Ecological risk characterisation of novel soil amendments: assessing impacts on soil properties, earthworms and soil microbial functions

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PhD in Environmental science

March 2023

Abstract:

Land application of water treatment residuals (WTRs), a by-product of drinking water treatment, is increasingly common due to tightening landfilling regulations and attempts to embrace greener practices. In this thesis a thorough literature review into WTRs was firstly conducted, with a focus on their end of life uses and any effects they might have on earthworms, soil microbial populations and element bioavailability. The review identified clear knowledge gaps in relation to any such effects, hence these were investigated through a series of interrelated field, semi-field and laboratory studies encompassing WTR-treated and control sites in Wales; indoor and outdoor mesocosms, column leaching and batch extraction experiments, and analysis of WTR and WTR treated soils' bacterial communities using 16S rRNA sequencing. Key receptors were observed and analysed throughout including earthworms, soil porewater chemistry, bacterial populations, and soil respiration. It can be concluded that the spreading of WTRs to land produced no meaningful, measured negative impacts on terrestrial ecology or soil chemistry when following common practise and respecting current regulatory limits. Key findings include confirming that Al leaching from WTRs is unlikely to occur under common natural conditions, identifying that WTRs can immobilise organic carbon in the soil column, finding that WTRs are unlikely to adversely affect microbial populations, and concluding that earthworm populations were not negatively affected by field or laboratory application of WTRs to soil. Most of the experiments within this thesis were short-term (<1 month), leaving room for longer-term experiments to confirm the permanence of this lack of negative effects. Further avenues for research include exploring the carbon capture potential of WTRs in soils, investigating the effects of WTRs on other terrestrial invertebrates, and exploring the effect of storage time and seasonality on WTRs properties.

Acknowledgements:

Firstly, I’d like to thank my ever-diligent supervisor Dr Ian Oliver for helping me all the way through the process of my PhD. He has always been there when I had stupid questions or when anything went wrong such as the great worm escapes of 2018 and 2019. I’ve learnt a lot from him on this journey and wouldn’t be where I am today without him.

I’d also like to thank our colleagues at 4R who have helped along the way with sample collection, being our port of call for industry related questions, and facilitating our communication with the water treatment sector and landowners, Becky Wheeler, Adam Stone, Paul Whyatt, Chris Ash and Richard Evans. In addition, I want to thank Becky Wheeler, Adam Stone, Richard Evans and Adam Hopley for looking after me during my work placement with 4R.

None of the mess that I’ve made, otherwise known as PhD experiments, would have been possible without the help of the support staff in the GGE department, Ian Wilshaw, Richard Burgess, Luke Hobson, Gwyn Jones, Adam Jeffreys, and David Wilde.

For their help with my 16S rRNA sequencing chapter I would like to thank Helen Glanville for her feedback and help with method development and Daniel Tonge for sharing his expertise in DNA extraction and data analysis.

I must also thank Huw and Rhian Price, and Jamie Mayo for allowing me to access their farmland to conduct earthworm collection, without which I would not have been able to complete a large portion of my PhD work.

For his help in training me in earthworm identification and allowing us the use of his electrical octet device I would like to thank Olaf Schmidt at the University College of Dublin.

This work is part of a research project funded by the UK National Productivity Investment Fund (NERC grant NE/R007314/1).

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Chapter 1 - Introduction

Thesis overview and rationale

Water treatment residuals (WTRs) are a by-product produced during the treatment of drinking water. These sludge-like materials form in the flocculation and coagulation process triggered by aluminium or iron based salt additions to raw drinking water, removing impurities from the water as they form. Traditionally, these WTRs were dredged from the reservoirs or treatment tanks where they were generated and were disposed of via landfill but, over recent decades, considerable research effort has gone into finding uses for them that are more environmentally beneficial. These include studies of WTRs use for nutrient sorption from solution (Gibbons and Gagnon, 2011, Maqbool et al., 2016, Chiang et al., 2012), incorporation into reed bed systems (Zhao et al., 2013, Doherty et al., 2015, Xu et al., 2016), construction (Shamsudin et al., 2017, Hegazy et al., 2012), land application as a soil amendment (Bugbee and Frink, 1985, Gallimore et al., 1999) and its subsequent effect on plant growth and grazing animals (Madison et al., 2009, Maurice et al., 1998). However, there are many research gaps and unknowns that persist, and which need to be addressed so that the full potential benefits of utilising these WTRs can be achieved and understood. For example, very few studies have explored or quantified the degree to which chemical and physical properties of WTRs vary over a large sample size or geographical range, even though such variations have been shown to be important (Babatunde and Zhao, 2007, Ippolito et al., 2011, Turner et al., 2019). From the point of view of using WTRs as a soil amendment, which is the focus of this thesis, there are many pressing questions including how applications of WTRs influence soil ecology, particularly earthworms but also soil microbial ecology, soil porewater chemistry, and how WTRs influence element mobility within soils.

Therefore, the work reported in this thesis aimed to explore the physical, chemical, and biological characteristics of WTRs from a variety of sources in the UK and, moreover, sought to investigate the influence of WTRs applications on soil chemistry and ecology via field site investigations and experiments in semi-field mesocosms and laboratory trials. The principal objectives of the project were to:

- Conduct investigations on field sites where WTRs have been applied (along with matching untreated control sites) to determine the extent to which WTR applications have influenced earthworm population density and species diversity and the general level of microbial activity when applied at typical agricultural rates.
- Investigate possible application effect thresholds by conducting laboratory and semi-field mesocosm experiments to identify any changes in earthworm survival and growth, soil porewater chemical parameters and microbial respiration when WTRs are applied to soils at different rates.
- Determine how WTRs from around the UK vary in their amounts of mobile (i.e. leachable and potentially bioavailable) elements that may contribute nutrients and/or toxicants to soil systems.
- Investigate how microbial diversity differs between WTRs from different sources and whether WTR applications to soils alters soil microbial diversity.

Each chapter focuses on addressing one or more of these aims and objectives, as outlined in the chapter-by-chapter summary below.

Chapter summaries

Chapter 2 is a comprehensive review of the literature on water treatment residuals and their beneficial reuse, encompassing production of WTRs, their physicochemical properties, disposal and reuse methods and possible environmental and ecological impacts, and concerns and unknowns when they are applied to land as soil amendments. Because of their importance to soils and because they feature prominently in the investigations and experiments reported in this thesis, there are also substantial components of the literature review devoted to earthworms and their ecology, element bioavailability, and soil-atmosphere gas fluxes related to bacterial and other soil processes. The earthworm sections discuss ecological subgroups and species distributions in the UK, earthworm sampling and identification methods, their interaction and role within soil systems, and earthworm ecotoxicology bioassays. The element bioavailability section discusses the concept of bioavailability, its different definitions, and measurement techniques. The soil-atmosphere gas flux section focusses on soil greenhouse gas emissions, pathways of soil-atmosphere gas flux interaction, and the measurement of soil gas fluxes. The WTR section of the literature review has been published as a review article (Turner et al., 2019) and has a high citation rate.

Chapter 3 is a separate, stand-alone literature review on the benefits and risks of land application of another waste product used as a soil amendment; paper pulp waste. This work was conducted during a period of government enforced cessation of field and laboratory work (lockdown) in response to the covid-19 pandemic. It is written in the style of a review article and has been accepted for publication (pending revisions) in the Journal of Environmental Management. The chapter discusses the feasibility, advantages, and limitations to further uptake of land spreading practises of the material with an emphasis on impacts on (and unknowns concerning) soil chemical and physical properties after land application.

Chapter 4 is based upon field studies exploring the impacts of land spreading WTRs. This investigation was conducted at three farm sites; two in North Wales and a third in South Wales. Both sites in north Wales were examples of sites where WTR spreading had recently begun within the prior 3 years, whereas the site in South Wales had a long history dating back 20+ years. At all of these sites there were adjacent control sites, either in the same field or the adjoining field, where WTRs had not been applied. During all the site visits, earthworm sampling and gas (CO_2) flux measurements were conducted to build a picture of the effects of spreading WTRs on soil ecology and general microbial activity level. Gas flux was measured using a chamber method. Two earthworm survey and collection methods were employed to extract earthworms from soil; the commonly used hand sorting method and the more specialised electrical octet extraction method. Adult and juvenile earthworms extracted from a sampling point were counted, with juveniles then released and adult earthworms subsequently preserved for identification to species level for community structure and diversity comparisons. Covid-19 disruptions badly impacted the planned activities for this part of the study, with the outcome being that work at two of the sites was limited to initial site surveys only. At the third site (one of the locations in North Wales) three site visits over a one-year period were completed hence the chapter focuses mainly on this location. Comparisons were be made between treated and untreated control areas in terms of earthworm abundance, diversity and community structure, as well as in relation to soil gas flux. Additionally, comparisons were made between the earthworm results generated by the two earthworm collection methods.

Chapter 5 focusses on the leachability (potential mobility) of elements in WTRs and in soils treated with them. Batch extraction procedures on a large range of WTRs obtained from different locations around the UK, which varied in raw water source type and principal flocculant salt used, were conducted using 0.01 M $CaCl_2$ as extracting reagent. Similar procedures were also conducted on treated and untreated farm soils. Additionally, column leaching experiments were conducted on soils treated with WTRs (and on untreated controls) to examine element mobility in a setting more closely approximating field conditions (i.e. as opposed to the potential mobility determined in the batch extraction procedure). For the column experiments, two rates of amendment of WTRs (5% and 10% w/w) were applied to Kettering Loam soil using two application methods; surface application only and surface application followed by incorporation (both of these methods are commonly employed in agriculture depending on land usage and WTR water content). The chemical composition of leachate was determined via inductively coupled plasma (ICP) spectrometry to assess whether there were any elements of concern being leached from WTRs and at what concentration these were present in leachate. Potential toxicity of leachate constituents were also directly assessed via ecotoxicological assays with aquatic invertebrates. Furthermore, in addition to evaluating mobility of potential toxicants, the leaching of nutrients from WTRs was explored as the materials are often promoted as providing a nutrient addition to soil. The column leaching experiments also allowed for the comparison of element sorption capabilities of WTRs which are widely reported in the literature. These column leaching experiments are also complimented by total organic carbon measurements of leachates, thus enabling an assessment of carbon mobility and export following soil treatment with WTRs. This study is pertinent, as previous studies have highlighted the variability in WTRs' composition and within the current literature no studies exist which have analysed such a large variety of WTRs.

Chapter 6 is based upon a series of mesocosm experiments, which were conducted to investigate the effects of WTR application on earthworms, microbial activity and porewater chemistry. Experiments were split between an outdoor enclosure and an indoor climate-controlled cabinet. The initial outdoor experiment is written in the style of a journal article and has been published (Turner et al., 2021). The two experiments (outdoor and indoor mesocosms) were complimentary to each other; the outdoor experiment better simulated field conditions, while the climate control chamber experiments allowed for more constraint on variables such as temperature, humidity, and light cycles for better determination of the controlling variables if differences were discovered between untreated soil and the various treatments. In the outdoor experiment, mesocosms were filled with 1.5kg of substrate which consisted of Kettering Loam treated with 0, 5 and 10% w/w of two different WTRs, one aluminium based and one iron based, there were also additional mesocosms containing treated and untreated farm soils collected from the field sites. During the indoor experiment Kettering loam was treated with 5, 10 and 20 % w/w of Al or Fe WTRs. Using regression analysis, t-test and ANOVA tests, and through examining trends and differences in earthworm weight change, soil CO_2 efflux and porewater chemistry parameters, variations between laboratory amended and unamended soils and between treated and untreated farm soils were explored.

Chapter 7 is dedicated to exploring the results of sequencing extracted DNA from WTRs and WTR treated farm soils (with a control soil for comparison) in order to identify and evaluate variability in bacterial community composition in WTRs from different locations and to determine whether WTR applications lead to bacterial composition shifts in treated soils. The extracted DNA had the V4 region of the bacterial 16S rRNA gene amplified and analysed by Illumina MiSeq sequencing. The structure of bacterial communities was compared between WTR samples and between treated and untreated soil samples through correspondence analysis and the influence of environmental variables of WTRs such as the water source and main coagulant used in treatment were explored. Samples were checked for the presence of potentially harmful pathogens and other bacteria. Additionally, canonical correspondence analysis was conducted on WTR samples to explore the influence of WTR available elemental concentration and pH on the bacterial community structure.

Chapter 8 is a general conclusions chapter that brings together and presents the main findings of the study. It also sets out the many future avenues of research that have been identified as important through the course of this study.

The project was funded via a UK National Productivity Investment Fund grant (Natural Environment Research Council, NERC, grant NE/R007314/1) with additional financial and in-kind support from project partner 4R (a waste recovery and land management company) hence, under the framework of the funding scheme, the project included a placement at 4R to participate in multiple aspects of the company's operations. Further planned physical placements were necessarily cancelled on account of government stipulated covid-19 response lockdowns, and in their place a remote desk study was conducted on the company's behalf that examined and summarised the literature on the use of another waste product of interest; coal mine ochre waste. That report does not form part of the thesis but may be obtainable via request to 4R.

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Chapter 2 - Literature review

Potential alternative reuse pathways for water treatment residuals: remaining barriers and questions – A review

Abstract: Water treatment residuals (WTRs) are by-products of the coagulation and flocculation phase of the drinking water treatment process that is employed in the vast majority of water treatment plants globally. Production of WTRs are liable to increase as clean drinking water becomes a standard resource. One of the largest disposal routes of these WTRs was via landfill, and the related disposal costs are a key driver behind the operational cost of the water treatment process. WTRs have many physical and chemical properties that lend them to potential positive reuse routes. Therefore, a large quantity of literature has been published on alternative reuse strategies. Existing or suggested alternative disposal routes for WTRs can be considered to fall within several categories: use as a pollutant and excess nutrient absorbent in soils and waters, bulk land application to agricultural soils, use in construction materials, and reuse through elemental recovery or as a wastewater coagulant. The main concerns and limitations restricting current and future beneficial uses of WTRs are discussed within. This includes those limitations linked to issues that have received much research attention such as perceived risks of undesirable phosphorus immobilisation and aluminium toxicity in soils, as well as areas that have received little coverage such as implications for terrestrial ecosystems following land application of WTRs.

Keywords: Water treatment residuals, Reuse, Sorption, Alum sludge, Land spreading

Water treatment residuals and their end of life uses

2.1. Introduction

To meet the water requirements of a growing population, and with escalating efforts to deliver clean drinking water to the estimated 29% of the global population that do not currently have access to it (WHO, 2017), there is an ever-increasing demand for clean and safe drinking water. In order to supply this increasing demand, raw water must be treated to remove any waterborne microorganisms, excess mineral content and suspended sediment. One of the most common methods employed to remove suspended particles and colloids from raw water is the addition of metal salts to initiate a coagulation-flocculation process. However, this process results in the generation of vast quantities (generally between 10-30 ml of WTRs for every litre of water clarified) of a sludge-like waste (or by-product) known as water treatment residuals (WTRs), which require an outlet for their disposal or end use (Dassanayake *et al.*, 2015).

Previous reviews have described some of the potential beneficial uses for WTRs considered up to that time, the most recent of which being in 2011, along with the research that had been conducted into their uses (e.g. Babatunde and Zhao (2007); Ippolito *et al.* (2011)). However, considerable advancements in the testing and application of WTRs have been made in the past 10 years. To illustrate, a search on the Web of Science Core Collection for "water treatment residual*" OR "water treatment sludge" for the period 2008-2018 returned >400 articles. Recent reviews have focussed on various aspects of the WTR reuse; ranging from use as a sorbent (Ippolito *et al.*, 2011), coagulant recovery (Keeley *et al.*, 2014) and the broader scope of WTR utilisation at international levels (Ahmad *et al.*, 2016; Zhao *et al.*, 2018). Therefore, this review aims to expand on these previous works and to produce a comprehensive picture of the generation of WTRs and possible options for end markets, with a particular focus on options that result in environmentally beneficial use. The barriers to widespread adoption of these applications will be examined, including identification of the main knowledge gaps that maintain the uncertainty and concern surrounding potential environmental impacts.

2.2. Production

Being by-products of the coagulation-flocculation process used to remove suspended and colloidal particulates from drinking water, the composition of WTRs is principally determined by the type of coagulants employed and the constituents of the raw water that is being treated. The most common of these coagulants are aluminium sulfate ($Al_2(SO_4)_3{\cdot}14H_2O$), commonly known as alum, and the iron-based salts ferric chloride ($FeCl_3$) and ferric sulfate ($Fe_2(SO_4)_3{\cdot}9H_2O$). When these Al and Fe salts are applied as coagulants in the presence of alkaline conditions, their Al/Fe ions are hydrolysed to form hydroxide precipitates that remove impurities via co-precipitation, sorption, flocculation and settling (Eq 2.1-2.3) (Dassanayake *et al.*, 2015). The process involves formation of positively charged complexes that are able to sorb and flocculate negatively charged organic impurities effectively by overcoming their initial repelling characteristics (Figure 2-1). Depending on the design of a particular water treatment plant, removal of the impurities then proceeds via simple flocculation and settlement under gravity or via a more active process of filtration.

$$Al_2(SO_4)_3 \cdot\ 14\,H_2O + 6(HCO_3)^- \rightleftharpoons\ 2Al(OH)_3 + 6CO_2 + 14H_2O + 3SO_4^{2-} \qquad (2.1)$$

$$Fe_2(SO_4)_3 \cdot 9H_2O + 6(HCO_3)^- \rightleftharpoons 2Fe(OH)_3 + 6CO_2 + 9H_2O + 3SO_4^{2-} \qquad (2.2)$$

$$FeCl_3 \cdot 6H_2O + 3(HCO_3)^- \rightleftharpoons\ Fe(OH)_3 + 3CO_2 + 3Cl^- + 6H_2O \qquad (2.3)$$

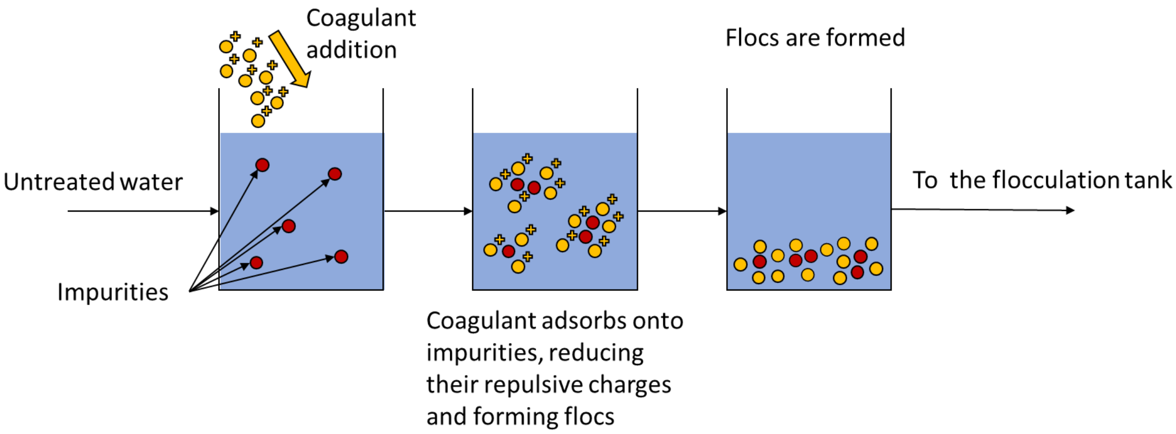

Figure 2-1. A simplified diagram of the coagulation process employed during drinking water treatment

Freshly produced WTRs are in a liquid state with high water content (2- 4% solids) making them expensive to transport and challenging to handle, particularly in the volumes generated by large-scale water treatment plants (i.e. >1000 t y^{-1} of liquid WTRs produced), hence dewatering or thickening processes are commonly employed (Dassanayake *et al.*, 2015). Where space availability permits, common dewatering strategies include the use of drying lagoons or beds (Walsh, 2009). Where space is less available, dewatering via centrifugation and/or belt presses is often employed. Generally, after the full mechanical dewatering process (e.g. Figure 2-2) the solids content of these WTRs increases to between 17% and 35% solids (Dassanayake *et al.*, 2015). However, this treatment tends to be a more energy intensive and thus more expensive process, leading to many smaller water treatment works (those without the space for drying lagoons) leaving WTRs in a liquid (2- 4% solids) state. The term 'water treatment residual' is a holistic term that applies both to the material when it contains 2 to 4% solids as well as when in a dried or partially dried condition. These terms are generally used as such by engineers and waste management companies. However, within the scientific literature it tends to be the dewatered product only that is most widely referred to as WTRs. This discrepancy in definition highlights some of the uncertainties associated with the use and application of WTRs and with how they are discussed in the literature.

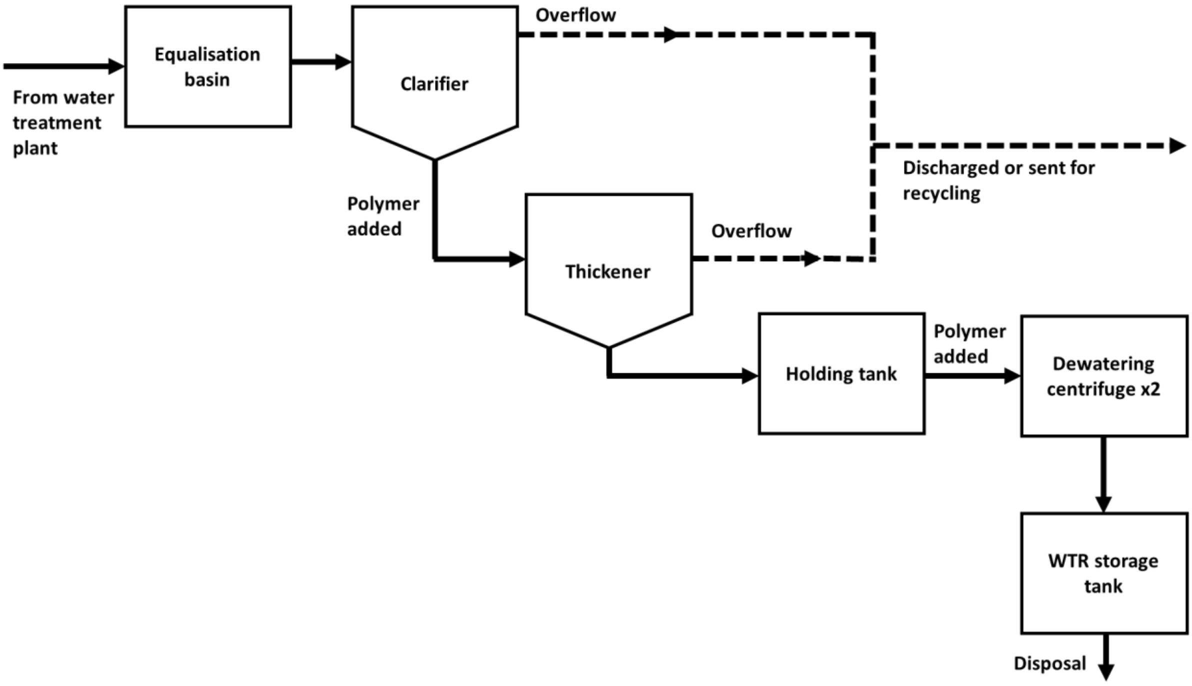

Figure 2-2. A typical drying process for treatment residuals, (adapted from USEPA (2011)The utilisation of coagulants during the water treatment process is very common, e.g. it has been adopted by approximately 70% of the drinking water treatment works in the USA (Keeley et al., 2014). This widespread use of the approach has led to streamlining for cost effectiveness. For example, dose optimisation has reduced Al coagulant inputs from ~50 mg l^{-1} *in the 1970s to concentrations as low as 2–5 mg* l^{-1} *today, while modern Fe coagulant dosages range from 4–10 mg* l^{-1}*. These doses depend on the specific coagulant and the turbidity and pH of the source water (Keeley et al., 2014; WHO, 2017). While the literature on this topic generally omits or only mentions the addition of further coagulant aids, such as activated silica and organic polyelectrolytes, these also play a key role in aiding the coagulation-flocculation process (Matilainen et al., 2010). As the process has been optimised over time, it is difficult to see any future reductions in the quantities of WTRs produced and thus their generation is only likely to increase with the increasing demand for clean drinking water.*

In the UK for example, 107,000 t and 165,000 t of alum and ferric based coagulants are used per annum respectively (Keeley *et al.*, 2014). However, there is limited information available in the form of national production figures for WTRs, with recent estimates only available for their production in a few countries or regions (Figure 2-3). Unfortunately, many of these figures are, as previously mentioned, only estimates by government bodies, NGOs or water companies that may not be indicative of the full extent of production. Limited production data may be due to a variety of factors, such as i) differing practices across treatment plants and regions that result in WTRs of differing water contents that cannot easily be directly compared or summed in terms of total mass, ii) varying reporting requirements in different regions and countries, and iii) commercial interest issues that encourage resistance in releasing WTR production, disposal and recovery figures. Additionally, production figures rarely discuss the water content of the WTRs produced, leaving the weight of solids ambiguous. Nevertheless, some very useful sub-national case studies do exist in the literature (Table 2-1).

Table 2-1. Examples of sub-national water treatment residual production figures.

Location of WTP	Coagulant	Raw water source	Water treated (Ml d^{-1})	WTR production (t y^{-1})	Water content %	Reference
Ghaziabad and Noida, India	Poly-aluminium chloride	River Ganges	120 Ml d^{-1}	28,100-29,700	2.30–10.65	(Ahmad *et al.*, 2017)
Puglia and Campania, Italy	Aluminium and polyvinyl organic flocculent	Reservoir, river and aquifer	950 Ml d^{-1}	25,200	70-80	(Makris *et al.*, 2004a; Caniani *et al.*, 2013)
Dublin, County Kildare	Aluminium sulphate	Reservoir	230 Ml d^{-1}	16400-27400	72–75	(Yang *et al.*, 2006b; Babatunde and Zhao, 2010)
New York State, USA	Aluminium sulphate	Lake	190 Ml d^{-1}	990	Not specified, calculated total suspended solids	(Gruninger, 1975)

These figures highlight the varying amount of waste produced in different regional settings; in the case of Ghaziabad and Noida, where greater quantities of WTRs are produced per litre of water treated (after accounting for water content), a large proportion of WTRs are produced during the monsoon season when the amount of suspended sediment in raw water is higher (Ahmad *et al.*, 2017). This makes estimation of global production volumes of WTRs very difficult. Many past reviews have also referred to a global wet WTR production figure of 10,000 t d^{-1}, however this figure is from the grey literature of the 1990s and the original report (Waite and Dharmappa, 1993), and therefore the methods of estimation, are no longer readily accessible. However, it is highly likely that global production now exceeds that 10,000 t d^{-1} estimate.

