.0	1	0.0	0.2
15	2.4	6.3	3.0	0.0	0.0	4.1	0.0	0.4	0.0	0	0.0	0.2
16	1.0	0.2	0.0	0.6	3.9	3.0	0.0	1.0	18.6	0	0.0	0.8
17	0.0	1.6	0.2	1.2	0.4	5.7	0.0	0.0	1.2	3	0.0	0.0
18	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	5.4	1.2	0.0	0.0
19	0.2	0.4	0.0	5.5	1.0	0.0	0.0	2.4	2.0	0	25.0	2.2
20	0.0	0.0	0.6	4.7	0.0	8.5	0.0	0.0	4.8	0.2	2.6	0.0
21	1.2	0.0	2.8	8.5	0.0	0.0	0.0	16.6	0.0	2.8	0.8	3.4
22	0.0	0.4	3.7	1.0	0.0	0.0	0.0	2.6	0.0	0.2	0.4	6.4
23	2.0	0.0	4.9	0.0	0.0	0.0	0.0	1.0	0.0	0	0.2	0.0
24	0.0	0.0	2.0	0.0	1.4	0.0	0.0	0.0	0.0	3.2	3.6	1.0
25	1.2	0.0	2.8	0.0	1.0	0.0	1.8	8.4	0.0	0.8	0.0	2.0
26	0.0	0.0	5.9	0.0	0.0	0.2	0.0	0.6	0.0	0	0.0	2.0
27	0.0	3.2	4.3	0.0	1.4	0.0	4.5	1.2	0.0	9.4	0.2	0.6
28	0.0	0.8	7.1	3.2	0.0	2.0	0.6	11.2	0.0	0	1.0	0.2
29	0.0	4.5	-	1.4	0.0	0.6	5.1	2.2	0.2	0.4	0.0	0.0
30	0.0	0.4	-	0.4	10.4	0.0	0.2	3.6	0.4	6.2	0.0	0.0
31	2.2	12.6	-	1.0	-	0.0	-	0.6	1.6	-	0.0	-
Average	0.9	1.9	1.8	1.6	0.8	1.1	1.5	2.4	2.5	1.5	1.2	1.7
Total	28.8	57.5	50.3	50.1	23.5	32.9	43.9	74.0	78.8	44.6	38.6	51.8