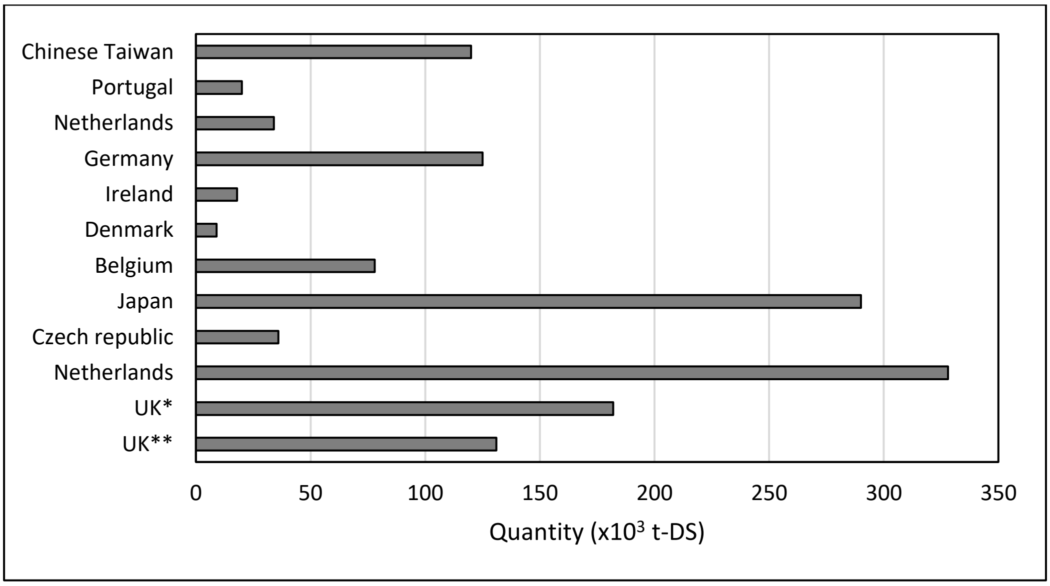

*Figure 2-3. A selection of publicly available annual, national clean drinking water treatment residual (WTR) production figures in tonnes of dissolved solids, based upon Babatunde and Zhao (2007) and Zhao et al. (2018) and references therein. *figure from 2003, ** figure from 2013.*

2.3. Physicochemical properties

Scanning electron microscope (SEM) studies of WTRs have shown that they have varying particle sizes and are highly porous (Makris *et al.*, 2004a; Ippolito *et al.*, 2011) which, together with the reactive surfaces that Al- and Fe- hydroxides typically have, account for their high sorption capacity. For example, sodium displacement testing revealed WTRs from Oklahoma, USA, to have a high cation exchange capacity in the order of 13.6 to 56.5 $cmol^+ kg^{-1}$, compared to 3.5 to 35.6 $cmol^+ kg^{-1}$ for typical soils (Dayton and Basta, 2001). Chemical examination of WTRs using x-ray diffusion (XRD) has revealed that WTRs are amorphous in nature, and therefore lacking a crystalline structure. Nevertheless, the presence of quartz, feldspar, calcite, illite/smectite, feroxyhyte, albite and kaolinite within WTRs has been confirmed (Ippolito *et al.*, 2003; Ippolito *et al.*, 2009b; Ociński *et al.*, 2016b; Ahmad *et al.*, 2018). Further detail on the high sorption capacity of WTRs, which has driven much of the research into beneficial use of the materials, is provided in Section 2.5.1.

Elemental concentrations, organic matter content and pH of WTRs can vary greatly depending on the characteristics of raw water treated, coagulant choice and treatment method (Table 2-2). The total concentration of elements and how readily they leach from WTRs are an important consideration when disposing of or utilising the materials. Elliott *et al.* (1990) analysed eight $FeCl_3$ and alum based WTRs from Pennsylvania (USA) and found that the concentrations of Cd, Cu, Cr, Ni, Pb and Zn were all well below the local permissible concentrations for land application. The authors noted that most metals were present in a weakly mobile, non-leachable form (determined by a sequential extraction procedure based on Tessier *et al.* (1979)). Wang *et al.* (2014) similarly found that, for six WTRs from various regions in China, the concentrations of As, Ba, Cd, Cr and Pb were below the threshold limits of the US Environmental Protection Agency's (USEPA) Toxicity Characteristic Leaching Procedure assessment that is used to determine whether the leachate of a material is hazardous. Example studies in which leachability of elements within WTRs have been assessed using weak salt solutions and weak acids are shown in Table 2-3. Zhao *et al.* (2018) considered the possibility that the chemical composition of WTRs may have changed through time due to increased environmental contamination (and therefore increased contaminant incorporation during raw water treatment). They found, in the past papers that they reviewed, that a majority of parameters had remained constant between 1990 and 2017 but that there had been a marked increase in Mn, As and Cr in WTRs since the early 2000s. However, the concentration data Zhao and co-workers reviewed was highly variable and so the increase noted for these elements was associated with much uncertainty. The perceived increase reported may also have been influenced by the very limited number of publications available for review that had relevant data pre-2000.

*Table 2-2. Typical components and properties of dry water treatment residuals (WTRs) reported in the literature**

Al (g kg^{-1})	**Fe (g kg^{-1})**	**P (g kg^{-1})**	**Ca (g kg^{-1})**	**Mn (g kg^{-1})**	**Pb (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Ni (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Organic matter (%)**	**pH**
6.7-180	1.1-277	0.2-10	0.18-32	0.4-31.6	2.5-69	0.12-246	10.9-60	35-624	5.8-24.5	5.12-8.0

* compiled from [(Lin and Green, 1987; Dayton and Basta, 2001; Makris *et al.*, 2004b; Makris *et al.*, 2006; Yang *et al.*, 2006a; Agyin-Birikorang and O'Connor, 2007; Makris *et al.*, 2007; Agyin-Birikorang and O'Connor, 2009; Hovsepyan and Bonzongo, 2009; Ippolito *et al.*, 2009b; Lombi *et al.*, 2010; Gibbons and Gagnon, 2011; Ippolito *et al.*, 2011; Oliver *et al.*, 2011; Putra and Tanaka, 2011; Ulén *et al.*, 2012; Wang *et al.*, 2012b; Castaldi *et al.*, 2014)].

Table 2-3. Extractability of elements/ ions (mg kg-1 ± standard error) in water treatment residuals (WTRs).

WTR type	**pH**	**Al**	**Fe**	**As**	**Cd**	**Co**	**Ni**	**Pb**	**Zn**	**PO_4^{3-}**	**SO_4^{2-}**	**Extraction method**	**Reference**
		(mg kg^{-1})											
Fe sludge	8.1	N/A*	N/A	0.38	0.13	0.02	0.31	0.03	4	<0.01	730	0.01 M CaCl2	Chiang (2012)
Fe	7.4	2.8± 0.2	0.6±0.1	<1.0	<1.0	N/A	N/A	<1.0	<1.0	N/A	N/A	0.001 M $CaCl_2$	Howells et al. (2018)
Al	7.3	4.4± 0.4	<1.0	<1.0	<1.0	N/A	N/A	<1.0	1.4±0.2	N/A	N/A	0.001 M $CaCl_2$	Howells et al. (2018)
Al sludge	N/A	10.3	N/A	N/A	0.02	N/A	N/A	<0.20	N/A	N/A	N/A	0.5 M acetic acid	(Caniani et al., 2013)

*data not available

Geotechnical analysis of partially dried but otherwise untreated (~10-40% w/w dry matter) WTRs established that they have high plasticity, high compressibility, and very low permeability (O'Kelly, 2008; O'kelly, 2010) (Table 2-4). These characteristics would suggest that untreated WTRs are unsuitable for use in aggregates for engineering, however some work has been done exploring the use of treated WTRs (i.e. dried, heat treated and ground) in construction (see section 5.5.).

Table 2-4. Geotechnical characteristics of Al WTRs (O'kelly, 2010).

Parameter	**Value**
Liquid limit	100-550
Plastic limit %	80-250
Specific gravity of solids	1.8-2.2
Total volatile solid %	10-60
Bulk density tonne m^{-3}	1.0-1.2
Dry density tonne m^{-3}	0.12-0.36
Effective cohesion kPa	0
Effective angle of shearing resistance (^{o})	28-44

2.4. Disposal via landfill

Incineration and biological digestion of WTRs are not viable options because of their low combustibility and nutritional value, hence disposal via landfill remains common. For example, a study from 2006 estimated that 40% of the dried WTRs produced in the USA were disposed of via landfill while another from 2011 estimated that in Japan the figure was 21%, and in 1999 it was suggested that 57% of the WTRs in the UK went to landfill (Keeley *et al.*, 2014). However, more recent data compiled from between 2014 and 2016 by Zhao *et al.* (2018) paints a more positive picture, estimating that 98%, 55%, and 75% of WTRs are recycled or re-used in the Netherlands, Czech Republic, and Denmark, respectively. Anecdotal evidence would suggest that it is highly likely that this large proportion of WTRs being recycled also occurs across other European countries. Within Europe, under the European Waste Code (2014/955/EU), WTRs and related sludges are classified by the code 19 09 02 in the European Waste Catalogue (European Commission, 2014), defined as *'Preparation of water intended for human consumption or water for industrial use – sludges from water clarification*'. Within the sovereignty of individual EU countries, various environmental regulations further govern the disposal or recovery and use of WTRs.

The disposal of WTRs is a large contributing factor to the overall cost of the drinking water treatment process. For instance, the Netherlands reportedly spends €30-40 million on disposal of WTRs annually, while in the UK the most recent data from 2000 suggests an annual expenditure of £5.5m (Babatunde and Zhao, 2007). The expenses associated with landfill disposal are set to increase further as legislation and taxation in many countries is becoming increasingly stringent in regards to landfill, e.g. the UK government has increased inert landfill waste fees to ~£90 per tonne in 2018 compared to the 1996 rate of £8 per tonne (Simpson *et al.*, 2002; Keeley *et al.*, 2014; HM Revenue & Customs, 2018). This gives an indication of the scale of increasing costs related to WTR disposal by landfill and identifies a clear economic driver for recycling WTRs for environmentally beneficial uses. Additionally, the European Landfill Directive commits its participating states to reducing the amount of biodegradable municipal waste that goes to landfill by certain dates, and WTRs fall into this category (CEC, 1999). However, in some regions the economic tipping point has not yet been reached. For example, Miyanoshita *et al.* (2009) conducted an economic evaluation of the disposal of WTRs in Japan and reached the conclusion that, based on their modelling, it was still more economically viable at that time to dispose of WTRs via the sewage system than to dewater and use the WTRs. Similarly, to production estimates, annual figures for WTR disposal/alternative end use and their associated costs are difficult to obtain. Nevertheless, beneficial use of the material, across a range of applications, appears to be growing.

2.5. Alternative disposal and beneficial use

Alternative disposal practises and research into their benefits, limitations and restrictions can be grouped into four broad categories: i) use in treatment of water or soil to remove or immobilise (manage) contaminants or excess nutrients; ii) other soil/land related applications; iii) as a constituent of construction materials; and lastly, iv) reuse and recycling for the drinking/waste water treatment process.

2.5.1. Sorption of pollutants or excess nutrients from solution

The chemical composition, amorphous nature and porosity of WTRs imparts them with a relatively large and highly reactive surface area when compared to typical soils (Ippolito *et al.*, 2003; Babatunde and Zhao, 2007). Therefore, numerous investigations have been conducted to determine the extent to which WTRs can be exploited to sorb certain potentially harmful elements, or elements in excess of desired concentrations, to reduce impacts on the environment. Initial work with WTRs as a sorbent focussed on the removal of excess phosphorus from solution (Ippolito *et al.*, 2003) but many other elements and chemical compounds have since been the target of investigation, including copper, lead, arsenic and certain pesticides and industrial chemicals (Chiang *et al.*, 2012; Elkhatib and Moharem, 2015; Ociński *et al.*, 2016b); the sorption capacities for various elements and compounds reported in the literature are summarised in Table 2-5.

The sorption capacity of WTRs is a function of particle size, surface area and surface charge (i.e. WTRs with a smaller mean particle size can sorb greater quantities of P (Yang *et al.*, 2006a)). Furthermore, the adsorption of P and arsenic onto Fe hydroxide surfaces of WTRs was thought to be reasonably described by Eq 2.4 and 2.5 (Parks *et al.*, 2003). While these equations are useful for describing P and As sorption onto WTRs at a general level, it is generally recognised that there are likely many other additional process involved such as ligand exchange reactions, hydroxide exchange reactions, surface complexation reactions, and co-precipitation reactions (Parks *et al.*, 2003; Yang *et al.*, 2006a; Gibbons and Gagnon, 2011).

$$2\mathrm{Al}-\mathrm{OH}+\mathrm{H_2PO_4^-}\rightleftharpoons(\mathrm{Al})_2\mathrm{HPO_4}+\mathrm{H_2O}+\mathrm{OH^-} \tag{2.4}$$

$$Fe-OH+H_2AsO_4^-+H^+\rightarrow Fe-H_2AsO_4+H_2O \tag{2.5}$$

Maqbool *et al.* (2016) successfully used Al based WTRs to remove 90% of the orthophosphorus (the simplest form of inorganic P, i.e. PO_4^{3-}) and 70-80% of the condensed phosphorus (a more complex chain of orthophosphate units) from synthetic and municipal wastewater using WTRs. While it has been suggested that alum sludge has a greater tendency for uptake of inorganic P than organic P (Gon Kim *et al.*, 2002).Wang *et al.* (2016) produced filters using Al based WTRs as a substrate in a WTR: kaolin: humic acid ratio of 10:7:2. Their tests with synthetic solutions found that an adsorption capacity of 1.31 mg P g^{-1} could be achieved and after an initial adsorb–desorb cycle using 0.25 mol L^{-1} NaOH, 80.0% of the P could then be recovered, though this did reduce to 31.4% after three more cycles. The uptake of P by WTRs was characterised by Bai *et al.* (2014) as being proportional to oxalate extractable Fe and Al, as well as surface area. It has been found that oxidation state has a negligible effect on P sorption and retention by WTRs, however pH has been found to have a significant impact on these factors (Oliver *et al.*, 2011; Maqbool *et al.*, 2016). WTRs can also remove fluoride from aqueous solution in a process which is characterised by a decreasing sorption rate after 5 minutes (Sujana *et al.*, 1998) presumably due to saturation of the most effective fluoride binding sites.

Potentially toxic element (PTE) adsorption by WTRs is also well documented in the literature (McBride, 1995; Hovsepyan and Bonzongo, 2009; Zhou and Haynes, 2011; Chiang *et al.*, 2012). For example, sorption tests conducted on sediments amended with WTRs found high amounts of Cd, Co, Ni, Pb and Zn were removed from solution, performing 240% better than sediments containing equivalent alternative additions of goethite in multi contaminant testing (Chiang *et al.*, 2012). One comparative study of PTE binding capacity of Fe WTRs and Al WTRs found that, while both forms sorbed large quantities of the metals, Fe WTRs bound more Zn and Cd from solution than Al WTRs did across a pH range between 4.5 and 7.0 (e.g. ~0.200 and ~0.100 mmol g^{-1} of each metal sorbed by Fe and Al WTRs respectively at a pH of 7.0) when applied at a WTR:solution ratio of 0.1:25 (Silvetti *et al.*, 2015) which is in agreement with unpublished results for Cd generated in our laboratory (Keele University) (Table 2-5).

Arsenic removal from water using WTRs has also been widely investigated (Makris *et al.*, 2006; Makris *et al.*, 2008; Kim *et al.*, 2012; Caporale *et al.*, 2013; Elkhatib *et al.*, 2015a; Ociński *et al.*, 2016b). Makris *et al.* (2006) applied both Fe and Al based WTRs separately to solutions containing As (III) and As (V) during batch sorption experiments (15,000 mg kg^{1} solution equilibrated for 2 days at a pH of 5.3-6.3). Their results suggested that Fe WTRs showed a greater sorption capacity for As (III) than As (V) (99% As (III) sorbed vs 67% As (V)), while Al WTRs effectively removed greater quantities of As (V) than As (III) (55% As (III) vs 93% As (V)) (Makris *et al.*, 2006). However, Kim *et al.* (2012) found that Al WTRs adsorbed greater quantities of As (III) than As (V), and that As (V) adsorption rapidly decreases when the pH increases above 6.

Because of its potential as a low cost alternative for removing contaminants, and because As contamination of drinking and waste water is a major environmental and health issue, there have been multiple attempts to calculate the maximum sorption capacities of As and its various forms (i.e. As (III) and As (V)) (Makris *et al.*, 2009; Gibbons and Gagnon, 2010; Nagar *et al.*, 2010; Gibbons and Gagnon, 2011; Ociński *et al.*, 2016b) (Table 2-5). Ociński *et al.* (2016a) incorporated WTRs into an alginate based polymer in order to reduce the clogging potential of using WTRs in aquatic bed systems, but this resulted in greatly reduced maximum adsorption capacities (3.4 mg g^{-1} As (III) and 2.9 mg g^{-1} As (V)) which the authors believed was due to impediment by carboxylic groups in polymer chains of the alginate.

The applicability of WTRs for remediating multi-element contaminated stormwater has been explored by multiple studies. Soleimanifar *et al.* (2016) produced encouraging results during batch and column tests by applying WTRs to the surface of mulch, with adsorption rates from synthetic storm water reported as high as 97% for Pb (initial concentration, 100 mg L^{-1}), 76% for Zn (initial concentration 800 mg L^{-1}), 81% for Cu (initial concentration 100 mg L^{-1}) and 97% for P (initial concentration 2.30 mg L^{-1}) over a 120 min timeframe. Deng *et al.* (2016) also produced promising results when using a combination of WTRs and scrap tyre rubber to remove Cu and Pb from synthetic stormwater, while limiting the leaching of Zn from the tyres to some extent. Both of these approaches were employed in order to reduce the clogging that could occur when using solely WTRs.

Treatment of other non-metals and non-nutrient contaminants have also been examined, *e.g.* Makris *et al.* (2006) applied Al WTRs to 10 mg L^{-1} perchlorate solution in a ratio of 1 g: 5 mL; reductions in perchlorate of up to 65% were described over 2h, increasing to 76% after 24h, the authors inferred that the mechanism of removal was the reduction of perchlorate to chloride (Makris *et al.*, 2006). There has been particular success achieved in the application of WTRs to textile industry wastewater for the removal of textile dyes (Chu, 2001). In one study, around

88% of a hydrophobic dye (Dianix Blue) was removed from wastewater by additions of 75 mg l^{-1} of alum at pH 9.13 (Chu, 2001), while Vaezi and Batebi (2001) applied ferric sulphate recovered from WTRs to two types of textile industry wastewaters with positive results. More recently, dye removal efficiencies of 53-95% for alum based WTRs and 97% for ferric WTRs have been reported (Moghaddam *et al.*, 2010; Gadekar and Ahammed, 2016; Butani and Mane, 2017). Following these studies, Yusuff *et al.* (2017) went a step further and optimised the removal of methylene blue textile dye, achieving a 100% removal rate by exposing the treatment process to a temperature of 450 ^{0}C and a pH of 6. This level of treatment success demonstrates the great potential for environmental benefits that could be gained through this type of WTRs application, particularly in developing countries where vast quantities of textiles are produced and waste water treatment options are often limited (or even absent) because of technical access and cost issues. However, this application of WTRs is not effective for treating wastewater containing hydrophilic dyes due to their high solubility (Chu, 2001). Another limitation to this application is the question of further use of WTRs after they have been used to sorb excess dyes and other chemicals in textile-related waste streams; they may be unsuitable for other re-use options and may need to be disposed of via specialist landfill (Asif *et al.*, 2016). Basibuyuk and Kalat (2004) considered the use of WTR sludge for remediating vegetable oil refinery wastewater. Jar experiments revealed that WTRs were as effective as ferric chloride and alum salts. Optimum conditions resulted in the removal of 99% of the oil and grease, 99% of total suspended solids and 83% of the chemical oxygen demand (COD).

Success has also been found in using WTRs to adsorb and immobilise organic pollutants. (2015); Punamiya *et al.* (2016) successfully removed large quantities of two veterinary antibiotics (tetracycline hydrochloride and oxytetracycline hydrochloride) from solution and immobilised them in manure treated soils using WTRs. Importantly, in terms of environmental applications, the optimum pH range for tetracycline sorption was found to be across the range 4.0 to 8.0, i.e.

typical conditions encountered in the natural environment (Punamiya *et al.*, 2015). However, the effectiveness of sorption was reduced in the presence of elevated P, due to competitive ligands. Water treatment residuals have also been shown to be effective in removing cyanobacteria, which are known to contribute to freshwater algal blooms and the production of cyanotoxins, from waterborne cultures; e.g. unicellular and colonial *Microcystis aeruginosa*, by 89% and 75% respectively having had an initial density of 106 cells mL^{-1} (Wang *et al.*, 2017a).

There have been a handful of studies investigating the application of WTRs that were first milled to <100 nm diameter and thus referred to in the literature as nano particle WTRs (nWTRs) (Elkhatib *et al.*, 2015a; Elkhatib *et al.*, 2016; Mahdy *et al.*, 2017). This is done to increase their surface area, and thus their sorption capacity. One study produced a 2- to 3-fold increase in surface area over that of non-milled WTRs (e.g. 129 m^{2} g^{-1} vs 53 m^{2} g^{-1}; (Elkhatib *et al.*, 2015b)). Sorption studies have shown that nWTRs have maximum sorption capacities for As, Cd and P that are greater than those of normal WTRs (e.g. 50 mg As g^{-1} compared to 3 mg g^{-1}, 47 mg Cd g^{-1} compared to 2.80 mg g^{-1}, and 50 mg P g^{-1} compared to 1.67 mg P g^{-1}) (Elkhatib and Moharem, 2015; Elkhatib *et al.*, 2016). Similar results were produced in another nWTR in batch study experiment, with ~95% of P removed from a starting concentration of 5– 1,000 mg P l^{-1} over a period of 100 min (Elkhatib *et al.*, 2015b). The study by Elkhatib *et al.* (2016) also reported the subsequent release of less than 0.2% of Cd that was initially bound to the WTRs after 4 consecutive desorption cycles. However, although the practicalities, cost implications and long-term effectiveness of milling WTRs to nano-sized particles for use in sorption and water treatment applications are yet to be properly evaluated, it seems very unlikely that this approach would ever become a commercially viable option for mainstream recovery and use of WTRs.

Table 2-5. Reported maximum sorption values for WTRs.

Element/compound	**WTR type**	**WTR origin**	**Solution Matrix**	**WTR: solution ratio**	**Maximum sorption reported**	**Reference(s)**
As (V)	Fe	West Flanders, Belgium	2% Nitric acid solution	1 g L^{-1}	0.04 mg g^{-1}	(Chiang *et al.*, 2012)
As (V)	Fe	‘‘Na Grobli” Water Treatment Plant in Wrocław, Poland.	Deionised water solutions	1 g L^{-1}	0.077 mg g^{-1} As (V)	(Ociński *et al.*, 2016b)
As (III)					0.132 mg g^{-1} As (III)	
As (V)	Al	Bradenton, FL, USA.	KCl solution of As(V)	100 g L^{-1}	7.68 mg g^{-1} of As (V)	(Makris *et al.*, 2009)
As (III)	Al		KCl solution of As (III)	100 g L^{-1}	6.00 mg g^{-1} load of As(V)	
Arsenate (AsO_4^{3-})	Al and Fe	A range of water treatment plants in the United States and Canada	Dionised water solution (100 µg L^{-1} AsO_4^{3-})	Varying	−0.07 to 0.34 mg g^{-1} and 0.44 to 2.35 mg g^{-1} for Al and Fe WTRs respectively	(Gibbons *et al.*, 2009) (Gibbons and Gagnon, 2010) (Gibbons and Gagnon, 2011)
			Ground water (43 µg L^{-1} AsO_4^{3-})		−0.01 to 0.003 mg g^{-1} and 2.23 to 42.91 mg g^{-1} for Al and Fe WTRs respectively	
As	Fe and Al	The Fe- WTRs from Tampa, Florida and Al-based WTR from Bradenton, Florida, USA	1M HCl and NaOH solutions (375, 750, 1,500 and 3,000 mg L^{-1} As)	50, 100, and 200 g L^{-1}	3.00 mg g^{-1} Al WTR, maximum was not reached however. 1.35 mg g^{-1} Fe WTR	(Nagar *et al.*, 2010)
P	Fe	Ballymore Eustace in Co. Kildare, South Dublin and Leixlip in west Dublin	Water solution (100 mg L^{-1} P)	0.1-0.5 g L^{-1}	22.4 mg g^{-1} and 20.1 mg g^{-1} for two different sludges	(Zhao and Yang, 2010)
Phosphate (PO_4^{3-})	Al and Fe	A range of water treatment plants in the United States and Canada	Dionised water (2 mg L^{-1} PO_4^{3-}) Municipal waste water (4.2 mg L^{-1} PO_4^{3-})	0.5, 1, 5, 10 and 20 g 100 L^{-1} 25 mg to 2,000 mg L^{-1}	1,030 to 1,110 mg kg^{-1} and 2,960 mg kg^{-1} for Al and Fe WTRs respectively 890 to 1,590 mg kg^{-1} and 2,250 mg kg^{-1} for Al and Fe WTRs respectively	(Gibbons *et al.*, 2009) (Gibbons and Gagnon, 2010) (Gibbons and Gagnon, 2011)
Orthophosphorus	Al	Simly and Rawal water treatment plants in Islamabad, Pakistan	Synthetic waste water	30 g L^{-1}	1.58 mg g^{-1} to 4.86 mg g^{-1}	(Maqbool *et al.*, 2016)
Condensed phosphorus					4.21 mg g^{-1} to 4.71 mg g^{-1}	
Pb^{2+}	Fe	West Flanders, Belgium	2% Nitric acid solution	1 g L^{-1}	120.00 mg g^{-1}	(Chiang *et al.*, 2012)
Cd^{2+}	Fe			1 g L^{-1}	21.02 mg g^{-1}	
Zn^{2+}	Fe			1 g L^{-1}	40.01 mg g^{-1}	
Multi-element (As (V), Cd^{2+}, Pb^{2+} and Zn^{2+})	Fe			4 g L^{-1}	50.05 mg g^{-1} As (V), 100.08 mg g^{-1} Pb^{2+}, 11.02 mg g^{-1} Cd^{2+}, 10.00 mg g^{-1} Zn^{2+}	
Cr (III) and Pb (II)	Al	Seqwater Mount Crosby and North Pine, Brisbane	0.01 M $NaNO_3$ solutions (0.4 mM to 8 mM of Cr(III) and Pb(II))	10 g L^{-1}	100% of both elements from the highest concentration solution at pH of 6	(Zhou and Haynes, 2011)
Cd, Zn	Fe and Al	Al-WTR from Abbanoa industry plant and Fe-WTR from Bidighinzu, Truncu Reale, Sassari (Italy)	1.0 mM $NaNO_3$ solution (1.0 to 20.0 µmol in 25 mL^{-1})	4 g L^{-1}	Fe-WTR [13.47 and 22.37 mg g^{-1} of Zn(II) and Cd(II) respectively] and Al-WTR [6.28 and 11.92 mg g^{-1} of Zn(II) and Cd(II) respectively] at pH 7.0	(Silvetti *et al.*, 2015)
Cd	Fe and Al	Water treatment plants in the English midlands	0-1,000 mg L^{-1} Cd deionised water matrix	10 g mL^{-1}	Fe WTR, 9.91 mg g^{-1}	Unpublished data from our laboratory
					Al WTR, max sorption 4.76 mg g^{-1}	

Hg	Al	Bradenton, FL, USA	Nanopure water solution (30 to 80ppm)	600 g L^-	79 mg g^-1 Hg	(Hovsepyan and Bonzongo, 2009)
Co	Al Fe combination	9th water treatment plant in Beijing, China,	Distilled water (0–800 mg L^{-1} Co(II))	16.7 g L^{-1}	17.307 mg g^{-1}	(Jiao *et al.*, 2017)
Perchlorate (ClO_4)	Al	Bradenton, Florida	KCl solution (10, 50, 100 and 200 mg L^{-1})	200 g L^{-1}	76% of 10 mg L^{-1}, 31% at 200 mg L^{-1}	(Makris *et al.*, 2006)

2.5.2. Use in constructed wetlands, reed beds and filter beds

An area that has received considerable attention in the literature is the use of WTRs as a reactive media in constructed wetlands (CW) or filter beds (Figure 2-4). Zhao *et al.* (2009) reported the results of a long-term (730 days) trial of WTR sludge cake as a reed bed substrate as part of an agricultural wastewater treatment process. Removal efficiencies of 73% for COD, 83% biological oxygen demand (BOD), 86% reactive phosphorus, 89% soluble (<0.45 µm filtered) reactive phosphorus and 78% suspended solids were achieved. Of this 42% of the P removal was directly related to sorption which is dependent upon the availability of reactive surfaces of WTRs, leading the authors to estimate a working lifetime for WTR based CWs of 9-40 years, excluding other limiting factors such as clogging (Zhao *et al.*, 2009). In a follow-up study into the efficiency of COD, BOD, total nitrogen (TN), total phosphorus (TP) and NH_4-N removal, Zhao *et al.* (2011) observed monthly reductions of these values in the ranges of 36–84%, 57–84%, 11–78%, 75–94% and 49–93% respectively. In another study, Babatunde *et al.* (2010) reported a reduction of 91% for BOD and 72% for COD and a 98% reduction in soluble reactive P in a four-stage laboratory-scale constructed wetland which utilized alum WTR as the absorbent medium.

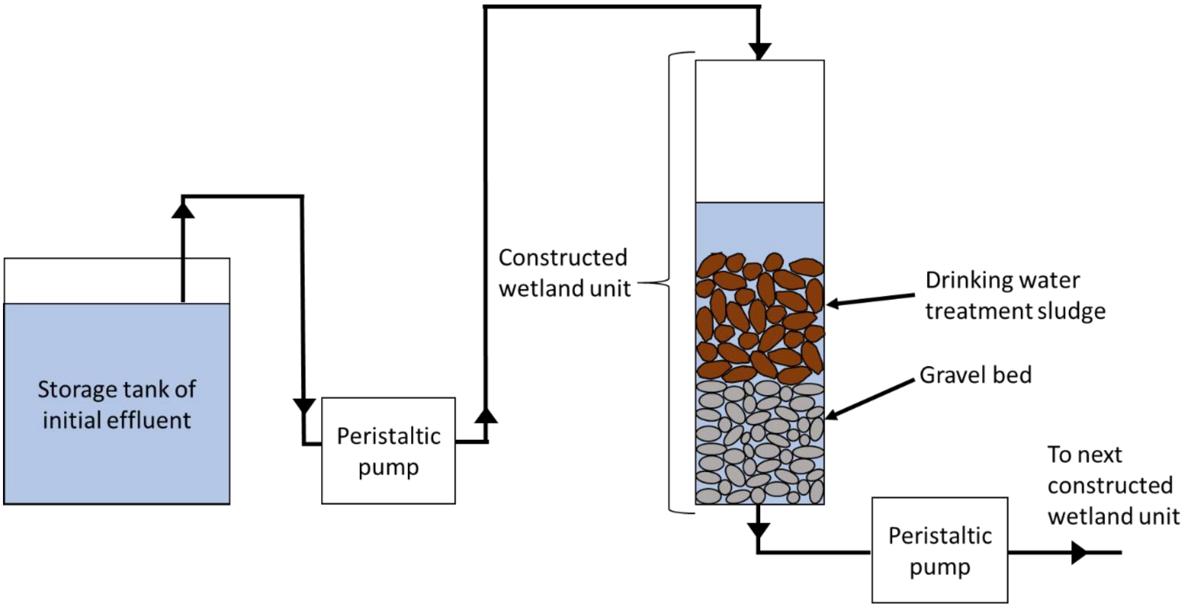

Figure 2-4. A generalisation of the WTR constructed wetlands experimental setup, as used in multiple studies (Razali et al., 2007; Babatunde et al., 2009; Babatunde et al., 2010; Zhao et al., 2011).

New advances are constantly improving on the existing WTR CW model. For example, Wang *et al.* (2017b) trialled incorporating Al WTR based haydites (a heat expanded aggregate) into wetland cells. This resulted in average removal rates of 90.1%, 23.3%, 86.1%, and 97.2% of COD, NH_4–N_4, TN and TP respectively. In another study, Hu *et al.* (2012) achieved an increased N removal rate of 83% under a high nitrogen load (19.1 g N m^{-2} d^{-1}) by means of a step feeding strategy in a tidal fed WTR CW, compared to 23-59% in earlier tidal fed WTR based CWs. More recently the incorporation of microbial fuel cells into WTR based CWs has been investigated in several studies (Zhao *et al.*, 2013b; Doherty *et al.*, 2015; Xu *et al.*, 2016a) (Table 2-6). These systems aim to produce bioelectricity through harnessing microbial breakdown of organic matter, therefore increasing the environmental payoff through energy generation. The reported maximum power outputs for such units are similar to those produced by microbial fuel cells in CWs made from conventional bedding material (i.e. not WTRs) (Zhao *et al.*, 2013b), showing that the use of WTRs incurs no trade-off cost in this regard.

Table 2-6. Results from attempts at incorporating microbial fuel cells into WTR based constructed wetlands (CWs).

Type	**Type of wastewater**	**COD (mg L^{-1})**	**COD removal (%)**	**Max power (mW m^{-2})**	**Reference**	**Notes**
Vertical upflow	Swine	1058	71.5	12.83	(Zhao *et al.*, 2013b)	Without aeration
			76.5	9.4		With aeration
Simultaneous upflow downflow	Swine	583	64	276	(Doherty *et al.*, 2015)	
Vertical upflow	Swine	484	70	36.58	(Xu *et al.*, 2016a)	Alum cathode
			81	87.79		10% additions of powder activated carbon to the cathode

Aluminium WTR CWs have also been incorporated into the aeration tanks of conventional activated sewerage sludge systems, in order to produce green bio-sorption reactors (GBRs) (Liu *et al.*, 2017). Removal rates of 96%, 99% and 90% for BOD, TP and TN were achieved in a GBR system (Liu *et al.*, 2017). While concerns have arisen that the occurrence of natural organic matter may lead to a deterioration in the P adsorption rates, this technique still offers a possible pathway for upgrading outdated activated sludge CW systems (Liu *et al.*, 2017).

The largest issue facing WTR CWs over the long-term is clogging, having been reported to occur around 14 months into WTR based CW's lifecycles. However, this can be partially alleviated by the use of an anti-sized gravel bed (i.e. smaller gravel at the base of the bed and larger at the top) and did not occur over a period of 18 months while running a CW based on the use of WTRs in the form of haydites (Zhao *et al.*, 2015; Wang *et al.*, 2017b). In contrast with an earlier study by Oliver *et al.* (2011) that reported the release of organic matter under anaerobic conditions as a possible cause for concern in relation to water quality effects, Liu *et al.* (2016) assessed natural organic matter release from a WTR based CW and found it to be of low risk to human health. As an additional environmental benefit, once the WTR cakes in CWs have reached the end of their working lifespan it is possible to recover the Al and P, in the form of $AlPO_4$, through a process of P extraction by H_2SO_4 addition and $AlPO_4$ precipitation through pH adjustment (Zhao *et al.*, 2013a). Employing this method resulted in reported recovery rates of 97% and 99% for P and Al respectively (Zhao *et al.*, 2013a). Overall, the use of WTRs in CWs and filter beds is highly promising, particularly with the potential of incorporation of microbial fuel cells. However, the release of certain substances, such as Al and Fe, under the varying models still requires further research. Further development and trial of anti-clogging techniques would also enhance their efficiency, longevity and likelihood of use.

2.5.3. Use in lakes or reservoirs

In addition to application in constructed wetlands and reedbeds, use of WTRs as a P sorbing material in lakebeds has also been trialled. First suggested by Young *et al.* (1988), multiple recent studies have expanded on this idea (Wang *et al.*, 2012a; Wang *et al.*, 2013a; Takashima *et al.*, 2015; Yuan *et al.*, 2016a). The most common approach is to mix 10% WTRs by weight with lake sediments (Wang *et al.*, 2012a; Wang *et al.*, 2013a; Yuan *et al.*, 2016b), however some studies have varied this amount, such as Yuan *et al.* (2016a). WTRs have the potential to limit internal P loading within lakes, leading to a reduction in algal growth (Yuan *et al.*, 2016a). This is achieved by preventing P release from sediment, via the sorption and retention mechanisms described in previous sections. However, WTRs have been found to increase overlaying waters Al and Fe concentrations. For example, Wang *et al.* (2013a) noted that Fe and Al concentrations in the overlaying water were marginally increased (<0.03 mg l^{-1}) in all experiments. Furthermore, another study found that 0.0064–0.0073 mg g^{-1} of Al from WTRs is released into waters when 1g was added to 80ml of water at its surface and allowed to settle for 2 hours (Wang and Pei, 2013). While these increases in overlaying water Fe and Al concentrations are notable, their impact on ecological receptors will likely be negligible.

Although there are no studies quantifying the P sorption of WTRs when actively deployed in lakes, Phoslock® (bentonite clay containing ion exchanged La), a commercially available material used in the treatment of excess P in lakes and which has a similar sorption capacity for P to that of Fe WTRs (i.e. Phoslock 21,670 mg P kg^{-1}, Fe WTRs 22,400 mg P kg^{-1} and 20,100 mg P kg^{-1} for two different sludges (Zhao and Yang, 2010; Meis *et al.*, 2012)), can increase P sorption of a reservoir by ~28 kg ha^{-1} when applied at 2.7 T ha^{-1} and hence similar levels of performance might be achievable with WTRs. An estimation of the P immobilisation capabilities of WTRs in lake sediments can be expressed by the following equation 2.6 (Wang *et al.*, 2013b).

$$(Al_{ox}+Fe_{ox})_{WTR} = 83 \times P_m - 40 \qquad (Eq. 2.6)$$

Where $(Al_{ox} + Fe_{ox})_{WTR}$ is the sum of oxalate extractable Al and Fe content of the WTR and the P_m is the P concentration in the lake sediments, all terms are expressed in mol g^{-1} (Wang *et al.*, 2013b). Therefore, the required volume of WTR for P immobilisation can be estimated if the $(Al_{ox} + Fe_{ox})_{WTR}$ and P concentration in sediments are known.

Yuan *et al.* (2016b) evaluated the risk of pollution related to WTRs in lake water with regard to environmental regulatory limits and human health risk assessment. WTRs were mixed with sediments (~10% WTR by weight) and incubated aerobically and anaerobically in beakers. It was found that, while both Fe and Mn exceeded regulatory standards (the Environmental Quality Standard for Surface Water in China, and the National Recommended Water Quality Criteria for fresh water in the USA) after anaerobic incubation, amended sediments were considered to be non-hazardous to aquatic plants.

2.5.4. Application to land

Land application of WTRs is increasing and may eventually become the most common reuse route. For example, the Sydney Water corporation (New South Wales, Australia) stores and then reuses 100% of the produced WTRs via land spreading in their catchment area (equating to 5228 dry t during 2011-2012) (Sydney Water, 2011). Application of WTRs onto clean (i.e. no previous history of contamination) agricultural and forestry land has become a well-established practise in some regions (e.g. parts of the USA and UK) and has been a topic of periodic research activity (e.g. Bugbee and Frink (1985); Geertsema *et al.* (1994); Oladeji *et al.* (2007)). It is difficult to obtain precise figures to support the assertion that land application is growing because of the lack of cohesive data from reliable sources, but there is anecdotal evidence; for example, in the English Midlands and Wales (where the authors of this review are based) two major water treatment companies recover and spread ~86,000 t of WTRs per year to land. It is easy to recognise the potential benefits of land applying WTRs, for example, they have a high organic matter content, often a degree of liming capacity, and are a potential source of nutrients. Moreover, recovery and use in this way promotes the waste hierarchy concept as per the EU Waste Framework Directive (2008/98/EC) and facilitates development of a circular economy. Indeed, land application of WTRs has reportedly resulted in yield increases in various plants and other soil benefits such as increased aeration and hydraulic conductivity (e.g. Geertsema *et al.* (1994), Ahmed *et al.* (1998)), although the limitation of plant available P has occasionally been observed (see section 5.4.2).

There are many variables to consider in the application of WTRs to agricultural land including the cropping system, crop nutrient requirement, time of year, and existing soil conditions. Methods of application tend to be split according to whether the WTRs are solid (approximately 14% dry matter or above) or liquid. Solid WTRs are generally spread using tractors using conventional manure spreaders, and trajectory splash plates or precision injection techniques such as a trailing shoe or dribble bar while spreading wet material. The rates of application are typically linked to plant or soil requirements, with maximum limits set according to local governing regulations. In the UK for example, the maximum application rate permitted is 250 t ha^{-1} with a further limit on liquid applications of 50 m^3 ha^{-1} at any one time, and additionally no more than 250 kg N ha^{-1} may be applied to land (DEFRA, 2011; Environment Agency, 2013).

Land application for general soil improvement

The use of WTRs in potting mix and other plant growth media has also been explored, for example Bugbee and Frink (1985) included experimentation with Al-WTRs as a component of potting mixtures at rates of 25%, 50%, 75% and 100% combined with soil. The authors noted a general increase in aeration of the potting mix that was related to the WTR additions while the impacts on available water, pore space and bulk density variations were minimal or negligible. However, they did note reduced growth of marigolds (*Tagetes sp.*) and that lettuce (*lactuca sativa*) had developed a purple hue when grown in a WTR containing potting media which they associated with P deficiency at these high proportions of WTR (as per the discussion in section 5.4.2).

Heil and Barbarick (1989) found that low (5g kg^{-1}) application rates of Al and Fe WTRs to soils increased yield of sorghum-sudangrass (*Sorghum bicolor* L. Moench), attributed to their Fe contributions and pH increasing abilities. During trials of co-application of WTRs and vermicomposts, Ibrahim *et al.* (2015) found that a 2:1 ratio of WTR to vermicompost with a combined application rate of 5 g kg^{-1} resulted in greater wheat yields than all other treatments tested, which included pure vermicompost and control (i.e. dried and sieved soil only) treatments. WTRs were found to serve as a better planting medium for peppers (*Capsicum annuum* 'Takanotsume') than granite parent material based soils when both were amended with 10% additions of compost (Park *et al.*, 2010). This was believed by the authors to be related to the higher gas diffusivity, saturated hydraulic conductivity, N content and cation–exchange capacity following WTR addition. During a study into the effects of aging soils treated with WTRs for 1.5 years (7.5 t ha^{-1} application rate), Mukherjee *et al.* (2014) found that many physical properties of the soils remained the same as those of control soils, however there were minor but significant increases in electrical conductivity from 7.1 μS m^{-1} to 7.5 μS m^{-1} and a penetration resistance (i.e. soil strength) increase of 87%. However, these impacted factors were unlikely to have any major implications for soil use. One study did report reductions in Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) biomass when WTR application rates above 2% (w/w) despite fertiliser addition, highlighting the possibility that high WTR addition rates can render the conditions of the treated soil sub-optimal for growth of some plants (Tay *et al.*, 2017).

The use of WTRs as an amendment to soil substrates that are used for plant growth (i.e. as opposed to land upon which buildings are to be constructed) appears to be a positive reuse of WTRs that has multiple benefits for the environment. There is evidence of improved plant growth in a majority of cases when WTRs are applied alongside a P source (e.g. fertiliser or compost), which is often the way applications are made in real world agricultural settings. The added potential benefit of reducing plant uptake of some PTEs could make this use of WTRs of particular benefit to agricultural production in areas that have contaminated soils requiring management and remediation, and this is addressed in section 5.4.3. An additional benefit perceived by land owners is that the sulfur content of WTRs may also provide a nutrient benefit to plant production, but this has not been examined or reported in the literature to date and is thus an area warranting investigation.

Grazing animals and poultry

Grazing animals obtain a large proportion of their foodstuffs from the soil surface, and it is known that grazers such as sheep typically ingest considerable quantities of soil while grazing (e.g. 1-6 g soil / kg body mass/ day, or up to 400 g kg^{-1} body mass across a growing season (McGrath *et al.*, 1982)), this raises considerations about potential implications for grazers in relation to surface application of WTRs.

Van Alstyne *et al.* (2007) investigated the impacts of feed containing Al-WTRs on lambs when compared with feed containing a sand control or a more available Al source ($AlCl_3$). The highest WTR treatment rate imposed (10% of the feed by mass; 8,000 mg Al kg^{-1} total feed) was considered as a proxy for a very high WTR field application rate ~225 dry metric tonnes ha^{-1}. It was concluded that any minor fluctuations in measured parameters (feed intake, body weight, P absorption, or P availability in blood plasma) between some WTR treatments and the controls were not a risk to lamb health, although it was suggested that P should also be supplied at 0.25 w%. Madison *et al.* (2009) investigated the effects of land application of WTRs on grazing cattle over a 2-year period. Over that time, 75.8 t ha^{-1} of WTRs were surface applied. It was found that cattle were unaffected (in terms of weight gain and tissue mineral concentrations) due to the low bioavailability of the components in the WTRs. Maurice *et al.* (1998) mixed 20% alum WTR and 10% zeolite into chicken feed and found that there were no significant impacts on health indicators (i.e. body weight, feed conversion, or leg scores) of 3 to 6-week-old chickens. Additionally, litter characteristics including ammonia, pH, N, P, and Cu were all also unaffected.

Immobilisation of contaminants and excess nutrients in soil

In addition to application onto unspoiled soils, WTRs have been investigated for their use in managing or restoring soils with excess nutrient or contamination problems. In this approach, the capability of WTRs to sorb contaminants and excess nutrients in soils is utilised to immobilise them *in situ*. Immobilisation of P and N in soil, with the aim of reducing nutrient run-off and subsequent accelerated eutrophication in receiving waters, is an area that has received much attention. For example, Gallimore *et al.* (1999) reported small plot trials in which Al WTRs were applied in two ways (broadcast, at rates equivalent to 11 and 45 t ha^{-1}, and in buffer strips) to poultry manure amended soils. They found that run-off from simulated rainfall had P concentrations of 14 mg l^{-1} from control plots, 6 mg l^{-1} from 45 t ha^{-1} broadcast treatments and 7 mg l^{-1} from buffer strip plots while the 11 t ha^{-1} treatment caused no significant decrease when compared to control plots. Similarly, Codling *et al.* (2002) found that WTR application rates of 50 g kg^{-1} decreased water-soluble P and Mehlich 3 extractable phosphorus concentration by 98-99% and 87 -90% respectively. Likewise, Silveira *et al.* (2013) found that the treatments trialled (30 and 75 t ha^{-1}) reduced water extractable P in soil A and E horizons by more than 60% (e.g. 18.4 mg kg^{-1} in control soil A horizon, down to 7.3 and 2.8 mg kg^{-1} in 35 and 70 t ha^{-1} treatments respectively). The study by Silveira and co-workers also reported reductions in shallow ground water P (i.e. 60 cm depth), reducing P from 1.0 mg l^{-1} to 0.43 mg l^{-1} and 0.20 mg l^{-1} in 35 and 70 t ha^{-1} treatments respectively, while in plots which were ploughed in order to incorporate WTRs produced reductions from 3.4 mg l^{-1} down to 1.4 mg l^{-1} and 0.5 mg l^{-1} respectively. This indicates that the act of ploughing, in this periodically anoxic soil, disturbed the soil matrix sufficiently to expose leachable P in control as well as treated plots. Comparable results have been produced in a laboratory setting also. For instance, work by Oladeji *et al.* (2008) demonstrated that surface application of Al WTRs at 10 g kg^{-1} reduced the leachate P concentration by 46- 54% in sandy soils amended with either poultry manure or sewage biosolids. In soil column experiments, Al WTRs surface applications of 124 and 248 t ha^{-1} have proven to assist in the reduction of leached P from urban runoff (Ippolito, 2015). Similarly, field studies found that Al WTR application at 22.4 dry t ha^{-1} can reduce leaching of P into shallow groundwaters (Agyin-Birikorang *et al.*, 2009).

A number of studies have identified the potential for over efficient phosphorus sorption as a long-standing potential concern when it comes to land application of WTRs to agricultural soils, as it could restrict P supply to plants and subsequently risk sub-optimum nutrient levels or even P deficiency (Bugbee and Frink, 1985; Elliott and Dempsey, 1991). Lucas *et al.* (1994) linked this effect with yield reductions in tall fescue grass (*Festuca arundinacea*) when Al WTRs were applied above 1% or 2% by mass. More recently, Lombi *et al.* (2010) reported that a pot trial using two soils from South Australia amended with Al WTRs in which plant tissue P concentrations fell below critical levels at application rates approximating 10 t ha^{-1} and above. Other studies (e.g. Oladeji *et al.* (2007)) have also reported reduced plant tissue P concentrations in WTR amended soil. However, the situation is not straightforward, as numerous studies have contrastingly shown no such P limitation or deficiency following WTR application, e.g. Geertsema *et al.* (1994); Ahmed *et al.* (1998), and certain reference sources cited in Elliott and Dempsey (1991). Long term studies investigating this effect are rare, but a 3-year study reported by Geertsema *et al.* (1994) found that there were no significant differences in the P (or any other element) content of pine tree tissues grown in Al WTR amended soils.

Anecdotal evidence from the vast acreage of agricultural land across many countries that is now regularly amended with WTR applications, evidently without adverse effects that would deter land managers from continuing the practise, would support the notion that excessive P immobilisation in soil is not typically a problem. Nevertheless, the potential for excessive P restriction, particularly in already P deficient agricultural soils, remains a factor that must be considered in relation to planning land application of WTRs. One potential solution in such a situation was presented by Okuda *et al.* (2014), who demonstrated that by treating WTRs with sulphuric acid (principally to reduce the Al concentration via leaching) the plant availability of P within the WTRs increased. The authors found that Japanese mustard spinach (*Brassica rapa* var. *perviridis*, Komatsuna) P uptake was enhanced by 40% when grown in a treated WTR: peat mixture (50:50 ratio) when compared to a mixture containing untreated WTR sludge.

On the other hand, the use of WTRs as a fertiliser has also been explored. Rigby *et al.* (2013) explored the viability of using Al WTRs as a fertiliser in P and N deficient sand. They found that WTRs supplied adequate quantities of plant available N and that their application resulted in grain yield increases for wheat (*Triticum aestivum* L. cv. Wilgoyne) relative to untreated control plots. However, produced yields were only 62% and 69% (year 1 and 2 respectively) of grain grown in plots which had received standard inorganic P and N fertiliser applications (Rigby *et al.*, 2013). In order to address these issues, Zohar *et al.* (2017) produced a WTR-organic composite that could potentially find use as a slow-release P fertiliser; this was achieved by initially using WTRs to treat soil leachate and dairy wastewater to remove P. A nine-week desorption experiment found that P was slowly released, initially at a rate of 30 mg kg^{-1}, which decreased to 10 mg kg^{-1} after 28 days and remained constant until the experiment concluded at 60 days. However, this preparation may not always be necessary as Silveira *et al.* (2013) highlighted that WTRs were an adequate substitute for organic rich fertiliser in all but P deficient soils when growing bahiagrass (*Paspalum notatum* Flüggé).

There have also been attempts to utilise WTRs to remediate or make safer soils known to be contaminated with PTEs such as As, Cd and Pb. Garau *et al.* (2014) trialled Fe WTRs and other waste materials in a study designed to assess immobilisation of PTEs (i.e. Cd, Cu, Pb, Zn) in a circum-neutral pH soil contaminated with As (2105 mg kg^{-1}), Cd (18 mg kg^{-1}), Cu (264 mg kg^{-1}), Pb (710 mg kg^{-1}), Zn (522 mg kg^{-1}). The amendments were added at 3% by mass and equilibrated for 6 months after which plant growth, element mobility, microbial diversity and microbial function were assessed. They found a 27% reduction in the non-specifically adsorbed (i.e. readily mobile) As in Fe WTR treatments, as well as reductions in $Ca(NO_3)_2$ extractable Cd, Pb and Zn. The authors observed a 2.5-fold increase in plant yield for the common bean (*Phaseolus vulgaris*) and a 1.8-fold increase for wheat (*Triticum vulgare*) grown in WTR treated soil and, importantly, the yield increases could be directly attributed to reduced toxicity through contaminant immobilisation rather than to any changes in pH or nutrient addition. However, while WTR treatment resulted in a significant decrease in shoot As concentration in wheat (from ~39 to 21 µg-^{1}), a conflicting result was noted for bean where the shoot As concentration increased (from ~50 to 60 µg-^{1}).

Nagar *et al.* (2015) investigated the long-term (three-year) effect of incorporating Al and Fe WTRs, separately, on As bioaccessibility in two As spiked soils with contrasting properties (a sandy soil with pH 4.05 and a sandy clay loam with pH 7.85). The authors found that bioaccessible As, determined via the in vitro gastrointestinal simulation was reduced by 50 to 80% when compared with non-WTR treated soils for both Al and Fe WTRs at both rates of application tested (5% and 10% w/w). It was also reported by the same research group that As sorption decreased as pH increases under As loads of 1875 and 3750 mg kg^{-1} applied to a sandy soil, while this effect was undetectable under a lower As load of 125 mg kg^{-1} (Nagar *et al.*, 2013). Contrary to the findings of Gibbons and Gagnon (2011) and Castaldi *et al.* (2014), who both observed Fe WTRs to have greater As and P sorbing capacity than Al WTRs, a study by Nagar *et* *al.* (2013) reported Al WTRs to have achieved a greater As sorption than Fe WTRs in the soils they tested (see Table 2-5).

Other studies focusing on contaminant metals in soil have also found reductions in mobility or availability following WTR application. For example, Elkhatib and Moharem (2015) explored the effect of WTR application on the non-residual fraction (i.e. the fraction considered potentially mobile or bioavailable), determined by the Tessier *et al.* (1979) sequential extraction method, of Cu, Pb and Ni in calcareous and sandy agricultural soils, the results of this study can be seen in Figure 2-5.

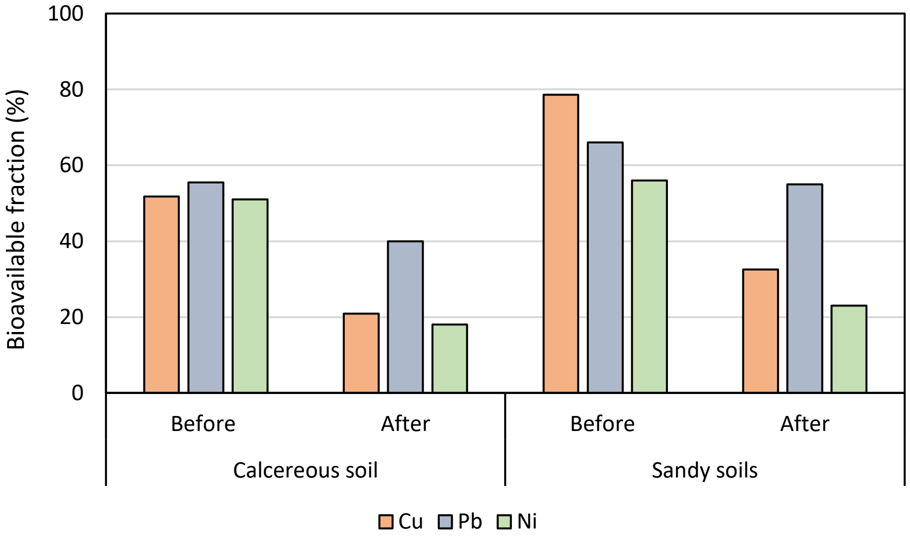

Figure 2-5. The bioavailable fraction of Cu, Pb and Zn, as determined by the Tessier et al. (1979) sequential extraction method, before and after WTR application to soils. Data extracted from Elkhatib and Moharem (2015).

As with water studies, there have also been attempts to incorporate nWTRs into soils. When mixed with Cd and Pb spiked soils (spiked at 50, 100 and 150% of the soil metal concentration limits suggested by USEPA), incorporation of 0.3% by mass nWTRS led to a >99% reduction in phytoavailability (*Brassica napus* L.) of both Pb and Cd (Elkhatib *et al.*, 2018).

There have been several attempts to co-apply WTRs alongside other organic wastes such as sewage biosolids (Maurice *et al.*, 1998; Ippolito *et al.*, 2009a; Mahdy *et al.*, 2009). Co-application of WTRs and biosolids was shown to increase corn yield proportionally to WTR concentration up to an application rate of 3% by weight, above this point yield decreased (Mahdy *et al.*, 2009). This was matched by a corresponding effect on P uptake, while no phytotoxicity was observed (Mahdy *et al.*, 2009). The long-term (13-15 y) impacts of a single application and short-term (2-4 y) impacts of repeated coapplications of WTRs and biosolid amendments to semi-arid grasslands were investigated by Ippolito *et al.* (2009a). They found that ammonium bicarbonate-diethylenetriaminepentaacetic acid extractable Cd, Cu, Fe, Mn, Ni, Zn and soil pH, EC, NO_3–N, NH_4–N, total C, and total N showed no significant change under a single WTR-biosolid application, however a majority of these increased under repeated applications (Ippolito *et al.*, 2009a). Unfortunately, this study did not compare results to those of a plot containing only biosolid amendments and thus it is difficult to distinguish the influence of each amendment. Tay *et al.* (2017) outlined a project that aimed to maximise the benefits of WTR amendments while minimising P immobilisation by coapplying P fertilisers, as had been recommended previously (Elliott and Dempsey, 1991; Lucas *et al.*, 1994). This combined application produced no significant difference in total plant tissue P concentration relative to controls but did reduce plant tissue As and Cd concentrations when WTR amendments to the sandy loam soil tested remained between 2-4%.

Land application of WTRs, or use as a component in plant growing media, is therefore well justified as an increasingly common recovery and reuse option. As well as for use on productive agricultural land, the literature indicates that WTR application on degraded or contaminated soils can help realise benefits through reducing plant uptake and mobility of PTEs and other contaminants. However, there are remaining questions that need to be answered in terms of the full range of influences and benefits that WTRs can have on soil chemistry and ecology once applied. This is explored further in section 6.

2.5.5. Use in construction materials

Research into the use of WTRs in construction materials has been limited when compared with other potential beneficial uses and this is likely due to concerns that arise from the variability of physical properties of products produced from WTRs (Babatunde and Zhao, 2007). This is brought about by their variability in chemical composition, water content and organic matter content. However, a general geotechnical evaluation of alum sludge found that, when mixed with zeolite Al sludge produces a material which is suitable for use in a variety of geotechnical and geoenvironmental applications due to its high shear strength, mechanical stability and low permeability (Balkaya, 2015). This along with other studies suggest that WTRs can be used in construction, as long as the correct pre-treatment (i.e. dewatering and drying at 105^{o}C) is applied and they are mixed with the correct materials.

Clay bricks

Due to the similarities in the chemical composition of brick clay and WTRs, production of bricks with a partial replacement of clay with WTRs could offer a large reuse pathway for WTRs. Multiple experiments have explored incorporating WTRs into bricks (Table 2-7). Initial studies explored the combined additions of WTRs and sewage sludge ash (Anderson *et al.*, 2003; Golbold *et al.*, 2003). Anderson *et al.* (2003) documented the use of WTR and sewage sludge incineration ash as a combined 5% by weight addition to brick production. Preliminary results of that study facilitated the rollout of a full-scale factory trial producing 100,000 bricks. Multiple other studies have suggested that 15-20% WTR additions are optimum to comply with a variety of national regulations, including those in India, Taiwan, Britain and Malaysia (Huang *et al.*, 2005; Elangovan and Subramanian, 2011; Shamsudin *et al.*, 2017). Leaching testing has also confirmed that bricks produced with up to 2.5% WTR content complied with the relevant national (British) Standards (Anderson *et al.*, 2003). Bricks containing much higher WTR sludge content, 50-80% sludge, and those containing solely WTRs, rice husk ash and silica fume were found to generally be of superior quality to those available locally in some areas of Egypt (Ramadan *et al.*, 2008; Hegazy *et al.*, 2012). Therefore, in areas where brick production standards are less stringent or the availability of quality bricks is limited, the use of WTRs in brick production could be of particular benefit. However, in order to produce bricks containing higher amounts of WTRs, the sintering temperature of the bricks must be increased to ~1050-1100 ^{o}C due to the lower silica content and higher water content of WTRs, thus making the brick production process more energy intensive at these rates and therefore making the process less economically and environmentally appealing. Furthermore, Anderson *et al.* (2003) noted that additional investment in equipment was required by the brick making industry in order to achieve the required blending of materials.

Table 2-7. The outcomes of studies into the incorporation of WTRs into the brick manufacturing process.

Country of study	Sludge content	Other component materials	Sintering temperature (°C)	Outcome	Reference
United Kingdom	0-2.5%	2.5% sewage sludge incineration ash and 95% clay.	1050	Additions had no significant impact on compressive strength, water absorption or efflorescence.	(Anderson *et al.*, 2003)
India	0-50%	Clay	700 - 950	20% WTR was optimum, although compressive strength drops by 15-50% beyond 15% additions.	(Elangovan and Subramanian, 2011)
Malaysia	0-30%	The brick mixture was 1:2:4 of cement: sand: soil + WTP residual solution.	Not specified	20% WTR content was optimum for bricks in order to comply with British and Malaysian standards (BS 3921: 1985 and MS 7.6: 1972 respectively).	(Shamsudin *et al.*, 2017)
Taiwan	0-30%	The remainder of the brick mixture was made up of excavation waste soil.	850-1050	Up to 15% WTRs could be included in bricks that still meet the Taiwanese National Science Council's specification for a first-grade brick, while additions between 15% and 30% can be used to produce second or third class bricks. However, the sintering temperature of the bricks must be increased from 950 to 1050 ^{o}C.	(Huang *et al.*, 2005)
Egypt	50-80%	Clay	950-1100	50% WTR content was optimum although no control was tested.	(Ramadan *et al.*, 2008)

Egypt	0-50%	The remainder of the brick mixtures contained 50-75% combinations of silica fume and rice husk ash. Whilst the control consisted of 100% clay.	950- 1100	WTR content increased compressive strength at higher sintering temperatures (>1100 C) compared to 100% clay bricks.	(Hegazy *et al.*, 2012)
Portugal	0-5%	Clay	Not specified	WTR additions improved thermal transmittance and therefore better for insulation purposes.	(Santos *et al.*, 2015)

Cement, concrete, and aggregates

There have also been attempts to incorporate WTRs into cements (Huang *et al.*, 2005; Chen *et al.*, 2010; Yen *et al.*, 2011) and concretes (Kaosol, 2010; Sales *et al.*, 2011; Lee *et al.*, 2012; Owaid *et al.*, 2017) (Table 2-8). The production processes related to these materials do not include a sintering step, consequently Rodríguez *et al.* (2010) found that additions as small as 10% were enough to retard the hydration rates of mortars which in turn increased the duration of the production process by over 12 hours. Multiple studies reported that increasing WTR additions reduced mechanical or compressive strength (Rodríguez *et al.*, 2010; Owaid *et al.*, 2017), although Chen *et al.* (2010) noted that these changes only occurred above 7% additions while increases in strength were produced below 7% additions. In order to address this issue, Lee *et al.* (2012) produced concrete with 30% sludge with additions and 2% of a solidification agent (main components $CaSO_4$ (61.5%) and CaO (31.4%)) and achieved an acceptable mechanical strength according to the ASTM International standards (ASTM C117 - 04). As with bricks produced from WTRs, the leachates of these bricks had a low content of many common PTEs, specifically Cd, Cr, Cu, Ni, Pb and Zn (Huang *et al.*, 2005; Chen *et al.*, 2010). It has been suggested that the high proportion of silica (SiO_2) in some WTRs allows for the formation of calcium silicate (Ca_2SiO_4) when combined with lime, this process provides the majority of set cements strength. The production of WTR based products has a clear economic driver, as hollow concrete blocks produced containing 10%- 50% WTRs were found to reduce the cost of production by 12%- 41 respectively (Kaosol, 2010).

Table 2-8. A summary of past studies in producing cement, concrete and aggregates produced from WTRs.

Country of study	Material produced	Sludge content	Other component materials	Outcome	Reference
China	Artificial aggregate and cement	Not specified	Excavation waste soil.	Aggregates required sintering at high temperatures to achieve construction standards.	(Huang *et al.*, 2005)
Malaysia	Concrete	0-25%	Silica fume, Ground Granulated Blast Furnace Slag, palm oil fuel ash and cement in varying ratios.	Decline in compressive strength of concrete containing thermally activated alum sludge ash (AASA) was found to correlate with the concentrations of AASA.	(Owaid *et al.*, 2017)
Thailand	Concrete blocks	0-50%	A mixture of powdered Portland cement, sand and crushed stone dust.	10-20% WTRs could be used in hollow load bearing concrete blocks, while up to 50% can be used in hollow non-load bearing concrete block.	(Kaosol, 2010)
Brazil	Concrete	52%	A composite was made of sawdust, sludge and water (1:6:4.5) and added to concrete made of cement: sand: composite: water mass ratios of 1:2.5:0.67:0.6.	Suitable for application in non-structural elements.	(Sales *et al.*, 2011)
Taiwan	Cement	10%	39.4% marble sludge, 10% water treatment sludge, 1.8% basic oxygen furnace, 39.4% limestone and 9.5% sand.	Sludge containing WTRs had higher compressive strength than conventionally produced cement after 28 days of aging.	(Yen *et al.*, 2011)
China	Cement	0-10%	Shale at 0-15%, 80.9-81.5% limestone, 0-5.8% sand, 2.5-2.9% copper waste and 0.4-0.5% aluminium hydroxide.	Cement containing up to 5.5% sludge had a higher compressive strength than conventionally produced cement, however additions of ≥7% led to a significant decrease in compressive strength after aging for 28 days.	(Chen *et al.*, 2010)
Taiwan	Concrete	0-30%	0-6% of a solidification agent (main components $CaSO_4$ (61.5%) and CaO (31.4%).	Achieved an acceptable mechanical strength according to the ASTM International standards (ASTM C117 - 04) at the maximum WTR content with as low as 2% solidification agent.	(Lee *et al.*, 2012)

Ceramics

In the production of ceramics, the addition of alum sludge up to 30% by weight alongside coapplication of a silicate based fluxing agent has been found to produce physically similar ceramics to the regular production process, additionally these new WTR ceramics were resistant to leaching (Vicenzi *et al.*, 2005). However, another study by Teixeira *et al.* (2011) found that additions of 10-20% of Fe-WTRs led to a reduction in flexural strength and specific mass, while also increasing water absorption, highlighting the need for a fluxing agent. Kizinievič and Kizinievič (2017) and Kizinievič *et al.* (2013) found that increasing the Fe-WTRs content of ceramics had the following impacts on its properties: lower density and compressive strength, while increasing shrinkage, water adsorption and porosity. Additionally, it has been commented that Fe WTRs have an impact on the colouration of ceramics, therefore they may be used as a substitute for dark red pigment. Wang *et al.* (1998) also found that leachates from WTR containing ceramics complied with the Taiwan EPA guidelines.

Landfill coating and daily cover

A more novel construction use is that of a hydraulic barrier and as a daily cover material for landfill which is applied to minimise interaction between wastes and the air whilst also producing a solid base for vehicles to manoeuvre. WTRs can be used effectively for these purposes and provide similar results to other commonly used materials such as soil, paper sludge or tyre derived aggregates (Balkaya, 2016). In keeping with this idea, Caniani *et al.* (2013) produced a bio-soil from the stabilized organic fraction of municipal solid waste, Al sludge and Clinoptilotite (a type of zeolite) which proved to be a successful barrier layer for landfill in terms of chemical and physical properties and leaching tests.

Construction summary

To summarise, the use of WTRs in construction is a potentially useful and beneficial end use, with perhaps the greatest scope for this being found in regions or countries where high quality building materials are less available, as suggested by some brick based studies (Ramadan *et al.*, 2008; Elangovan and Subramanian, 2011). The additional requirements such as higher sintering temperature (Huang *et al.*, 2005), longer production times (Rodríguez *et al.*, 2010) and fluxing/solidifying agents (Vicenzi *et al.*, 2005; Lee *et al.*, 2012) when WTRs are used in high percentage rates in bricks means that additional development is required in this area.

Nevertheless, positive results have been obtained from research into the use of WTRs in brick and ceramic production that could have widespread ramifications for the reuse of WTRs.

2.5.6. Coagulant recovery and reuse

Coagulant recovery (CR) offers a viable alternative end-of-life use for WTRs, which has the additional benefit of reducing the amount of WTRs produced as well as reducing disposal costs (Keeley *et al.*, 2014). During CR, acid is applied to water treatment sludge to redissolve spent coagulant metals (predominantly Al or Fe). Following this step, undesirable contaminants are separated through varying separation processes. CR was popular during the 1970s and 1980s due to more lenient legislation for water quality (Keeley *et al.*, 2014). However, its use was eventually ended due to issues relating to the accumulation of acid soluble impurities and the overall variability of WTRs.

Current methods can reliably recover >70% coagulant metals, effectively reducing sludge volumes by 60% (Keeley *et al.*, 2014). Abdo *et al.* (1993) reported that recovered coagulants were only 80% as efficient as fresh coagulants during water treatment. Xu *et al.* (2009) also measured the efficiency of recovered alum and found that turbidity, UV_{254} and COD removal efficiencies were 96%, 46% and 53% respectively of those that fresh alum displays. One of the greatest difficulties facing CR is ensuring that the benefits of the process outweigh the chemical (mainly acids) and energy costs.

Alternatively, direct reuse of WTRs in the treatment of wastewater has shown promise (Xu *et al.*, 2016b). WTRs can be used as a substitute for 40% of the required fresh coagulant while producing no significant difference in all measured parameters of outlet water when applied to low turbidity water (Xu *et al.*, 2016b). Suman *et al.* (2017) found that WTRs produced better removal results than conventional coagulants when treating dairy wastewater. The two-pronged reduction in WTRs obtained through this process make it an ideal reuse option, however further study is required.

Further data on the full-scale possibilities of CR and coagulant reuse is required. Although CR may not currently be viable, it has potential in the future as recovery technologies advance and the process can be streamlined. Until that time, recovered coagulant and alum sludge can both be used in waste water treatment in regions where regulations are less strict (Keeley *et al.*, 2014; Xu *et al.*, 2016b).

2.6. Environmental and ecological impacts and concerns

Despite land application of WTRs now being a well-established and increasingly common practice, the most frequently raised concerns in published literature related to the use of WTRs in the open environment are the possibilities for the leaching of metals (especially Al) and the immobilisation of too much P (as elevated mobile P can cause eutrophication of waterbodies).

Aluminium is a nonessential metal which, in humans, has been related to a number of neurological and respiratory diseases, renal failure and bone damage when present above critical threshold concentrations (Krewski *et al.*, 2007). The negative effects of Al on fish and invertebrates are also well established (Herrmann and Frick, 1995). However, concerns about Al regarding the use of WTRs in land application do not centre around any risks to human health, but relate rather to the risk of Al toxicity to plants and soil biota. Aluminium is abundant in the environment, accounting for ~7-11% (by mass) of the Earth's upper crust (Hu and Gao, 2008) and forming one of the main constituents of many soils. However, Al toxicity generally becomes a concern when a soil pH is below 5 because of greater quantities of soluble aluminium (i.e. Al^{3+} and $AlOH^{2+}$) being released into the porewater (Brady and Weil, 2008). Howells *et al.* (2018) highlighted the importance of placing some restrictions on the conditions in which WTRs are used, finding that the leachable fraction of Al (i.e. 0.001 M $CaCl_2$ extractable Al) from Al WTRs increased from 4.5 mg kg^{-1} at a pH of 5.5 to 382 mg kg^{-1} at a pH of 4.4. This is reflected in legislation and environmental regulation of some regions, an example of which is in England and Wales where the application of Al WTRs is limited to soils above a pH of 6 due to the increased mobility of Al below a pH of 5 in soils, while Fe WTRs are limited to application to soils above a pH of 5 (Environment Agency, 2013). A number of studies have examined the leachability of Al from WTRs and the bioavailability of the Al contained within them, while other studies have investigated the influence WTR application to land has on soil porewater Al concentrations and plant assimilation of Al (Bugbee and Frink, 1985; Chiang *et al.*, 2012; Caniani *et al.*, 2013; Howells *et al.*, 2018). Upon aging WTRs through incubation, Agyin-Birikorang and O'Connor (2009) found that WTRs required ≥ 6 months of aging to stabilise the most reactive Al forms (determined as 5mM oxalate extractable), therefore suggesting that fresh WTRs should be aged before being applied to land to alleviate such concerns. However, this has not been examined in detail or supported by other findings and thus warrants further investigation.

As discussed in section 5.4.3., the sorption of P is a potential limiting factor when it comes to land application of WTRs, unless the WTRs are being deliberately applied to restrict mobility of excess P that arises from previous overuse of fertilisers and manures, etc. However, as alluded to previously, co-application of WTRs with mineral fertilizers or other nutrient input (i.e. manures, composts, or biosolids) can circumvent this concern. Indeed, support for this approach can also be found in the review of legislation at a European level on WTRs disposal by Hidalgo *et al.* (2016), which indicated that the most feasible use of WTRs from a regulatory standpoint is as an agricultural substrate when mixed with a nutrient source.

Despite these and other past studies that have shown little to no negative impact from WTR land application, WTRs are still considered hazardous wastes in some jurisdictions. For example, the Environmental Protection Agency (USA) ranks WTRs as having the second highest environmental impact potential (based on risk) of all effluent sources, contributing 10.7% of the national hazardous effluents production, although this figure is inflated by the hazard potential of chlorine residuals from other stages of the drinking water treatment process (EPA, 2016). Having this kind of status may be pragmatic in one sense, i.e. in facilitating restriction of access and applications of WTRs to approved processes and practises, but in other ways it is a barrier to use as it imparts negative associations with the material and creates administrative and regulatory obstacles that must be overcome to enable use of the material. Research that better clarifies and quantifies any risks involved in the land application (or other uses) of WTRs would help to address this.

In terms of impacts on biota and ecosystems, while multiple studies have explored the direct effects of WTRs on aquatic ecosystems (Hall and Hall, 1989; George *et al.*, 1991; George *et al.*, 1995; Kaggwa *et al.*, 2001) there is limited coverage in the literature regarding the effects of WTRs on terrestrial ecology. This is a notable gap, especially considering that land application is an increasingly important disposal route. The information that is available in the literature on this aspect is discussed below.

2.6.1. Microorganisms and invertebrates

Ippolito *et al.* (2009a) found that surface co-application of WTRs with biosolids at a rate of 5-21 t ha^{-1} caused a minor enrichment on a subset of microbial community fatty acids, including markers for Gram-positive and Gram-negative bacteria after a period of 15 years, although it was suggested this increase was more closely related to biosolid addition than that of WTRs. However, the overall effect on plants and soil biology was minimal and was considered to pose no overall threat to the environment. Garau *et al.* (2014) found that Fe-WTR amendments (3% w/w addition rate) led to increased culturable heterotrophic bacteria and actinomycetes while having the opposite effect on heterotrophic fungi. Overall, they found the soil microbial biomass remained constant.

WTRs themselves are a source of some microorganisms, for example Oliver *et al.* (2011) verified that the abundance of microbes capable of proliferation under anaerobic conditions was comparable with that observed for soils, while Xu *et al.* (2018) determined that *Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia*, and *Planctomycetes* were the dominant phyla among the six Chinese WTRs they examined. Xu and co-workers also identified the presence of three genera of potentially toxic cyanobacteria (*Planktothrix, Microcystis* and *Cylindrospermopsis*), and four potential pathogens (*Escherichia coli, Bacteroides ovatus, Prevotella copri* and *Rickettsia*), with their abundances heavily influenced by the nutrient and Fe contents of the raw waters whose treatment generated the WTRs. This was in agreement with an earlier German study (Würzer *et al.*, 1995) that determined WTRs could contain pathogenic bacteria, viruses and Protista.

However, Pecku *et al.* (2006) investigated the influence of 300 t ha^{-1} applications of WTRs on microbial indicators of soil quality (soil respiration, denaturing gradient gel electrophoresis and DNA analysis) and found no detrimental effects on any of the measured values. Nevertheless, although no negative impacts have been observed, the long-term influence of WTR addition on microbial population dynamics remains unknown. Howells *et al.* (2018) conducted survival, reproduction and avoidance tests with earthworms exposed to 0-20% WTRs containing soils. Their study found that earthworm biomass, survival and reproduction were unaffected, although avoidance of soils containing ≥ 10% of Fe WTR and 20% Al WTRs was noted.

2.7. Summary, conclusions and recommendations

Despite a well-established continued level of WTR use in land applications, there are remaining questions regarding their utilisation in this sector. A key issue is the amount of published literature from utility companies and commercial trials in the public domain. Other end use markets exist but are less well researched and documented. With mounting environmental and economic pressure from the public and regulatory bodies, and the move away from landfill disposal, the need for sustainable and efficient outlets for WTRs continues. However, while research and publicly available data for other organic materials such as sewage sludge, is widespread, information relating to by-products from the drinking water treatment process are not nearly as extensive. The gap in published data relating to WTRs and impacts associated with application to land in the UK highlights a need for an up-to-date review of information held by utility companies. The benefits, disadvantages and limitations of the various WTR use options examined in this paper are summarised in Table 2-9.

Table 2-9. A summary of the advantages and disadvantages of varying disposal routed for WTRs

Potential Market for End Use			**Advantages**	**Disadvantages**
Sorption	Water remediation	Elemental contaminants	• Sorbs high amounts of individual or multiple contaminants • nWTRs have even greater sorption capacities	• Possible excessive P sorption • Leaching of some elements and compounds are still a concern
		Textile dye	• Very high removal rates	• Not economical currently • Disposal of produced product • Does not work for hydrophilic dyes
		Organic contaminants	• Possibly remediation method for emerging contaminants • Could reduce chance of eutrophication	• Lack of research
	Constructed wetlands		• High removal efficiencies • Proven success in incorporating bio-sorption reactors and microbial fuel cells	• Clogging • Low demand for WTRs
	Lakes and reservoirs		• Could reduce chance of eutrophication through nutrient control	• Leaching of some elements still under question
	Soil remediation		• Can sorb high amounts of organic and inorganic pollutants • Reduction of P runoff could reduce eutrophication	• Potentially excessive P sorption • May require coapplication to negate crop yield reduction in certain circumstances • Potential impacts relating to leaching of Al and Fe from coagulants
Bulk land application			• Increases aeration • Provides sufficient N for plant growth • Can increase plant yield • Increased hydraulic conductivity	• Excessive P sorption • Coapplication of P source may be required • Worries regarding the leaching of metals
Incorporation into construction materials	Bricks		• Could offer a disposal route for a large quantity of WTRs • Can reduce production costs • Up to 15-20% WTR content will pass a majority of standards	• Higher sintering temperature may be required • Some leachates of concern • Reduction in strength above 15% WTR content
	Concrete and cement		• Can reduce production costs • Could offer a disposal route for a large quantity of WTRs	• Higher sintering temperature may be required • May require solidification agent • Reduction in strength at higher WTR content
	Ceramics		• May be used as a pigment	• Can have an unwanted effect on colouration • Lower compressive strength • Greater shrinkage of products
Coagulant recover and reuse	Recovery of metals/coagulant		• Reduction of WTR production • Has been economically viable in the past • Can recover >70% of coagulants	• Expensive due to chemical costs and processes involved • Not economically viable currently • Recovered coagulants are not as efficient as fresh coagulants
	Reuse in wastewater treatment		• Reduction of WTR production • Efficient removal rates • Reduces coagulant requirements for the process	• Regulations may limit use

In summary, while it is known that WTRs can be utilised in multiple ways, there are still unexplored issues and potentially limiting factors. For example, while the use of WTRs in CWs is a disposal route that is viable, the lack of development of anti-clogging techniques is a prominent issue in the literature. Unfortunately, WTR based CWs are unlikely to provide a large demand for WTRs due to the systems long lifecycles. The adsorption qualities of WTRs are well documented, and while they are reportedly dependent on certain conditions such as pH (Hovsepyan and Bonzongo, 2009; Zhou and Haynes, 2011; Silvetti *et al.*, 2015), the use of WTRs as a sorbent is still applicable in a majority of natural soils sufficing that their application is carefully assessed. Leaching of pollutants has been shown to be an unlikely problem in most environmental settings, however there appears to remain somewhat of a disconnect between research findings, governing environmental policy and business enterprise regarding WTRs. Such disconnects can only be solved through the availability of more scientific evidence and through the communication of that evidence to policy makers. Nevertheless, land application has been identified as a commercially viable and, to the best available knowledge, an ecologically sound disposal route that can be further enhanced by the co-application of a P source when required. Alternatively, the Al removal methods suggested by Okuda *et al.* (2014) may offer another method of reducing P sorption by Al-WTRs when required. The short- and long-term influence on the ecology and biogeochemical processes in soils treated with WTRs do however remain avenues that require further research. Construction is a mass disposal route for WTRs, which appears to be feasible (as seen in Anderson *et al.* (2003)); however, variations in the physical properties of WTRs makes their incorporation into these materials difficult, therefore further innovation is required to bring this disposal route into the commercial sector. Finally, recycling of the coagulant components of WTRs may not currently be economically feasible, but it offers a proven avenue for disposal if the costs related to the process become more favourable.

The nature of WTRs, with their highly variable physical and chemical structure, means that even after previous studies, further investigation of WTRs from a variety of water treatment plants and from different regions is required before any definitive conclusions can be drawn and applied widely. It may be the case that WTRs reuse would be best assessed on a case-by-case basis. Additional end use options should also be explored. Any issues related to WTRs are only further compounded by a lack of published figures relating to their production and disposal. A more comprehensive review of this data is required in order to give a more accurate picture of the required end use capacity for WTRs.

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British earthworms: their collection and identification

2.8. Introduction

Earthworms (*Lumbricidae, Annelida*) have long been known to play a key role in the formation and development of soils. Darwin (1892) stated:

> *The plough is one of the most ancient and most valuable of man's inventions; but long before he existed the land was in fact regularly ploughed, and still continues to be thus ploughed by earth-worms. It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creatures.*

Earthworms are the most abundant terrestrial invertebrate in the temperate regions, accounting for 60-80% of soil biomass (Roubalová *et al.*, 2015; Omouri *et al.*, 2018). Earthworms are important 'ecosystem engineers' which contribute greatly towards soil forming processes and the overall ecosystem services soils provide (e.g. for facilitating food production and water management), which have been estimated to be worth $33 Trillion to the global economy (Sharma *et al.*, 2017).

Most commonly, earthworms are categorised into three broad ecological types: anecics, epigeics and endogeics. Anecics have strong anterior digging musculature which allows them to produce deep permanent burrows up to a vertical depth of 3m (Lavelle, 1988). They rely on surface leaf litter as their food source, and therefore their burrows help connect deeper soil layers with the surface. They are generally large in size (>20cm length), making them more prone to the effects of agricultural practices than epigeics and endogeics (Gavinelli *et al.*, 2017). Endogeic earthworms are topsoil dwelling and use organic matter from the soil as their food source, this organic matter is typically of greater quality and more abundant at shallow depths and surrounding plant roots (Lavelle, 1988). Species in this ecological category do not form permanent burrows as they simultaneously ingest soil for their food and excrete casts which fill or partially fill any biopores that they formed (Lavelle, 1988). Epigeics in contrast subsist aboveground in leaf litter, and tend to be small and have high metabolic rates (Lavelle, 1988).

2.9. Distribution and species

This review focusses on those species found within the United Kingdom and Ireland. There are 27-29 earthworm species that are native to the UK and Ireland (Natural-England, 2014), with the slight ambiguity around the number arising from certain species (e.g. *Aporrectodea caliginosa*) having distinct morphs that are contested by some of the literature to be separate species. Earthworm communities normally consist of 8-12 of these species under favourable conditions (Lavelle, 1988).

Natural-England (2014) conducted a large-scale study into the distribution and abundance of Earthworms in England and Scotland. The study was conducted at 333 sites covering 15 habitat types, with a focus on previously under-sampled habitats. The results of the study were then incorporated with a database including results from previous earthworm studies. Results from the final database highlighted that the ten most abundant earthworm species account for 95% of all identified specimens; *Allolobophora chlorotica* was the most abundant accounting for 34% of identified specimens, followed by *Aporrectodea caliginosa* at 19% and *Lumbricus castaneus* at 12%. It has been noted that endogeic species are more commonly found in spring, while epigeic and anecic species are more likely to be found in autumn (Paoletti, Sommaggio et al. 1998).

2.9.1. Identification

The key features of earthworms, which are used in their identification are the following: Head-lines (head shape), male pore, clitellum/saddle, tubercula pubertatis (TP) and Setae (Figure 2-6). Their heads are located at the end closest to the clitellum, and the head shape, identified by lines on the first segment at the head end (which are best viewed dorsally), is used to subdivide earthworms into two groups, tanylobic and epilobic. The tanylobic head shape has two distinct lines running down from the cap of the first segment all the way to the second segment, while the epilobic head shape's lines or grooves do not run all the way to the second segment (Figure 2-7). The male pore is located on either segment 13 or 15 (measured from the head) of the earthworm. It can either appear to be a small slit or encompass the surrounding segments. The clitellum (or saddle) is indicative of the earthworm having reached maturity, it also produces the mucus used during cocoon formation. Juvenile earthworms are very difficult (if not impossible) to identify using standard observation methods because their identifying features have not yet fully developed (thus molecular/DNA diagnosis would be required), and are therefore are not often identified as part of ecological earthworm studies. The TP take the form of either a marking, lumps, lines, swellings or sucker like discs found under the clitellum. Setae are the hair like structures which can be found on every segment of, their spacing (i.e. whether they are closely paired or widely paired) is of particular interest during identification. An in depth identification guide and key can be found in Sherlock (2012).

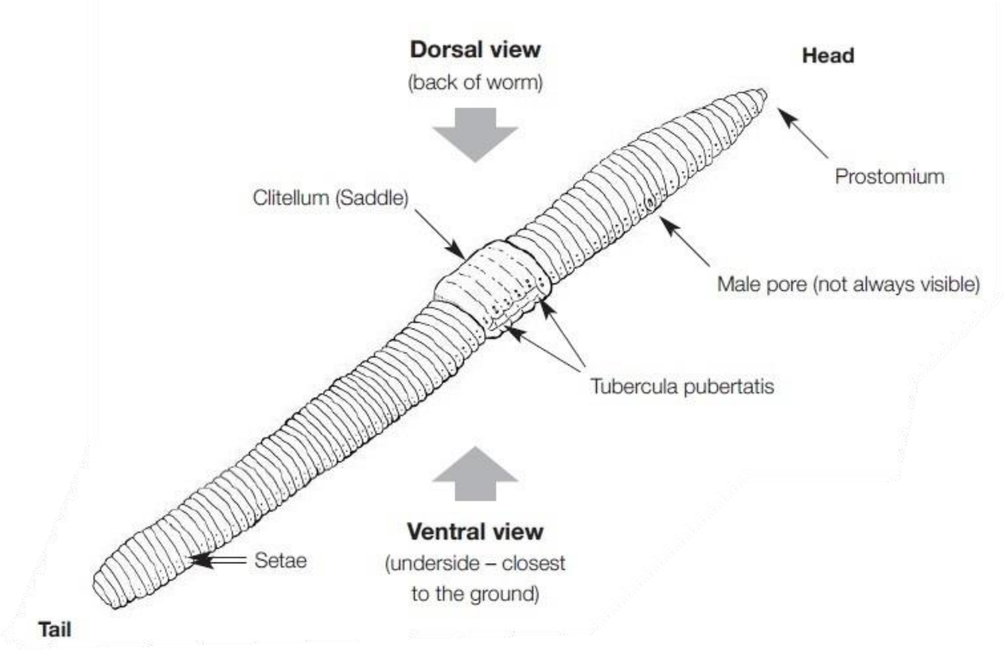

Figure 2-6. A diagram of the common adult earthworm, highlighting the main diagnostic features used during species identification (Sherlock, 2012).

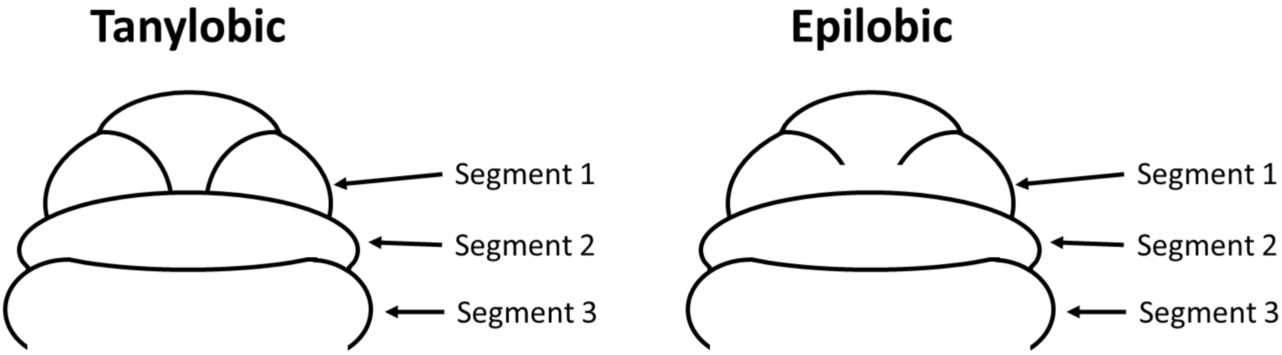

Figure 2-7. A diagram displaying the Identifying features of epilobic and tanylobic earthworms when viewed dorsally.

2.10. Role in soil processes

2.10.1. Soil structure and bulk density

The role that earthworms play in soil evolution is focussed around their influence on four main soil characteristics; porosity, aeration, water dynamics and structural stability (Lavelle, 1988). Estimates of cast (excretion) production vary between 30 to 100 tonnes ha^{-1} yr^{-1} (Evans, 1948; Sharpley and Syers, 1976; Sharma *et al.*, 2017), indicating the extent of soil and organic matter processing and turnover earthworms provide. The development of soils texture by earthworms was initially thought to be due to their general favoured ingestion of smaller particles (Lavelle, 1988). Blanchart *et al.* (1999) expanded on this idea, suggesting endogeic earthworms be categorised into a further two subcategories relating to soil structure: firstly, compacting species which egest large clasts, increasing bulk density of soils (e.g. *Pontoscolex corethrurus* or *Millsonia anomala*); secondly, decompacting species that consume large aggregates and egest smaller, fragile aggregates (e.g. Eudrilidae), reducing overall bulk density in soils. Decompacting species have the effect of increasing infiltration rates and decreasing water retention, while compacting species have the reverse effect on both of these properties. Blanchart *et al.* (1999) also highlighted that earthworm impact on soils may persists for up to 3 years in plots where earthworms are now absent. To give an indication of the magnitude of this effect, two studies using the endogeic *Reginaldia omodeoi* reported increases in soil bulk density from 1.37 to 1.48 g cm^{-3} and from 1.24 to 1.31 g cm^{-3} (Lavelle *et al.*, 2004). The effect earthworms have on soil porosity is also stratified; shallow burrows are unlined, increasing the porosity of the soil, whereas at depth their burrows often have a ~1mm lining of organic cast material which can obstruct soil pores and affect water flow (Lavelle, 1988).

2.10.2. Organic matter and nutrients

Earthworm digestion is widely thought to increase both mineralisation and humification of organic matter (Lavelle, 1988). It has been noted to enhance C incorporation into soil (Villenave *et al.*, 1999). Although, Don *et al.* (2008) found that there was no substantial increase in belowground C relating to earthworms, however earthworm burrows produced a route for the fast transfer of C to deeper soil horizons. Lubbers *et al.* (2013) conducted a meta review and found that SOC was not directly impacted by earthworm activity.

Counterintuitively, an increase of C emissions from soils has been widely reported in relation to earthworm activity (Sharma *et al.*, 2017). Lubbers *et al.* (2013) investigated this phenomena by reviewing literature on soil C content in relation to earthworms. Their results suggest that while earthworms sequester C in microaggregates, they also increase CO_2 and N_2O emission by 33% and 42%, respectively. However, these effects vary and in some cases, oppose each other (e.g. increased CO_2 emissions with simultaneous decreased N_2O emissions) due to the complicated relationships between earthworm activity, soil processes and greenhouse gas emissions (Lubbers *et al.*, 2013). The authors did note however, that the increases in CO_2 flux diminished over time, with studies longer than 200 days resulting in a negligible variation from initial fluxes.

For N_2O fluxes, increases became observable after ~30 days and these continued to increase over time, although unlike CO_2 measurements the N_2O flux studies never extended as long as 200 days. Of the studies reviewed by Lubbers and co-workers in relation to assessing earthworm effects, no change in soil organic carbon (SOC) content was reported in studies conducted over a period of greater than 200 days.

Increased plant biomass has been linked with soil earthworm activity in studies conducted in the tropics, with reported increases of 56.3% ± 9.3% in shoots and 35.8 ± 8.9% in grain (Brown *et al.*, 1999). The activity of Earthworms is thought to be beneficial as it enhances soil nutrient cycling by accelerating the incorporation of litter into mineral soils and increasing orthophosphate release through enhancement of the interactions between organic matter and reactive metals (Bhadauria and Saxena, 2010; Ros *et al.*, 2017). The cast material produced by earthworms produces a source of easily assimilated nutrients (N, P, K and Ca) for plants, which are in some cases greatly increased in concentration relative to surrounding soil concentrations (Bhadauria and Saxena, 2010; Ros *et al.*, 2017). Earthworms have generally been shown to increase P utilisation of plants and increase plant biomass production (Brown *et al.*, 1999; Vos *et al.*, 2014; Ros *et al.*, 2017). Le Bayon and Binet (2006), concluded that earthworms achieve this by significantly altering the biogeochemical status of P. It has however, been reported that surface casts can increase the amount of P lost to surface runoff (Sharpley and Syers, 1976).

Earthworms also exhibit the characteristic of increasing N mineralisation (Sharma *et al.*, 2017). The annual N excretions of earthworms in a corn agroecosystem was measured to be between 10 and 41.5 kg N ha^{-1}, equivalent to 22% of plant total uptake for the highest figure (Rosswall and Paustian, 1984; Whalen *et al.*, 2000). The efficacy of the nutrient stabilisation potential of earthworms is influenced by the land management practices in place (Bhadauria and Saxena, 2010).

2.11. Sampling techniques

There are multiple methods of earthworm sampling, however they can be categorised into two main groups: i) passive techniques where the earthworms are removed from the soil via sifting and hand-sorting; and ii) behavioural techniques which focus on capturing earthworms that have been encouraged to actively exit the soil profile (Valckx *et al.*, 2011).

Hand sorting has long been used as a reference method, however implementing it is not always possible due to physical impediments to digging (e.g. root density or highly rocky soils), restrictions on soil disturbance at sensitive sites, and the high labour intensity. Additionally, hand sorting often under represents anecic species as they can burrow to a depth greater than that of the sampling block (Pelosi *et al.*, 2009; Bartlett *et al.*, 2010). Hand sorting has also been shown to have a bias towards smaller earthworm species (Pelosi *et al.*, 2009).

Schmidt (2001b) experimented with shortening the time spent hand sorting. Results propose that 81–87% of earthworm abundance and 94–97% of earthworm biomass could be determined in only 36% of the time required when following regular hand sorting protocols (Schmidt, 2001b). Suggesting that hand sorting could be utilised even when time is a constraint for sampling. Hand sorting additionally allows for the collection of earthworm cocoons if they are of interest.

Hand sorting sample collection methods vary, often depending on the aims of the study (Bartlett *et al.*, 2010). The typical approach is to excavate four to six square blocks of soil with horizontal dimensions of 20-40cm and a depth of 20-30cm (Schmidt, 2001b; Pelosi *et al.*, 2009; Amossé *et al.*, 2016; Singh *et al.*, 2016). This soil block is then transported to the laboratory for sorting. Time spent sorting through soil cores is often up to the researcher’s discretion, as highlighted by Schmidt (2001b).

Initial behavioural techniques focused on the use of extractants (also referred to as expellants or vermifuges) that percolate down through the soil profile and irritate earthworms, causing them to evacuate their burrows. An extractants efficiency depends on the species, i.e. they are most efficient for epigeics and decrease in effectiveness for anecics, and further decrease for endogeics, this reflects species behaviour and burrow orientation (Valckx *et al.*, 2011). Evans and Guild (1947) first described the method with the use of potassium permanganate as an extractant. However, Svendsen (1955) soon discredited the efficiency of potassium permanganate as an expellant, revealing that it was far inferior to the hand sorting method for estimating earthworm populations.

The next big development in behavioural methods occurred when Raw (1959) pioneered the use of formalin as an extractant. Formalin extracted two to three fold more earthworms compared to potassium permanganate (Raw, 1959). Formalin soon became the standard extractant for earthworm extraction, however the use of formalin is limited as it is known to be phytotoxic and a carcinogen to humans (Valckx *et al.*, 2011). In some countries formalin use is forbidden by national health and safety regulations (Valckx *et al.*, 2011). Raw (1959) also noted that formalin extraction is not as effective for extracting all species, showing a bias towards anecics.

More recently, mustard has seen use as an extractant (Gunn, 1992; Valckx *et al.*, 2011). Mustard benefits from being neither phytotoxic nor carcinogenic. Gunn (1992) was one of the first studies to explore the use of mustard as an extractant, finding it to provide better results than formalin and equal results to potassium permanganate. Gunn (1992) could not however speicify an optimum concentration of mustard due to difficulties in keeping it in solution at higher concentrations. Another difficulty that has been encountered by past studies using mustard as a extractant, is that the concentration of the active ingredient, allyl isothiocyanate (AITC), varies between samples due to the heterogeneity of commercial mustard (Valckx *et al.*, 2011).

Mustard (the spice) is produced from the combination of three species of mustard plant: *Brassica nigra;* brown mustard*, B. juncea;* and white mustard*, Sinapis alba* (Zaborski, 2003). East and Knight (1998) compared the use of mustard application to hand sorting, and concluded that hand sorting is far more efficient, and suggest mustard use be limited to comparative studies. Valckx *et al.* (2011) suggests the use of two initial applications of mustard at a lower concentration (3 g l^{-1}) followed by two applications at a higher concentration (6 g l^{-1}), produces results which are at least equally efficient to other extractants. Chan and Munro (2001) evaluated the use of mustard as an extractant, the findings were that mustard best estimated the anecic *Anisochaetae sp* while it was ineffective for the endogeic *Aporrectodea trapezoids*. They also suggested that ~1.6 g l^{-1} mustard solution be used (Chan and Munro, 2001). Bartlett *et al.* (2006) also found that mustard solution extraction does not accurately reflect the earthworm communities, favouring larger, mature anecic species.

AITC itself has been used as an extractant to overcome the concentration issues of mustard. The AITC method was developed by Zaborski (2003), which suggested that the ideal concentration of AITC is 100mg l^{-1}, and found it to be equal if not better as an extractant than formalin. Care must be taken when applying AITC as it is toxic to aquatic organisms, therefore groundwater flow and runoff must be considered (Valckx *et al.*, 2011). AITC is not readily soluble in water and therefore an emulsifier is required (Zaborski, 2003). Valckx *et al.* (2011) compared AITC, mustard and formalin extraction methods and concluded that AITC and mustard better extracted earthworms. However, earthworms collected by AITC sometimes emerged with a loosened epidermis, making identification more difficult.

One of the most recently suggested extractants is onion (*Allium cepa*) solution. Steffen *et al.* (2013) assessed onion solution's use as a extractant and found that at concentrations of 170-175g L^{-1} onion solution produced similar results to 0.5% formalin solution when applied to a clayey Oxisol and a sandy Ultisol (USDA soil taxonomy system). Thus far, there is a lack of literature on this method, therefore further investigation into its feasibility is required.

An alternative form of behavioural technique is the application of an electric current to soil. Experiments into the use of electrical currents in this capacity have long been conducted (Walton, 1933). However, a large advancement in the methods came about when Thielemann (1986) suggested the use of an electrical octet setup, which has become popular. As the name 'octet' suggest, for this method eight electrodes are inserted into the soil. This method was initially overlooked by a large proportion of the scientific community as its early dissemination was solely in the German language (Schmidt, 2001a). Schmidt (2001a) was one of the first papers published in English to assess the method. A comparison with hand sorting and formalin extraction found that the octet method extracted more specimens, although total biomass was not significantly different from that of formalin extraction. Over a two-year timespan of periodical use, results were similar in diversity and abundance to those obtained by hand sorting.

A variation of hand sorting is suggested by the The Earthworm Society of Britain (ESB), named the National Earthworm Recording Scheme (NERS) (ESB, 2018). NERS attempts to set out a simple sampling method that can help encourage citizen scientists to identify earthworms in their region. This method suggests digging 5 pits, with a standard pit size of 25cm x 25cm and a depth of 10cm (ESB, 2018). This hand sorting method employs the use of pits much shallower than those documented in a majority of the available literature. Therefore, variance in results should be expected, particularly for the anecic group, which hand sorting already struggles to properly represent (Bartlett *et al.*, 2010). However, projects such as this are an excellent means to involve the public in earthworm studies and increase exposure to the field of study. NERS also endorses mustard solution use in the cases where minimal soil disturbance is required.

Formalin extraction in conjunction with hand sorting is currently proposed by the International Organization for Standardization (ISO) (Römbke *et al.*, 2006). However, hand sorting after formalin or AITC application is not ideal due to human health risks related to the chemicals (Valckx *et al.*, 2011).

In a majority of studies samples collected in the field require preservation before being transported to the laboratory for identification as this process requires laboratory equipment (e.g microscopes, petri dish) and benefits from the cleanliness. In order to preserve earthworms for transport an ethanol solution is most commonly used (Sherlock, 2012).

Eisenhauer *et al.* (2008) ran a comparative study into the used of the electric octet method and mustard solution for earthworm extraction under dry and manually wetted conditions. The octet method was found to be lacking when estimating community structure and neither method showed any influence from pre-wetting the soil. Pelosi *et al.* (2009) compared results from formaldehyde, mustard and AITC extractants when conducted with and without follow-up hand sorting. The study concluded that due to biomass of earthworms from each method not being significantly different, AITC is the most feasible extractant due to mustards insolubility and formaldehydes toxicity. Singh *et al.* (2017), ran a comparative study of earthworm sampling using AITC, *Allium cepa* solution and formalin extractants on a sandy loam test site. Results suggest that *Allium cepa* extraction produces the greatest response from earthworms in terms of quantity and biomass, extracting 53% and 66% more earthworms than formalin and AITC respectively.

2.12. Earthworms bioassays

Earthworms are well suited to monitoring contamination due to the constant contact between their semi-permeable skin and the surrounding soil which makes them sensitive to chemical and physical soil properties (Paoletti *et al.*, 1998; Roubalová *et al.*, 2015). Earthworm’s uptake of contaminants by oral ingestion is generally of lesser importance, but this can be a major uptake route for hydrophobic chemicals in soils with high organic matter content (Belfroid *et al.*, 1995). The ease in rearing, and classifying Earthworms is an additional benefit to their use in bioassay studies. Two epigeic species, Eisenia fetida and Eisenia andrei, are most commonly used to measure ecotoxicity. There exist two sets of guidelines regarding the impacts of acute toxicity on earthworms and the effect on their reproduction, these come from the Organization for Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO) (OECD, 1984; ISO, 1993; OECD, 2004; ISO, 2012). However, both of these species are best described as 'compost worms', and are not naturally abundant in soils.

In summary, earthworms react to soil pollutants through a reduction in resistance to pathogens as a result of pollutant exposure, making them reliable biomarkers (Roubalová *et al.*, 2015). Though, exact defence mechanisms vary between earthworm species (Suleiman *et al.*, 2017).

2.12.1. Heavy metals

Kuehle (1984) and D. Cluzeau (1987), were instrumental in confirming that Earthworms are indicators of microclimate, nutrition and toxicity in vineyard soils. Earthworm growth is impaired by the presence of high concentration of certain heavy metals such as Cd, Cu, Pb and Zn (Rida, 1996; Spurgeon and Hopkin, 1999). Furthermore, earthworms have been shown to accumulate these elements when exposed to contaminated soils (Ireland, 1979; Ireland, 1983; Van Vliet *et al.*, 2005). Spurgeon and Hopkin (1999) showed how earthworm abundance and biomass decreases with proximity to a Pb/Zn/Cd smelting works in the UK. Paoletti *et al.* (1998) investigated the use of earthworms as bioindicators in 72 Italian vineyards and orchards focussing on their response to heavy metal concentration, particularly copper and zinc. Results convincingly suggest that earthworms can be used as a rural bioindicator. Composting earthworms, E. fetida, E. andrei and D. veneta, were all found to exhibit the following trend in uptaking heavy metals: Cd > Co > Cu > Zn > Ni > Pb > Cr.

2.12.2. Pesticides

Lofs-Holmin (1983) first identified that earthworms could be used as bioindicators of pesticide toxicity. It is now known that many pesticides cause toxicity to earthworms (Alves *et al.*, 2013; Rico *et al.*, 2016). The practice of fumigation also has a large impact on earthworm communities by reducing reproduction and growth (Fouché *et al.*, 2017). The use of a combination of multiple pesticides is common practice in agriculture, but may enhance their toxicity, leading to greater than expected impact on earthworms and soil health (Yang *et al.*, 2017).

2.12.3. Tillage

Initial studies into the impact of tillage on earthworms found that epigeic and endogeic populations are heavily reduced by tillage, while anecics were not statistically impacted in a majority of circumstances (Paoletti, Sommaggio et al. 1998). Although examples of impact on anecics do exists, for example Daugbjerg *et al.* (1988) found that populations of *Aporrectodea longa* were significantly different in field that had varying agricultural management practices applied. A global meta-analysis of 165 publications, from over 65 years found that no-tillage treatment can increase earthworm biomass and abundance by 196% and 137% respectively, while conservation agriculture led to a mean increase of 101% and 127% respectively (Briones and Schmidt, 2017). Furthermore, earthworm's response was enhanced by fine textured soils, pHs below 5.5, clay content >35%, time under reduced tillage, and being located in a warm temperate zone. This meta-analysis also highlighted that epigeic and larger anecic earthworms are most sensitive to conventional tillage. A comparative study into the impacts of differing tillage regimes found that conversion from no-till to conventional tillage produced the greatest decrease in earthworm abundance; however, moldboard plowing resulted in the most significant declines in earthworm populations (Fox *et al.*, 2017).

2.13. Conclusions on earthworms

Earthworm literature has covered their use as a bioassay (Paoletti *et al.*, 1998; Roubalová *et al.*, 2015) and the key role in soil processes (Lavelle, 1988; Lavelle *et al.*, 2004). The fact that there is so much available information on earthworms in the UK and Ireland makes them ideal regions to develop and compare earthworm extraction methods.

There is no 'best' method for earthworm sampling. The choice of a single sampling method is highly dependent on the goals of the study and the acceptable impact on the environment. Of the aforementioned methods, hand sorting, mustard solution, onion solution and electrical methods pose the smallest environmental impact. However, AITC shows high potential for overcoming some of the downfalls of mustard solution, while posing a lesser environmental impact than other, more toxic vermifuges (e.g. potassium permanganate and formaldehyde), providing an intermediate method. It is the opinion of the author that, regardless of its endorsement by the ISO, the use of formaldehyde should be limited to controlled studies as opposed to in field sampling due to its impact on the environment. Combining behavioural techniques with follow-up hand sorting appears to be the most all-encompassing method for earthworm population and diversity estimation. Onion solution in particular requires future study, following very promising initial results (Steffen *et al.*, 2013; Singh *et al.*, 2017). There is a need to establish an environmentally friendly standard method for earthworm sampling to ensure comparability between studies, however this may need to vary depending on factors such as climate, soils type and target earthworm species.

Measurements of bioavailability in ecology and soil science

2.14. Introduction

Metals, metalloids and other elements are naturally occurring constituents of all soils, existing in many forms and associations such as in minerals, clays, and within the organic fraction of soils (Gadd, 2007). Some soils naturally contain toxic concentrations of trace elements, which are derived from their parent materials (Violante *et al.*, 2010). Element concentrations in soils can also be greatly affected (i.e. elevated) by anthropogenic activity such as mining, the chemical industry, transport, agriculture and waste management. Howsoever their presence arises, elements gain mobility through the aqueous phase of a soil, and can subsequently be transferred into organisms and the aquatic environment.

The total amount of an element in a soil is an important parameter to determine during environmental studies because it provides information about the extent of pollution and the maximum exposure potential (or maximum nutrient supply potential) if all of the element is mobile and/or accessible to organisms. The total amount is typically determined by digestion in mineral acids (e.g. $HCl+HNO_3+HF$ for total elements, or $HCl+HNO_3$, i.e. *aqua regia*, for a 'pseudo total') (Aitang and Häni, 1983; Häni, 1990). However, in the vast majority of cases, only a portion of an element's total content is 'ecologically active' within soil, with the remainder bound to resistant minerals and/or recalcitrant organic materials that prevent release of the element into the porewater or into other forms readily accessible to organisms. Therefore, the environmentally relevant portion of a nutrient/pollutant needs to be determined in ordered to evaluate the nutrient supply potential and/or the toxicity risk in a soil. Indeed, it is now widely accepted that the risk assessment of soils should include both the total content of pollutants and their biologically available (bioavailable) fraction (Figure 2-8) (Tiensing *et al.*, 2001; Hobbelen *et al.*, 2004). For this reason, a vast amount of the soil science and soil management literature is devoted to developing, refining and employing a host of methods that aim to identify the bioavailable fraction in soils.

The fraction that is/becomes bioavailable is influenced or controlled by many processes, these include precipitation and dissolution, sorption and desorption, complexation and dissociation, and oxidation and reduction (Violante *et al.*, 2010). The degree to which each of these determine bioavailability is mainly dependent on the intrinsic properties of the pollutant of interest and how they respond to factors such as pH, soil characteristics (i.e. texture and organic matter content) and biological processes including chelation by plant and microbial exudates, and microbially catalysed reactions. For example, some microbes can reduce certain metal species, resulting in them having a lower mobility and bioavailability, and thus reduced toxicity (Violante *et al.*, 2010). These metals include U (VI), Cr (VI) to Cr (III), Fe (III) and Mn (IV) (Tebo and Obraztsova, 1998; McLean and Beveridge, 2001; Finneran *et al.*, 2002).

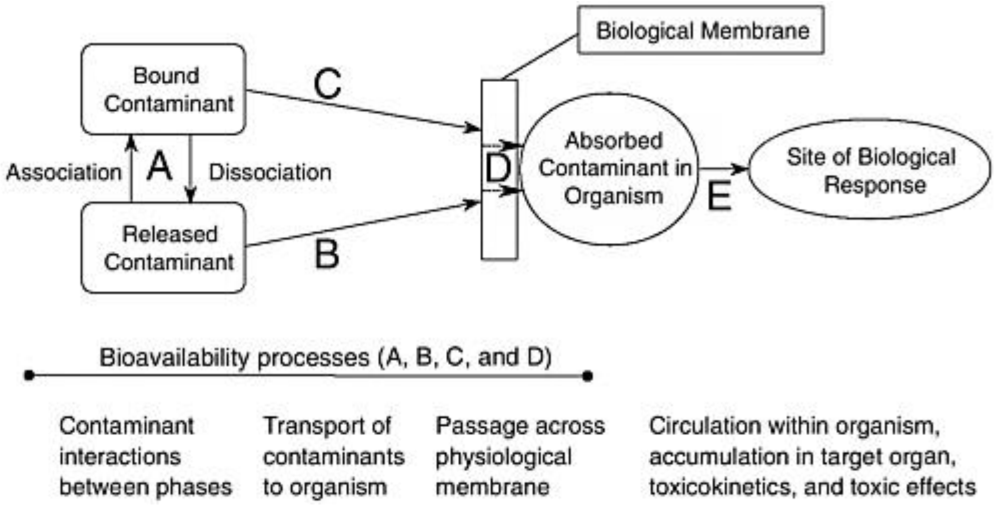

Figure 2-8. A simplified summary of the process of bioavailability in soils (National Research Council, 2003).

Further complication in the determination of the bioavailable fraction of elements arises from the fact that the term 'bioavailable fraction' itself is not always well-defined, partially due to its use over multiple fields of study. For example, Sposito (2008) defines bioavailability as "a chemical element is bioavailable if it is present as, or can be transformed readily to, the free-ion species, if it can move to plant roots on a time scale that is relevant to plant growth and development, and if, once absorbed by the root, it affects the life cycle of the plant". Caussy *et al.* (2003) notes however, that different papers refer to bioavailability in two distinct forms; the first being external bioavailability, which refers to the ability of metals to be solubilized and released from environmental media such as soil. This is synonymous with bioaccessibility, which is measured abiotically (i.e. a chemical measurement requiring no organism). The second form of bioavailability discussed by Caussy *et al.* (2003) can be thought of as internal bioavailability, which is the ability of the metals to be absorbed and transferred to a specific organ within an organism where it exerts its toxicity (or nutrient benefit). Evidently, to measure internal bioavailability requires quantification of this biotic process, hence an organism is required in the procedure.

Many authors have advocated more dynamic definitions, encompassing both external and internal bioavailability (Peijnenburg *et al.*, 1997; Sposito, 2008). Two large research bodies that focus on the subject of bioavailability, The International Organization for Standardization (ISO) and the US National Research Council (NRC), have adopted variations of these all-encompassing definitions of bioavailability (National Research Council, 2003; ISO, 2008). The NRC refer to the concept of a "bioavailability process", which consists of internal and external bioavailability, rather than that of bioavailability itself. The ISO, use a model based on external, internal bioavailability and the toxicological impact.

2.15. Measurement of bioavailability

There is currently no universally accepted method for estimating an elements bioavailable fraction in soils. Past research has highlighted that measuring only the total soil content is not a sufficient means to estimate the bioavailability of contaminants in a soil (Di Bonito *et al.*, 2008). Estimation of bioavailability of metals generally focusses on the hypothesis that plant available metals are represented by free metal ion and by isotopically exchangeable fractions of the soil metal pool; However, there is an increasing interest into the effect of ligands on bioavailability as there is some evidence that chelated forms may also be assimilated by organisms (Black *et al.*, 2011).

This review focusses on bioavailability estimation pertaining to plants, soil organisms and invertebrates, and not those relating to uptake by humans. Current methods for measuring bioavailability fall into three main categories: 1) the amount that can be extracted by single or sequential chemical extraction techniques; 2) the amount of a contaminant dissolved in the soil pore water phase; and 3) the amount adsorbed by a newly introduced medium.

2.15.1. Chemical extractions

Chemical extraction techniques can be approached in two ways, single extractions and sequential extraction procedures (SEP). Single extraction techniques were devised in the 1950s and 1970s due to an increasing interest in soil pollution and P availability. As the determination of the different phases that elements are present in is of great interest to risk assessment and ecotoxicology, SEPs were then developed to better understand the fractionation of possible harmful elements. SEPs were initially used to discern the various fractions of metals in sediments, before being expanded to also be used on soils. These techniques commonly consist of 3-8 (although some range up to 10) extractions of increasing strength, in order to determine increasingly immobile fractions of metals (Peijnenburg *et al.*, 2007; Hooda, 2010).

The most commonly used extractants for environmental and soil sciences are:

- Neutral salts, such as $CaCl_2$, MgCl2, NH_4NO_3, $NaNO_3$, KNO_3.
- Acid extractants, such as HNO_3 and HCl, and acetic acid.
- Organic complexing agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and triethylamine (TEA).

- Artificial rainwater mixtures produced by adding different concentrations of multiple elements to distilled or deionised water based upon the concentrations measured in the region.

Artificial rainwater may appear to be the ideal extractant, however it relies on additional data collection on regional rainwater chemistry, and is more time consuming to produce. Neutral salt and weak acid (e.g. acetic) extractions aim to approximate plant available (phytoavailable) elements. While there is no clear 'best' neutral salt technique, the use of $CaCl_2$ is promoted in Europe, USA, New Zealand and Australia (Menzies *et al.*, 2007). Fedotov *et al.* (2012) attributes the widespread use of 0.01 M $CaCl_2$ to the following factors:

- Ca^{2+} is preferable over some of its counterparts, e.g. Sr^{2+} and Ba^{2+}, as they have been known to enhance displacement of the easily exchangeable fraction, leading to possible overestimation of this fraction.
- The method allows for multiple trace metals to be assessed simultaneously, simplifying sample preparation and analysis.
- $CaCl_2$ extractants concentration, in terms of ionic strength, is broadly similar to that in the average soil solution.

There are many advocates for the use of $CaCl_2$ in the study of heavy metals in the phytoavailability literature (Novozamsky *et al.*, 1993; Houba *et al.*, 1996; Lebourg *et al.*, 1996; Houba *et al.*, 2000; Sahuquillo *et al.*, 2003; Pueyo *et al.*, 2004; Meers *et al.*, 2007; Menzies *et al.*, 2007). For example, Menzies *et al.* (2007) discovered via reviewing and correlating available literature, that neutral salt extracts (0.01 M $CaCl_2$ and 0.1 M $NaNO_3$) provide the most accurate representation of the plant available fraction. The use of $CaCl_2$ has also proven to be a time saving technique that could supersede having to use multiple extractants to determine a variety of elements, which was commonplace in some regions, while also increasing the amount of information obtained (Houba *et al.*, 1996). However, $CaCl_2$ is not without its drawbacks; indeed, chloride salts can form stable complexes with many metalloids and metals (especially Cd), leading to a shift in equilibrium towards the liquid phase, and therefore an overestimation of the exchangeable fraction (Leece *et al.*, 2000). Nitrate salts (e.g. $NaNO_3$ and $Sr(NO_3)_2$) on the other hand, do not suffer from this drawback (Fedotov *et al.*, 2012). Although, they have their own associated drawbacks, for example, NH_4NO_3 can reduce the pH in low buffered soils (Kim *et al.*, 2015).

The two most widely used organic complexing agents in bioavailability measurement are EDTA and DTPA. These are most commonly employed in studies into physiochemical process, such as chelation in soil. Reported results from chelating agent extraction studies are mixed. A meta-analysis found that while DPTA was the better of the two complexing agents, both DPTA and EDTA produce poor estimates ($R^2 \leq 0.50$) of Cd, Zn, Ni, Cu and Pb uptake, when results over a variety of soils were considered (Menzies *et al.*, 2007). These results were poor when compared to other commonly employed extraction methods (i.e. acid and neutral salt extracts) (Menzies *et al.*, 2007). However, there are examples of the success with the DTPA extraction method; Hseu (2006) found that DTPA extracts strongly correlate with uptake by the shoots of Chinese cabbage grown in tropical soils. Echevarria *et al.* (2006) assessed 100 soils, representing a worldwide range of Ni fate, and found that DTPA reasonably well assessed the labile fraction of Ni when compared to uptake by *Trifolium pratense* L. cv. Violetta (a Ni indicator plant), and *Alyssum murale* Waldst. & Kit (a Ni hyperaccumulator).

A majority of modern SEPs are based upon early work by Tessier *et al.* (1979) and Stover *et al.* (1976). Tessier *et al.* (1979) defines the following five fractions of elements and their corresponding reagents: 1) Exchangeable elements, which are bound to colloidal or particulate material (1 M $MgCl_2$) ; 2) Bound to Carbonates (1 M NaOAc, pH adjusted with acetic acid); 3) Bound to Iron and Manganese Oxides (either 0.3 M $Na_2S_2O_4$ + 0.175 M Na-citrate + 0.025 M H-citrate, or 0.04 M NH_2OH-HCl in 25% (v/v) HOAc at 96 ± 3 °C); 4) Bound to organic matter (0.02 M HNO_3 and 5 mL of 30% H_2O_2, adjusted to pH 2 with HNO_3, conducted at 85 ± 2); and, 5) Residuals, that are bound within crystalline structures and unlikely to be available to the environment (the residue from step 4 is digested in HF-$HClO_4$ mixture).

Similarly, to single extraction techniques, SEPs lack global standardisation. However, one of the most widely used SEPs in Europe, originally suggested by the Standards, Measurements and Testing Programme of European Community, formerly known as the Community Bureau of Reference (BCR) is commonly used in the literature (Quevauviller, 1998). This is a three-step process that fractionates elements into three fractions (extractable by 0.11 M CH_3COOH, 0.10 M $NH_2OH \cdot HCl$ and 30% H_2O_2/ 1.0 M CH_3COONH_4) and the remaining residual fraction (Hooda, 2010).

SEPs have led to an improved understanding of the behaviour of potentially harmful elements in a variety of media. While these sequential extraction schemes are widely used (e.g. a search on the Web of Science Core Collection of ‘Soil’ + ‘BCR extraction’ for the period 2000-2018 returned >750 articles), they have numerous limitations and are often criticised. One issue is that they are labour and time intensive, another issue that can arise is that metal(loid)s released by one extraction reagent can rebind with another undissolved phase, leading to underestimation of that phase, and the overestimation of the subsequent phases. (Fedotov *et al.*, 2012). Furthermore, it is widely reported that individual extraction techniques are not wholly selective towards one soil fraction, leading to possible overlap between phases (Bacon and Davidson, 2008).

2.15.2. Isotopic exchange

In the isotopic exchange method, a fraction (e.g. exchangeable, labile or available) of an element within a soil is determined based on the distribution of a small amount of an introduced isotope within that soil system. This process benefits from not requiring the extraction of the element/compound of interest.

Early isotopic exchange methods were developed in the interest of studying the availability of P in fertilisers. Initially, early isotopic exchange procedures used the *E* value as a measure of the isotopically exchangeable fraction of an element within a soil based upon the specific activity of phosphate ions ($^{32}PO_4/^{31}PO_4$) after additions of $^{32}PO_4$ ions were made (Frossard *et al.*, 1994). However, it became apparent that this method overestimated available P under certain circumstances (i.e. highly P fixing soils) (Frossard *et al.*, 1994). Therefore, (Larsen, 1952) developed the *L* value, which is based upon the specific activity of a plant grown in soil after the addition of $^{32}PO_4$. These methods have, over time, been applied to other elements and chemical of concern, including Cu, Cd and Zn (Oliver *et al.*, 2006; Sterckeman *et al.*, 2009; Degryse *et al.*, 2011).

The use of isotopic techniques is limited to situations where the quantity of isotopic tracer which is introduced is much smaller than the pool as a whole, the same constraint is also applied to the quantity of sample extracted from the soil (Fardeau *et al.*, 1995).

2.15.3. Diffusive gradients

There are two methods based on diffusive gradients which are commonly employed in bioavailability assessment, those are Diffusive gradients through thin films (DGT) and The Donnan membrane technique (DMT). DGT is a novel method of measuring bioavailability in which a layer of ion accumulating binding agent (Chelex resin is most common) is covered by a diffusive layer of hydrogel and a filter. When deployed, only the diffusive layer will be in contact with the soil making the transport of ions to the Chelex resin layer dependent on concentration gradients. The DGT unit causes a depletion of the chemical/element of interest in the surrounding pore water, leading to rejuvenation from the solid phase’s labile metal pool. A diffusive boundary layer is formed in between the filter membrane and surrounding soil (Figure

2-9). The process is similar to that of plant uptake and explained below. Provided that the concentration gradient remains constant then the flux of given metal ions from the soil to the resin (J) can be calculated by Fick's first law (Equation 2.1) (Fick, 1995).

$$J = -D\frac{dC}{dx} \qquad (Eq. 2.1)$$

Where D is the diffusion coefficient/diffusivity (expressed in $m^2\ s^{-1}$) and dC/dx is the concentration gradient. Under the assumption that the diffusion coefficients of the DBL and diffusive layer are the same, then the concentration gradient (dC/dx) can be calculated using Equation 2.2.

$$\frac{dC}{dx} = \frac{C - C^l}{\Delta_g + \delta} \qquad (Eq. 2.2)$$

Where C is the concentration of ions in bulk solution, C^l is the concentration of ions at the binding gel-diffusive gel boundary, Δ_g is the total thickness of the diffusive gel and filter layers and δ is the thickness of the DBL (Zhang *et al.*, 2014).

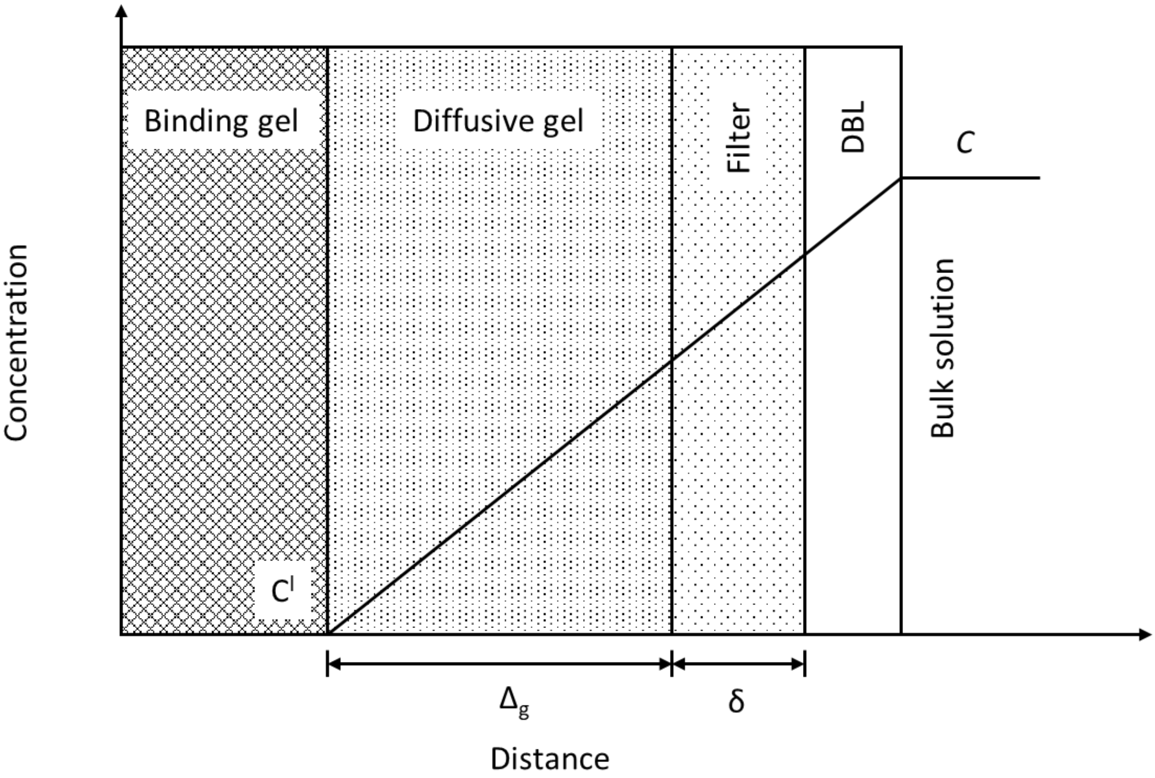

Figure 2-9. A diagram of the DGT-bulk solution boundary, based upon (Zhang et al., 2014).

Each individual DGT unit has a deployment time of 1 h to 3 months, depending on the saturation time of the binding agent which is determined by the amount of ions present (i.e. the binding agent will become saturated more quickly in a system with a high concentration of ions) (Zhang *et al.*, 2014). The measurements produced by this method can be considered independent of pH and ionic exchange while these factors remain within the range of 5-8.3 and 10 mM^{-1}-1 M respectively when chelex-100 is used as the binding agent (Zhang and Davison, 1995; Zhang *et al.*, 2014). Methods based on alternative binding gels have been developed to measure multiple elements simultaneously (Bennett *et al.*, 2010; Panther *et al.*, 2013).

The DGT method allows for high-resolution data to be collected on a 2D level for a host of ions and elements (e.g. P, As, Sulphides and Cs) (Zhang *et al.*, 2014). There is yet to be a standardisation of this method, and a degree of uncertainty caused by the compatibility of results between different binding gels. Further development of DGT binding gels with a higher adsorption capacity is required in order to expand the application of this method.

The Donnan membrane technique (DMT) is another popular diffusion technique which employs a cation exchange membrane to separate monomer and cationic free species from all other species. This setup consists of a donator and acceptor solution separated by the membrane which is assumed to be a Donnan phase (i.e. it has no mobile charge within its matrix and is placed within an electrolyte solution) (Figure 2-10). Once the cationic species concentrations have been measured they can be used to derive the concentration of free ions. The full mathematical foundations of this method are beyond the scope of this review, but a description of the process can be found in Weng *et al.* (2005).

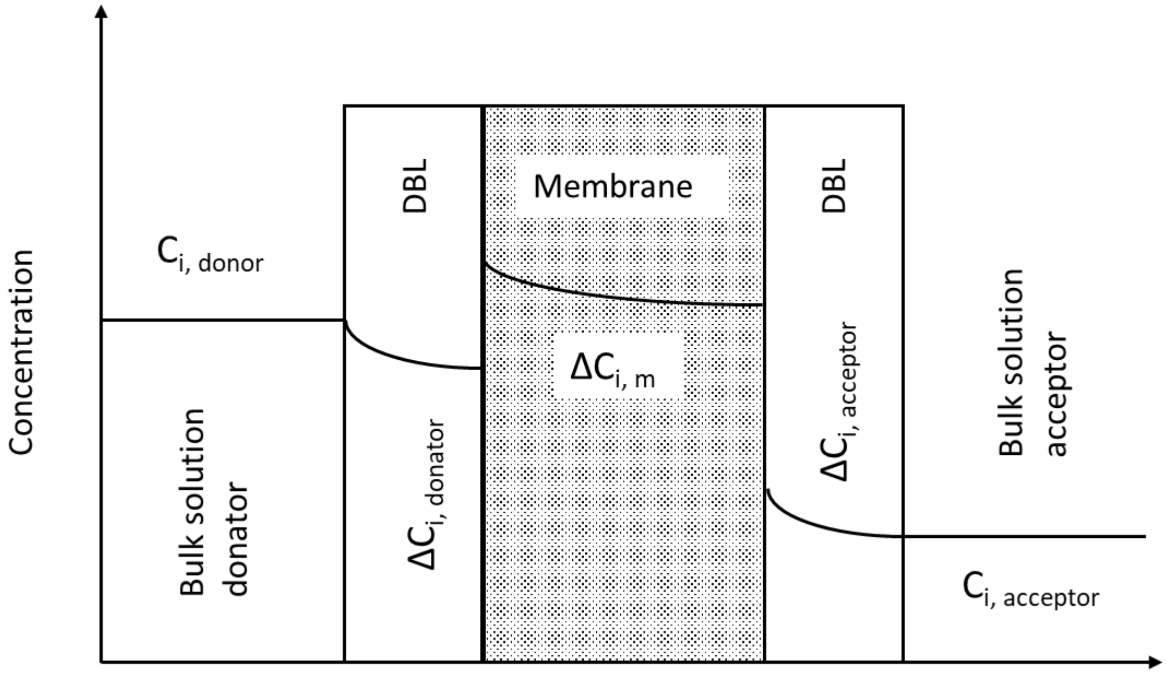

Figure 2-10. A simplified diagram of the DMT.

Weng *et al.* (2001) successfully measured Cu^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} activity simultaneously using the DMT. Nolan *et al.* (2005) found success in estimating Pb and Zn uptake by wheat (cv. frame), while Cd uptake was better estimated by $CaCl_2$ extraction. The use of DMT is however limited in soils with low levels of metal ions, due to detection limits (Weng *et al.*, 2005). There is possible scope for lowering detection limits by incorporating ion complexing ligands into the receiving medium, although this has not been fully explored in the literature.

2.15.4. Porewater Sampling

As mentioned in section 3.1., plants uptake a majority of their nutrients from the soil porewater (also known as ‘soil solution’), as the elements present in this phase are the most potentially bioavailable (Di Bonito *et al.*, 2008; Moreno-Jiménez *et al.*, 2011). Therefore, the sampling of porewater has become a prevalent technique for the estimation of bioavailability. Soil pore water encompasses two liquid phase regions, as described by (Yaron *et al.*, 1996). The first region is the near solid or adsorbed phase that occurs in the surface reaction zone and controls the diffusion of adsorbed solutes, this is commonly referred to as hygroscopic water. The second is the ‘free’ water phase which is key in the transport of solutes and water flow in the soil profile and resides in soil pores (Figure 2-11). Pore water sampling has many benefits compared to other measures of bioavailability, i.e. it directly samples the solution that plant roots and soil invertebrates experience, it does not rely on an artificial reagent to displace solutes and, depending on technique used, it can be done in a way that is non-destructive and non-disruptive to a soil system (e.g. using rhizon samplers, see below). However, it provides an indication of single moment element concentration only and does not measure flux or replenishment capacity, discrepancies can also arise as the result of varying rainfall and temperature conditions (Moreno-Jiménez *et al.*, 2011).

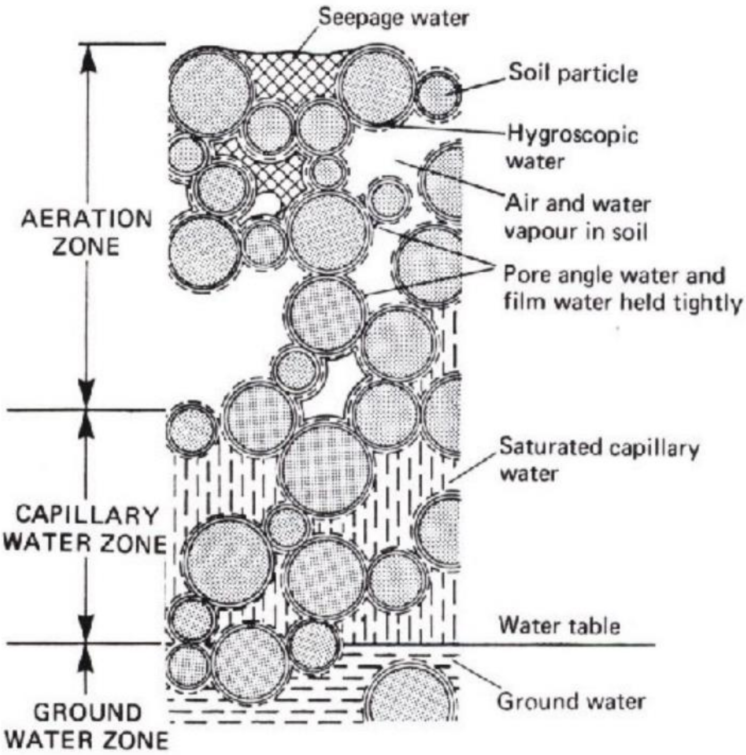

Figure 2-11. A diagram of the various pore water phases and states (Shaw, 2014).

Sampling methods

The sampling of pore water from an isolated block of soil is commonly termed lysimetry (Wolt, 1994). Samplers can be split into three groups, monolith, filled-in, ebermayer (Wolt, 1994). Monolith and filled-in (or backfilled) samplers hold columns or blocks of soil, these are undisturbed in the case of monolith sampling and disturbed in the case of filled in sampling. Ebermayer or pan lysimeters consists of an unwalled trough, pan, funnel, plate, or wick, which is placed under the in situ, undisturbed soils, and can be accessed via a trench, these lysimeters deployed in situ. Lysimeters are further subdivided depending on their drainage behaviour. Firstly, tension or suction lysimeters attempt to replicate the suction related to plant roots and are limited by the capillary pressure of the soil. Secondly, passive lysimeters which have no tension applied, and therefore samples are limited to the saturated water flux and rely on gravitational drainage. Lysimeters are also categorised as weighable or non-weighable; weighable lysimeters track the change in water storage over a time period; non-weighable lysimeters simply collect the water that is percolating through the soil column. Therefore, lysimeters can fall into multiple subcategories depending on the soil filling technique, weighability and the drainage behaviour (Figure 2-12).

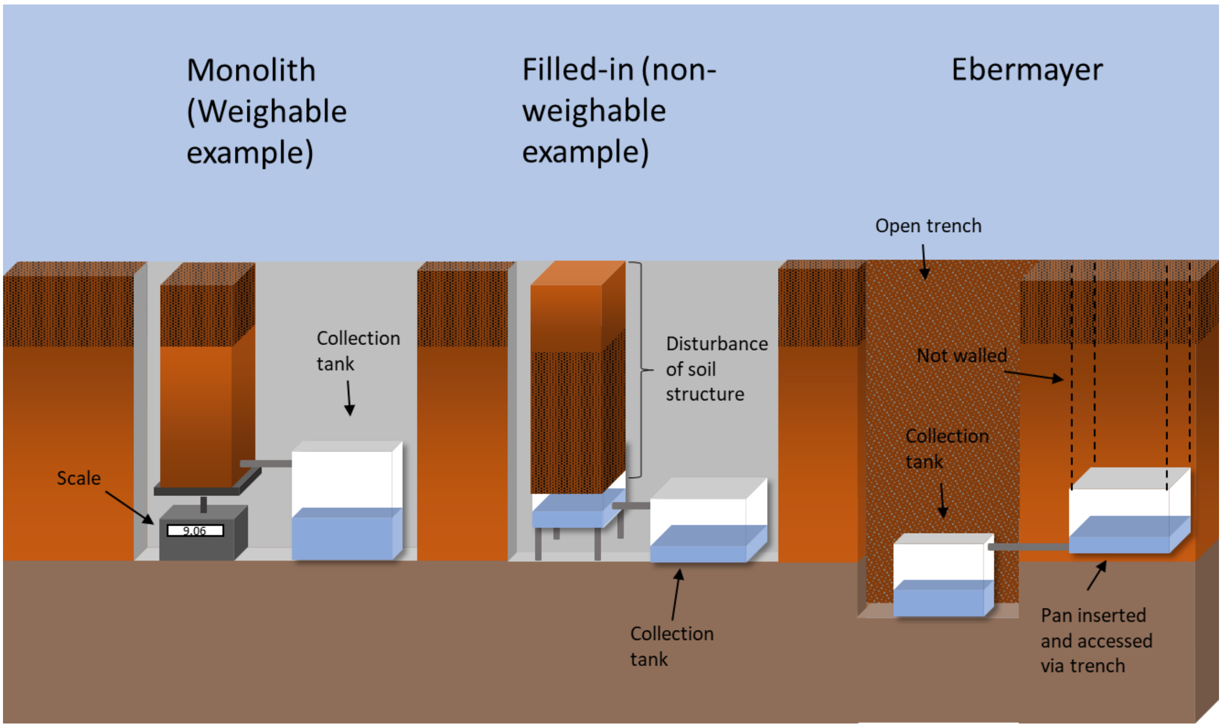

Figure 2-12. Passive variants of the three main types of lysimeter, monolith, filled-in and ebermayer.

There are also numerous laboratory-based sampling techniques that focus on pore water extraction. These can be grouped into column displacement methods, centrifugation, saturation extracts, water extracts, complexation and exchange techniques and finally lysimetric methods. Variations of the column displacement method are the most commonly adopted of these laboratory-based methods (Wolt, 1994)

Alternatively, if sampling directly from the field is of interest, rhizon samplers can be employed. Rhizon samplers consist of a semi porous sampling probe which is inserted into the soil, a sample is then obtained by applying a vacuum to the sampler through the use of either a syringe or a vacuum tube. This method allows for the extraction of 5-10ml of porewater without disturbing the soil chemistry, structure or biology. This method is also less laborious than lysimetery when a large number of samples are required.

Applicability

Sampling of soils in situ is preferential if the determination of pore water mobility is of interest (Moreno-Jiménez *et al.*, 2011). Moreno-Jiménez *et al.* (2011) found that sufficient pore water can be extracted even under semi-arid conditions by rhizon samplers which were horizontally inserted into the sides of excavated soil pits. Beesley *et al.* (2010) compared the results of pore water analysis to SEP and column leaching methods for the analysis of iron rich soils. Porewater samples were found to produce more accurate estimates of the short-term element mobility.

Hobbelen *et al.* (2004) sampled invertebrates (via hand sorting) and soil pore water from a floodplain in the Netherlands. It was found that pore water metal concentration correlated well with arthropod's uptake of all of the elements in the study, but the method was found lacking in the correlation with toxic concentrations of Cu in millipedes and Cu, Cd, Pb and Zn in earthworms.

2.15.5. Comparison of methods

Historically, methods that release a large portion of solid phase metals, such as EDTA and DPTA were favoured due to related detection limits (Hooda, 2010). However, as advancements in analytical techniques have been made, the use of weaker extractants such as organic acids (e.g. acetic or oxalic acid) or diluted mineral acids (e.g. HCl or HNO_3) has become more feasible. It is generally accepted that these milder extractants more accurately reflect the real-world amount of an element that is bioavailable in soils (Hooda, 2010).

Takeda *et al.* (2006) compared the results of multiple acid, neutral salt, chelating agents and pure water extractants for a suite of 28 elements in 16 Japanese agricultural soils. When compared to *Brassica rapa* L. var. *perviridis* and *Fagopyrum esculentum* M. uptake, the study found that milder extractants (e.g. 0.01 mol L^{-1} HNO_3, 1 mol L^{-1} NH_4NO_3 and 0.01 mol L^{-1} $CaCl_2$) better estimated bioavailability (Takeda *et al.*, 2006). Meers *et al.* (2007) compared the efficacy of 12 commonly used extraction and digestion methods (0.01 M $CaCl_2$, 0.1 M $NaNO_3$, 1 M NH_4NO_3, 1 M NH_4NOAc, 1 M $MgCl_2$, 0.11 M HOAc, 0.5 M HNO_3, 0.1 M HCl, DTPA–TEA–$CaCl_2$, EDTA-NH_4OAc, pore water from rhizon samplers and *aqua regia* [pseudo total]) for estimating heavy metal (Cd, Cu, Ni, Pb, and Zn) uptake in *Phaseolus vulgaris* grown in 21 soils of varying composition and degrees of contamination. It was found that Cd and Zn uptake both correlated well with porewater concentrations, $CaCl_2$ and extraction and methods based on unbuffered nitrate solution, with Zn uptake also correlating well with NH_4OAc and $MgCl_2$. Ni content was best predicted by $CaCl_2$ and NH_4NO_3. Cu and Pb uptake best correlated with vigorous extraction procedures and total contents in *aqua regia.* Overall extraction by 0.01 M $CaCl_2$ provided the most comprehensive phytoavailability estimates of the elements investigated in this study, supporting the findings of Menzies *et al.* (2007). Additionally, it was suggested that, in order to overcome issues relating to limits of detection of inductively coupled plasma-optical Emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS) analysis, a liquid to solid ratio of 5:1 should be used during $CaCl_2$ extraction, as opposed to previous suggestions of a 10:1 ratio (Meers *et al.*, 2007). On the other hand, Bakircioglu *et al.* (2011) found that 0.01 M $CaCl_2$ extraction produced a worse estimate of wheat uptake of Pb, and Ni from soils than 1.00 M HCl, 0.05 M DTPA and 0.05 M EDTA. They also found that a modified version of the BCR SEP inadequately estimated plant metal uptake.

Black *et al.* (2011), conducted a meta-analysis of commonly used methods for the estimation of metal bioavailability. The study covered 12 soil types over a period of 6 months to 13 years. The methods used included pseudo total concentration (HNO_3), EDTA, $Ca(NO_3)_2$, soil solution, DGT and free ion activity (determined using the Windermere Humic Aqueous Mode [WHAM]). Of these extractants, $Ca(NO_3)_2$ best estimated Ni and Zn suggesting the possibility of being used as a standard method. However, none of the methods could accurately explain more than 50% of the variations in Cu and Cd.

Madrid *et al.* (2007) investigated the use of 0.5 mol l^{-1} HCl and 0.05 mol l^{-1} EDTA extraction as a less laborious alternative to the first three steps of the BCR SEP method for a variety of urban and city soils. Results indicate that both HCl and EDTA underrepresented Cr, Cu, Fe, Mn, Ni, Pb and Zn when compared to the sum of the first three steps of the BCR technique suggesting they are not a viable alternative. Although, Tack and Verloo (1999) compared the early SEP technique described by Tessier *et al.* (1979) to single extractions on separate subsamples using the same reaction conditions as the SEP. Results from both methodologies were statistically determined to be "generally equivalent", therefore the separate single extraction method to be a viable alternative to the Tessier SEP if time is a constraint.

2.16. Conclusions on bioavailability of elements determinations

The assessment of bioavailability is of great importance in risk assessment and ecotoxicology. $CaCl_2$ appears to be the best candidate for estimating phytoavailability and establishing standardised methods for estimation of bioavailability due to its proven success and widespread use reported in the literature combined with the relative ease of the method. At present, chelating agents and acids are not as viable single extraction methods because they are cumbersome and not as widely used (making them less readily comparable across studies), however they may form part of a SEP. DGT and pore water sampling with rhizon samplers, lysimeters or equivalent, are best suited to studies where a change in soil in structure is unwanted. Porewater extractions are also desirable when the aim is to examine the soil solution itself, as experienced by plant roots and other soil biota, in any one moment.

Soil gas emissions and their measurement

2.17. Introduction

Soil gas fluxes have become a divisive topic since the rise of global warming science. However, the application of gas flux measurements predates this by a lengthy period (Romell, 1932). The three main gasses of interest, which are also the three most prevalent greenhouse gases (GHGs), are carbon dioxide (CO_2) and methane (CH_4) and nitrous oxide (N_2O). Soils are the source of approximately one fifth of global CO_2 emissions, one third of global CH_4 emissions, and two thirds of N_2O emissions (Lubbers *et al.*, 2015). Soils are also the largest terrestrial sink of carbon (2344 Gt in the top meter), second only to the oceans (38400 Gt) globally (Stockmann *et al.*, 2013). A change of just 10% in the soil organic carbon (SOC) pool would be equivalent to 30 years of anthropogenic emissions (Stockmann *et al.*, 2013). Many practices that aim to reduce one or multiple GHGs may impact other GHGs in a different and sometimes opposing manner, in these cases the net effect must be considered (Smith *et al.*, 2008).

In soils, carbon dioxide is primarily released through microbial decomposition of soil organic matter (SOM), root interactions (e.g. root respiration and rhizo-microbial respiration), and to a lesser extent by chemical oxidation and through the burning of organic matter (Raich and Schlesinger, 1992; Kuzyakov, 2006; Smith *et al.*, 2008). The release of CO_2 from soils through respiration is controlled by the CO_2 production rate, the concentration gradient between the soil and atmosphere, and physical properties such as pore size (Raich and Schlesinger, 1992). Soil respiration rates are estimated to be 50-75 Pg C yr^{-1} (Raich and Schlesinger, 1992).

There has been a rise in atmospheric CH_4 from 700ppb at the start of the industrial revolution in 1750 to current concentrations of 1800ppb (Tate, 2015). In soils, CH_4 is produced by the decay of organic matter under oxygen deprived conditions (Tate, 2015). Therefore, the highest contributors of CH_4 production are anaerobic soils. The soil-atmosphere CH_4 flux is controlled by two processes, methanogenesis (microbial production) and methanotrophy (microbial consumption) (Dutaur and Verchot, 2007). Soil methantrophy is incredibly important as it is estimated that 50% of CH_4 produced in soils is consumed before it exits the soil column (Dutaur and Verchot, 2007). During this process methanotrophs, in aerobic soils, oxidise CH_4, which leads to the eventual assimilation by biomass (Figure 2-13). There are two main taxonomic types of methantrophs: type i that assimilate formaldehyde through the ribulose monophosphate (RuMP) pathway; and type ii that assimilate formaldehyde via the serine pathway. Methantrophy is a relatively small CH_4 sink (20e45 Tg yr^{-1}), however this is the largest biotic sink in the global CH_4 budget (Dutaur and Verchot, 2007; Tate, 2015). This sink has however, been largely reduced through the expansion of agriculture and land use change (Lubbers *et al.*, 2013).

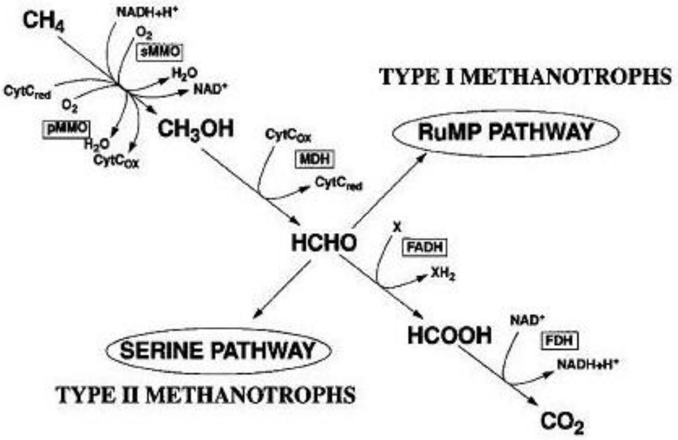

Figure 2-13. Methane oxidation pathways, and eventual formaldehyde assimilation (Hanson and Hanson, 1996).

As of 2004, atmospheric N_2O concentrations exceed preindustrial figures by 18% (Chapuis-Lardy *et al.*, 2007). This is due to a three to five-fold increase in N_2O emissions over the past century. This increase is in a large part because of agriculture intensification (Reay *et al.*, 2012). Aside from being a GHG, N_2O is additionally the single largest contributor to ozone depletion in the stratosphere (Ravishankara *et al.*, 2009). Soils are the largest contributor to N_2O emissions at 6.0 Tg yr^{-1} from natural soils and 4.2 Tg yr^{-1} from agricultural soils while forecasts predict that N_2O emissions related to agriculture are set to rise to 7.6 Tg N_2O-N yr^{-1} by 2030 (Chapuis-Lardy *et al.*, 2007; Reay *et al.*, 2012). N_2O production is governed mainly by two microbial processes, i.e. nitrification and denitrification (Chapuis-Lardy *et al.*, 2007). N_2O emissions are exacerbated by excess N in soil (Smith *et al.*, 2008). Similarly to CH_4, much of the N_2O that is produced within the soil column may be consumed before reaching the soil surface (Chapuis-Lardy *et al.*, 2007).

Smith *et al.* (2008) reviewed GHG mitigation in agricultural soils and found that the main controlling factors of GHG release are tillage, water treatment, nutrient management, grazing intensity, crop species and agronomy. However, gas fluxes from soil can also be used to quantify microbial populations and therefore to assess impacts of contaminants or additives to soil. For example, many studies have used soil respiration rate and/or soil metabolic quotient (qCO_2; the ratio of respiration to soil microbial biomass) to examine the impacts that metals or other contaminants have on the soil microflora. For example, Doelman and Haanstra (1984) found a relationship between a long-term (18 month) decrease of soil respiration to increasing amendments of Cd, Cr, Cu, Ni, Pb and Zn. Hupfauf *et al.* (2016) incorporated basal respiration (BR), substrate induced respiration (SIR) and qCO_2 measurements into the study of microbial community's reaction to the application of cattle slurry, co-digested cattle slurry, co-digested energy crops and mineral fertilisers. Smolders *et al.* (2004) used SIR measurements to identify the controlling factors on Zn toxicity to microbes.

However, the qCO_2 indices has also been criticised for its failure in highlighting certain disturbances (Bastida *et al.*, 2008). Additionally, the results of soil respiration data measurements are not individually conclusive, i.e. Chen *et al.* (2001) encountered difficulties during the interpretation of soil respiration due to a lack of correlation with soil enzyme markers (i.e soil dehydrogenase activity).

2.18. Sampling methods

Studies into soil gas fluxes have adopted various sampling methods. The most commonly accepted methods are: 1) closed static chambers (CSC), 2) closed dynamic chambers (CDC), 3) eddy covariance (EC) sampling, 4) flux gradient method (GM) (Figure 2-14).

2.18.1. The chamber methods

CSCs have been used for gas flux measurements since the early 20th century (Lundegårdh, 1927). For CSC, if done in the laboratory a subsample of soil is placed inside a sealable vessel, while if conducted in the field a frame is placed in the soil onto which a closed chamber is attached. The gas can be extracted by either using a syringe or alternatively it can be captured by an alkali or lime solution (in the case of CO_2) over a 30-60-minute incubation period (Liang *et al.*, 2004). The drawbacks of these methods are that the gas or liquid samples require storage and transport to a laboratory setting for use of gas chromatography, in the case of gas syringe collected samples, and acid titration/by weight change in the case of alkali trap solution. CDCs, on the other hand, can be used to analyse gas fluxes directly in the field. These also use a chamber mounted on the soil surface. CDCs can be subdivided into steady-state and non-steady-state chambers (Liang *et al.*, 2004). Steady-state CDCs are equipped with an infrared gas analyser (IRGA) that measures the gas concentration of a stream of ambient air entering the system and that of air that has been pumped through the chamber (Liang *et al.*, 2004). Non-steady state CDCs, are closed systems, in which the gas is pumped continually between the chamber and IRGA. These non-steady state CDCs quantify the change in gas concentration in the chamber headspace over time. The main benefit of CDCs over CSCs is that they can run continuously and autonomously (although sample runtime is limited) (Hall *et al.*, 2014). This eliminates the need for storage and transport of samples and reduces the associated errors. Both CSCs and CDCs take periodic measurements from which more long-term gas flux estimates are extrapolated. This process does not typically account for diurnal or longer-term variations in soil gas fluxes. Additionally, spatial variance must also be accounted for; past studies have shown large spatial variance in the flux of N_2O (Vourlitis and Oechel, 1997; von Arnold *et al.*, 2005; Chadwick *et al.*, 2014). All chamber methods suffer from the drawback that they only directly represent the portion of soil within them or upon which they are mounted, resulting in multiple sample sites being required in order to capture the spatial variability within a field and which consequently makes the process labour intensive (Jensen *et al.*, 1996). The general conclusion drawn from the comparison of both CDCs and CSCs is that CDCs produce more accurate results than CSCs (Rochette *et al.*, 1992; Nakadai *et al.*, 1993; Nay *et al.*, 1994; Janssens *et al.*, 2000). However, CSCs are more widely used due to their costs (Liang *et al.*, 2004). Overall, CSCs are mainly limited by available labour, while CDCs main limitation is the availability of funding/equipment.

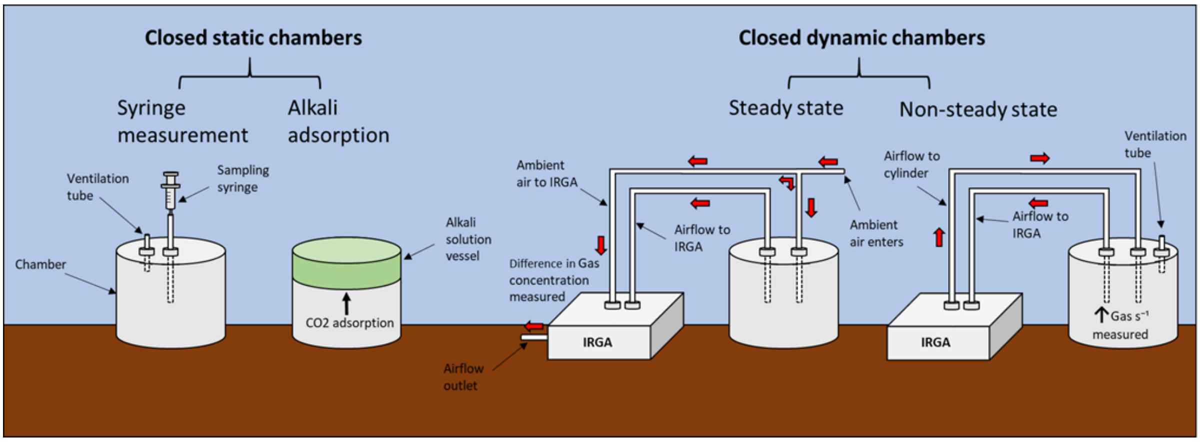

Figure 2-14. A simplified diagram of the range of chamber methods available for soil gas flux studies.

Pumpanen *et al.* (2004) compared the results of 20 different chamber CO_2 measurement methods to those of a reference calibration tank, and found that both steady-state and non-steady-state methods produced reliable results, with CSC generally underestimating CO_2 flux by 4-14% and CDCs showing no significant differences to the calibration tank values. The relationship between CDCs and CSCs CO_2 flux measurements was also investigated by Jensen *et al.* (1996). Their study found that the relationship between the different methods results was non-linear, with CSCs giving on average 12% higher flux rates below 100 mg CO_2-C m^{-2} h^{-1}, however CSC's flux rates were much lower above 100 mg CO_2-C m^{-2} h^{-1}. Measurements can however, have high variation (uncertainty) depending on the specific method being followed. One of the major sources of variations identified by Pumpanen *et al.* (2004) was whether collars were used as part of the chamber design because these can affect internal turbulence and headspace.

Bekku *et al.* (1997) found the alkali absorption method was inferior to both steady-state and non-steady-state CDCs and other CSC methods, due to its overestimation of CO_2 emissions of inoculated artificial soils. This was thought to be due to the chamber used in the alkali adsorption method enhancing respiration of *Trichoderma* sp. present in the soils caused by initially low internal CO_2 concentrations.

2.18.2. Eddy covariance method

One drawback with CSCs and CDCs is that they can alter the soil fluxes of the immediate area if used for an extended sampling period (Rochette *et al.*, 1992). An alternative, less intrusive method is EC sampling. EC is a micrometeorological method that can produce continuous data for a large spatial unit, although its use is limited by topography, vegetation coverage and its high cost (Collier *et al.*, 2014). The viability of EC has been proven during studies of CO_2 CH_4 and N_2O (Yuesi and Yinghong, 2003; Kroon *et al.*, 2007). Alberto *et al.* (2014) successfully used the EC technique to characterise diurnal and seasonal variations in CH_4 flux in rice paddies. EC is often used for measurements of ecosystem gas fluxes as the method inherently includes vegetation fluxes in its measurements, which inherently limits its use for soil gas flux studies.

The EC method works by measuring each component eddy, which as a whole characterise the entire horizontal airflow flow over a designated area (Figure 2-15). By calculating the sum of molecules that moved upward and downwards in eddies over time, the flux can be calculated for that time and place.

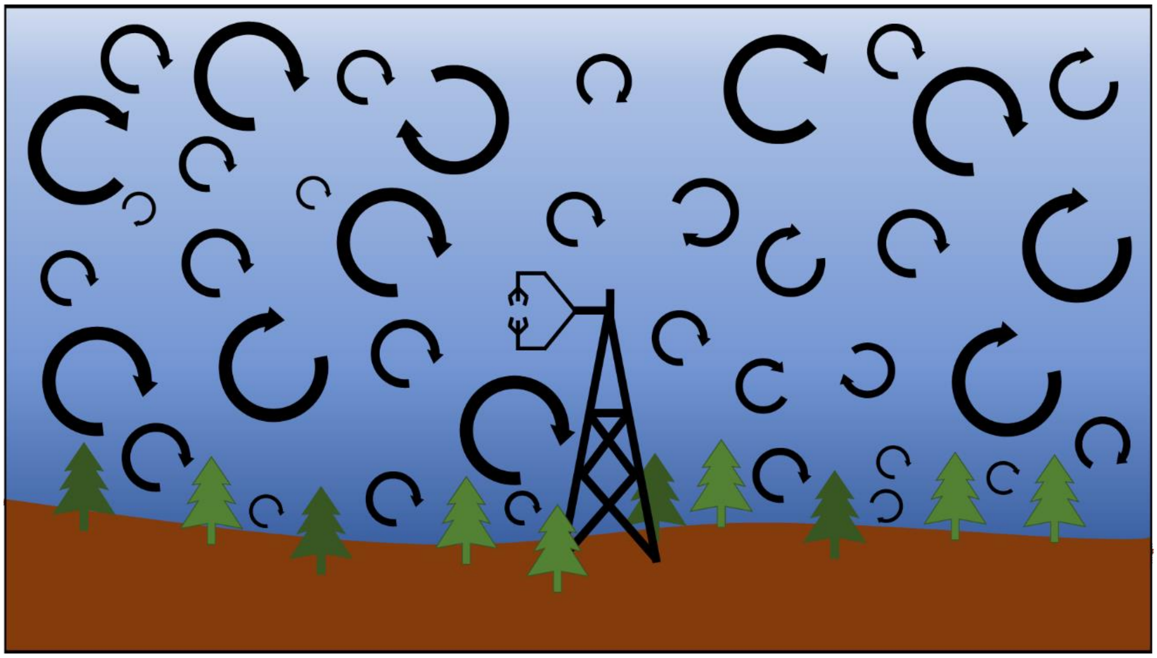

Figure 2-15. Diagram of the eddy covariance measurement tower and naturally occurring atmospheric eddies. It is noted that during the daytime measurements are usually taken below the canopy and at night measurements are taken above the canopy.

2.18.3. The soil gradient method

Another popular method of soil flux measurement is the soil gradient method (GM) (De Jong and Schappert, 1972). This method allows for continuous, and in some cases automatous data collection, through the installation of belowground samplers of concentrations gradients and gas transport properties. Although this method is more intrusive, minimal soil disturbance is experienced after the initial soil excavation during installation.

This method determines the soil CO_2 efflux from measurements of the CO_2 diffusion coefficient in the soil and the soil CO_2 concentration (Pingintha *et al.*, 2010). As molecular diffusion is the dominant mode of gas transport in porous media such as soil, gas flux (J) can be approximated using Fick's first law (Equation 2-3).

$$J = -D\frac{dC}{dz} \qquad (Eq\ 2.1)$$

Where D is the gas diffusion coefficient/diffusivity (expressed in $m^2 s^{-1}$), C is the change in gasses concentration and dz is the change in vertical position (m).

In order to ascertain the gas diffusivity and gas concentrations to input into this method, multiple active and passive methods have been developed. Active methods rely on direct collection of air from the soils pore spaces using tubes which are either inserted from the surface or through the walls of soil pits, or buried at depth. Installation pits can be optionally filled in, ideally an attempt is made to maintain original soil structure. However, if they are not filled in, horizontal diffusivity must be accounted for in calculations. A majority of these systems are kept in place for repeated measurements, although there are exceptions such as Richter (1972) where a single probe was repeatedly inserted successively deeper to produce measurements. These methods temporarily alter the surrounding areas soil gradient and therefore are best suited to studies with well-spaced sampling points and low temporal resolutions. Passive sampling methods consist of a tube or sensor which is inserted into the soil and allowed to equilibrate with the soil gas, they are less disruptive as the disturbance is negligible after the initial setup. This method can be combined with automated gas analysis (e.g. solid-state CO_2 sensors) to provide continuous measurements. The main limiting factor for this automated method is the heating of soils by the released heat of electrical mechanisms, when used over extended periods.

Although, previous GM studies have focussed on the measurement of CO_2, Kusa *et al.* (2008) produced positive results from N_2O measurements (Maier and Schack-Kirchner, 2014). Wolf *et al.* (2011) conducted GM application in a steppe environment for the measurement of CO_2, CH_4 and N_2O, finding the correlation between GM and chamber methods to be best for CO_2 followed by CH_4 and finally N_2O. Maier and Schack-Kirchner (2014) reviewed the use of the GM and concluded that it is best applied in well aerated soils for short term intensive studies. It was also noted that the GMs production of data about the depth profile of gas production or consumption in the soil make it uniquely suited to a selection of field studies (Maier and Schack-Kirchner, 2014).

A drawback of both passive and active methods is that measurements at soil surface are highly impacted by minor depth mismeasurements. Also, diurnal changes in atmospheric CO_2 can impact results and therefore these need monitoring ideally.

GM produces reliable results under well aerated conditions in soils which are horizontally homogenous, where the flux of gasses is driven solely by diffusion due to the limitation of Fick's law. Hendriks *et al.* (2010) found that GM methane measurements produced from soil where plant roots reached the water table were an order of magnitude lower than those produced by surface chamber methods. This was attributed to a change in the mechanisms by which CH_4 is transported to the surface, with ebullition (the formation of CH_4 rich bubbles that are transported upwards through waterlogged soils) and transport via plant systems becoming dominant.

2.18.4. Comparison of methods

One of the first comparative studies into soil gas flux measurements, De Jong *et al.* (1979), found that reported soil CO_2 flux measurements where highest from the carbon dioxide profile method (early variant of the GM) followed by micrometeorological methods (e.g. EC method), and that CDC produced the lowest measurements. Norman *et al.* (1997) compared six CO_2 flux measurement methods based on CDCs, CSCs and EC techniques. Adjustment factors of 0.93-1.45 were required to bring methods into alignment with an uncertainty of 10-15%. While, Pingintha *et al.* (2010) found that, when using GM, weighted harmonic averaging could be applied during the soil CO_2 diffusion coefficient calculations, resulting in measurements being much in agreement with those from the soil chamber method.

Liang *et al.* (2004) ran a comparative study on CO_2 flux measurements obtained from the CSC, CDC, GM and EC methodologies. They concluded that CDC (in a non-steady-state mode) and GM are best suited to the forest setting. Kusa *et al.* (2008) compared results from the chamber method to those from the GM when used in agricultural fields. Their findings were that the GM tended to be inaccurate (cases of both overestimation and underestimation) of CO_2 flux and N_2O fluxes when they are extremely high. However, the study found that the GM could be applied to N_2O studies where concentrations are not extremely high. Myklebust *et al.* (2008) conducted a comprehensive diurnal comparison of the GM, EC and chamber methods' CO_2 measurements in with varying land cover (living vegetation, straw and snow) over a year. All methods showed comparability within their results, however certain conditions produced inconsistencies in results for all methods. For example, night-time eddy covariance measurements were below uncertainty limits. GM encountered difficulties in producing measurements during summer rainfall. And finally, similarly to Pumpanen *et al.* (2004), it was found that the chamber method underrepresented fluxes in some situations due to interactions between the buried chamber collars and the surrounding environment (Myklebust *et al.*, 2008). Mills *et al.* (2011) compared collared and non-collared chamber methods and also found that they affected measurements, with a reported increase of 20-25% occurring when collars were used.

2.19. Conclusions on soil gas flux evaluations

Chamber methods offer the most cost-effective method for sampling soil gas flux, whilst other methods only warrant their costs when larger temporal or spatial studies are being conducted. Moreover, due to the widespread use of chamber methods, their results are more easily compared to other studies. However, attention must be drawn to the fact that many published papers do not discuss the finer details of their chamber methods (i.e. quantity of gas purged, equilibration time), leading to possible unattributable variations. For larger scale and more long-term studies, chamber methods are less viable due to the required labour input, although this is less of an issue for CDCs.

Summary

2.20. General conclusions

The main purpose of this review was to better understand the alternative disposal routes of WTRs. Furthermore, methods of quantifying the ecological impacts of soil amendments were reviewed with the aim of employing them during field studies of WTR land application in an agricultural setting.

Of all of the investigated alternative disposal routes of WTRs, land application is currently one of the most feasible. The application of WTRs to soils has been studied for a long time (e.g. Bugbee and Frink (1985)), however there is a clear lack of literature available on the effects of these amendments on soil microbes and terrestrial invertebrates, both of which are commonly used biomarkers for soil quality. These gaps in the literature highlights the fact that the ecological fraction of soils has been overlooked by many researchers in the field, and that there is a lack of disseminated knowledge surrounding this subject.

The debate on the 'best' method of estimating bioavailability has long been ongoing and is likely to long persist. However, pore water sampling offers the most unobtrusive method of bioavailability assessment, while extraction by the neutral salt, $CaCl_2$, is currently the most widely used chemical extraction method in Europe.

In the case of earthworm measurement, formalin extraction has been advocated, however there is still a strong opposition to the application of such a toxic and carcinogenic chemical to soils. With new 'greener' methods (i.e. mustard and onion solution) being conceived and developed, and reports of similar if not better success than that of formalin extraction, the move away from formalin extraction may occur in the near future. Most notably, the relatively new behavioural method of onion solution extraction has yet to be tested in more temperate regions, with previous research limited to the tropics (Steffen *et al.*, 2013; Singh *et al.*, 2017).

Meanwhile, although chamber methods are the generally accepted technique for gas flux, as mentioned in section 4.3., measurement in soils during smaller-scale studies, soil gas flux sampling falls short with regards to the reporting of the finer details of the sampling methodology.

Figure 2-16 summarises the conceptual framework of this thesis, and the chosen methods employed to explore the impacts on one or more of the key receptors: soil, porewater, earthworms and microbes. The application of WTR's main impacts are often derived from the increase of soil carbon stocks. This carbon can act as a food source for earthworms and an energy source for microbial respiration. This can result in a changed in earthworm or microbial population size, alter the population composition, or lead to increased activity. Microbial composition could also be directly altered by the introduced microbes which are present in the WTRs. The addition of pathogenic microbes to soil is of particular concern to researchers. In return, the activity of earthworms and soil microbes can have a feedback effect on soils, for example transforming their carbon stocks into CO_2 or other forms of C, and altering soil structure through bioturbation. WTR application also has the potential to change soil chemistry as discussed in sections 2.5.4 and 2.6 of this thesis. As Earthworms and soil microbes are sensitive bioreceptors, even small changed in soil chemistry could affect their populations negatively or positively, depending on the changes Any changes in microbial or earthworm populations could feedback into soil chemistry by altering nutrient cycling within soils either by incorporation into earthworm casts or transformation by microbes through processes such as respiration, nitrification and denitrification.

The main external influences that must be considered are climate and land management. Rainfall and temperature can make a major impact on earthworm and microbial populations. Earthworms will often burrow deeper to avoid extremely dry soils (Lowe and Butt, 2005; Reddy and Pasha, 1993). Similarly, microbes will not be as active during dry periods (Liang *et al.*, 2004).

Land management includes the choice of land use (e.g. arable or pasture), land use intensity, and the spreading of fertilizers or other products. An example of a land management practice that can negatively effect earthworm populations and alter soil structure is ploughing as disussed in section 2.12.3.

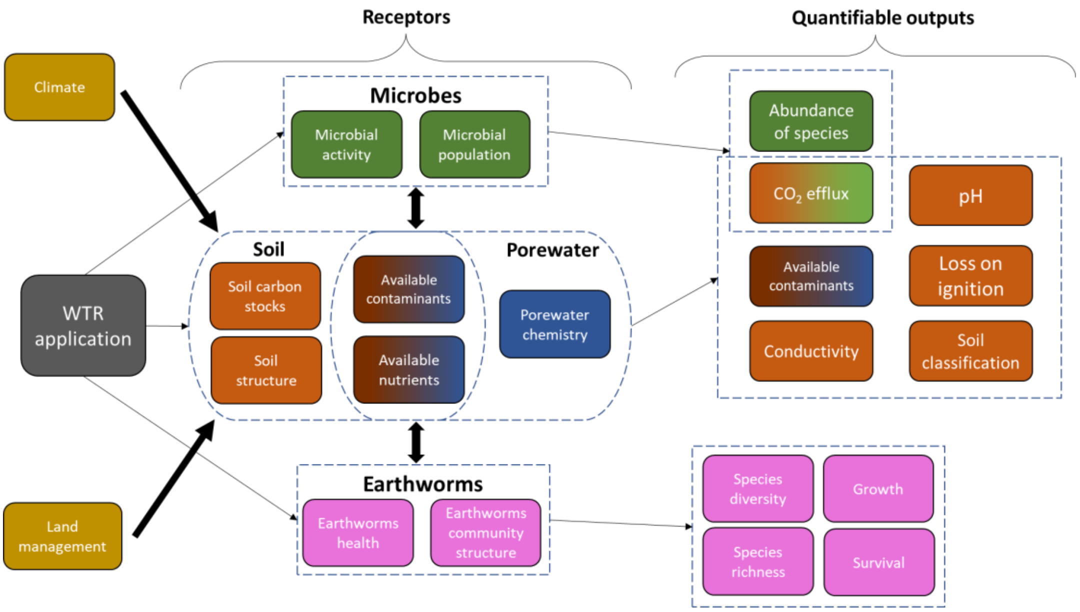

Figure 2-16. The conceptual framework of this research. Highlighting key interactions between WTR application and different environmental receptors. The quantifiable outputs are the chosen methods of quantifying WTRs impacts on receptors in this thesis.

2.21. Future aims

Having identified multiple gaps in the literature and established a conceptual framework, this research project intends to establish the overall impacts of land application of WTR on soil health characteristics, and aims to answer the following primary research question:

> *What impact do water treatment residual amendments have on soil health and ecology and is this a feasible future disposal route?*

In order to answer this question, the project will first establish the effect of WTR soil amendments on the four resceptors highlighted in figure 2-16, microbial populations, earthworms, soils and porewater.

One of the largest worries facing WTR land application is the release of sorbed contaminants and Al/Fe from the WTRs. Additionally, WTRs have been shown to be highly immobilising of P, leading to P deficiency. Attempts will be made to investigate these uncertainties, through the collection and analysis of WTRs extracts, porewaters from WTR treated soils, and waters leached from columns containing soils and WTR soils. This multi-pronged approach will explore multiple facets of WTR leaching and sorption under simulated field conditions.

As earthworms represent a large element of the soil biomass, and play a key role in soil formation and maintenance, they are an ideal bioindicator of soil quality. The addition of WTRs is could potentially increase earthworm biomass due to the addition of organic matter supplying them with a greater foodsource. Alternatively, this addition of WTRs could sorb nutrients or release potentially harmful elements, which in turn could be detrimental to earthworm populations. The abundance and biomass of earthworm communities will be measured through the employment of multiple collection and sampling techniques, allowing for comparison and reflection on which of these techniques is best suited to the aims of this study.

Thus far, inadequate literature is available on the impacts of WTRs on microbial populations. As discussed in section 2.20 of this thesis, the introduction of WTRs high organic matter to soils is likely to stimulate microbial populations. During this study microbial activity will be alluded to through the measurement of gas fluxes using the chamber method, and through amplification and illumina MiSeq sequencing of the 16S rRNA component of WTRs and treated soils.

In order to observer the potential impacts of WTR addition in both a natural and controlled environment a fieldwork campaign and multiple laboratory based experiments will be conducted. Fieldwork for this study will be conducted primarily at an agricultural field site in Gwynedd, West Wales, with additional fieldwork conducted in Monmouth, South Wales. Mesocosm, leaching column, batch leaching and DNA extraction studies will be conducted at Keele University.

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Chapter 3 - The potential and limitations of land spreading paper and pulp mill sludge to land

3.1. Introduction

During the production of paper and board, virgin or recovered timber and possibly a portion of recycled paper and board go through a series of processes at pulp mills to separate out the cellulose fibres and so produce a cellulose rich product known as pulp. Although mechanical pulping is still used in some areas, most large-scale pulping operations now use a chemical pulping process based on heat and pressure plus either an alkali treatment (known as the Kraft process, and is the most prevalent) or an acidic treatment (sulphite process) (Demuner et al., 2021; Monte et al., 2009). This pulp can then be used at an onsite paper mill, dried and bailed for transportation to offsite paper mills, or used in other industries such as a binding agent in pharmaceuticals and food products. At the paper mill this pulp is mixed with water and refined to the end product's specification before fillers such as clays, talc and calcium, as well as colouring agents, are added. This material is then processed to the desired specification of the end product (Figure 3-1). Globally, it is estimated that these processes produce 184.4 Mt of pulp and 402.790 Mt of paper and board annually (CEPI, 2018; Magnaghi, 2015). This leads to the production of approximately 400 million wet tonnes of PPMS globally every year (Faubert et al., 2016).

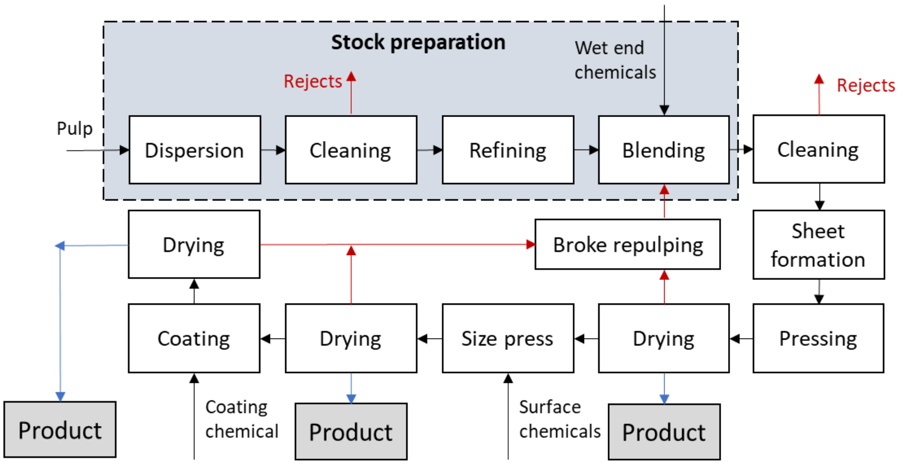

Figure 3-1 The typical processes employed at a paper mill, red lines indicate rejects and blue lines indicate products, based upon Webb (2003).

The production of both pulp and paper leads to the creation of multiple waste- and by-products, with around 87% of these materials being classified as pulp and paper mill sludges (PPMS), whilst the other 13% is accounted for by impurities, waste chemicals and gaseous emissions (Norrie and Fierro, 2020). The most commonly employed pulping process, the Kraft process, produces approximately 100 kg of waste per air dried tonne (ADt) of pulp, whilst other less common semichemical or physical methods produce around 60 kg ADt^{-1} (IPCC, 2001). The waste output can increase to 200 to 400 kg per tonne of product at mills where recycled paper is used as a feed stock (Balwaik and Raut, 2011; de Alda, 2008). This is important as 71.6% of paper is recycled in Confederation of European Paper Industries (CEPI) member states (Austria, Belgium, Czech Republic, Finland, France, Germany, Hungary, Italy, The Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom) and around 60 % in the U.S.A, both of which are major producers of paper and board, while comparable figures are reported in other parts of the world (CEPI, 2018; Scott, 2019).

This review focuses on the end of life disposal or reuse of PPMS, particularly spreading PPMS on agricultural land and the impacts that it has on soil chemistry, physical properties, and terrestrial ecology based upon past studies and available grey literature. The regulations on PPMS reuse within the UK will be discussed as a case study of the effective implementation of land spreading PPMS. The type of PPMS (i.e. pulp/paper or primary/secondary) will be emphasised as to distinguish the effects of each of these factors. Advantages and limitations to further adaption of this current recycling route will be highlighted.

3.2. Paper and pulp mill sludge production

Sludge is produced at both pulp and paper mills from the clarification of the liquid waste stream, although these wastes may be combined when both mills are integrated. As the main aim of pulping is to liberate the cellulose fibres, which are the foundation of paper products, from the lignin and other components found within wood, the pulp mill liquid waste stream is comprised of predominantly lignin and short chain cellulose fibres which are not suited for use in pulp production, while paper mill liquid waste is mainly composed of fines, added fillers and coatings, particularly kaolinite and calcium carbonate ($CaCO_3$), that are used in varying quantities depending on the end product of the paper mill.

The PPMS generated at pulp and paper mills can be categorised into Primary and Secondary sludges (Figure 3-2). Primary sludge refers to the material generated by the initial clarification of raw paper/pulp mill effluent via flotation or sedimentation. A proportion of this material can be reincorporated at paper mills for the production of lower quality end products such as board but is typically less suitable for reincorporation into higher end products. In order to reduce the volume, chemical oxygen demand and biochemical oxygen demand, the primary sludge may undergo further treatment. This commonly involves biological decomposition through aerobic activated sludge systems, aeration and mixing to oxidise, or a successive combination of these or other methods to generate a more processed waste material known as secondary sludge. Secondary sludge is more difficult to dewater due to the high biologically active content and thus is often combined with primary sludge before dewatering. These combined sludges generally contain approximately 70 % primary sludge and 30 % secondary sludge in developed countries, but will vary from mill to mill (Bajpai, 2015). Furthermore, some mills do not employ biological treatment leading to no secondary sludge being produced (Bajpai, 2015). At operations where both pulp and paper mills are integrated at the same site, sludge from both mills is sometimes combined (Hooda et al., 2018). Any of these sludges can be commonly referred to as paper and pulp mill sludge (PPMS).

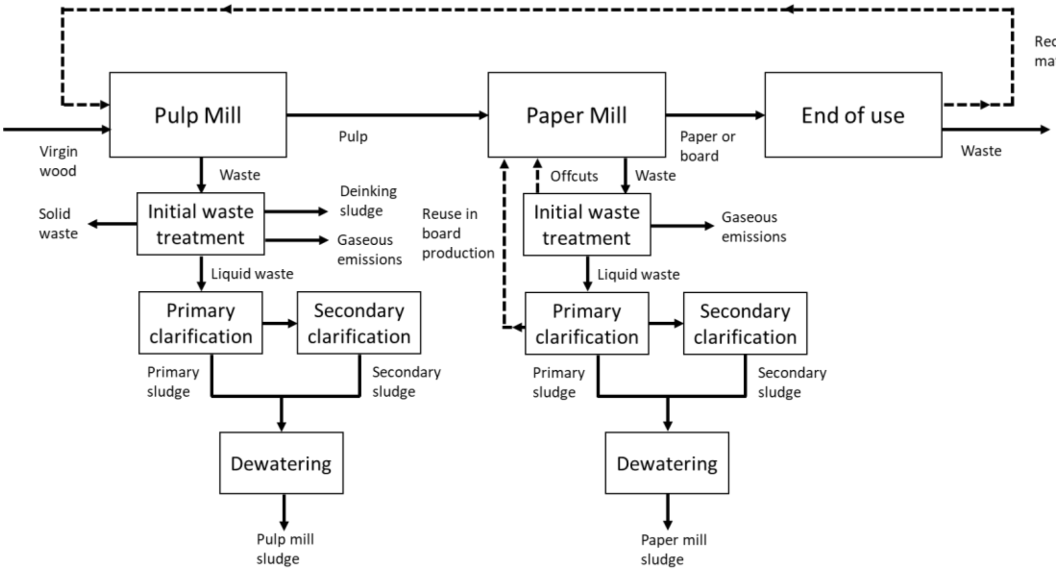

Figure 3-2. Waste streams produced during the production of pulp and paper.

3.2.2. The influence of recycled paper incorporation

Repulping of recycled paper at pulp and paper mills can vastly change the characteristics and quantity of sludge produced. As mentioned in section 1, the quantity of waste sludge produced at paper and pulp mills using virgin materials is relatively low, but this quantity increases (typically 2 to 4 fold; (Balwaik and Raut, 2011; de Alda, 2008)) where recycled paper is used in the production process. This increase in sludge production is due to the increased number of impurities. For example, when using recycled paper in pulping, components such as ink residue, coatings and fillers (some high grades of paper contain up to 40% filler (w/w) (de Alda, 2008)) must be removed and so these components end up in the sludge residue and therefore the sludge produced contains a lower proportion of organic content (CPI, 2015). The reuse of paper waste in pulp production is common, not only because it is environmentally beneficial but also because the cellulose fibres are already separated from the lignin present in wood and therefore reduce the need for that stage of processing. However, the recycling of paper cannot be repeated indefinitely as cellulose fibres are broken down and shortened with use, therefore eventually becoming unsuitable for paper making (García et al., 2008).

A separate, third kind of sludge, deinking sludge, can also be generated during the paper making process when using recycled paper as a feedstock. However, deinking sludges have different constituents and properties to the primary and secondary sludges (i.e. lower nutrient and organic matter content), which makes their potential land applications different to those of primary and secondary PPMS, and therefore they will not be discussed in this review. For a review of deinking sludge, see Camberato et al. (2006), while for a review of the wider suite of wastes from paper production, see that by (Simão et al., 2018). Additonally, for an example of governmental guidance on spreading of PPMS (UK example) see Gibbs et al. (2005).

3.2.3. Production figures

While pulp and paper mill companies do often report their waste production figures in annual reports, drawing comparisons is complicated because there is no common system for measurement or quantification of these wastes. That is, wide variations are observed such as to whether wet or dry masses are determined, the water content of wet materials, which other waste streams are included in the measurements, and at what point during the treatment process quantities are measured.

3.3. Physicochemical properties of PPMS

The physicochemical properties of PPMS produced at different mills can vary (Table 3-1) depending on the raw materials used, the treatment processes employed at the mill, and the nature of the end product (i.e. the grade of product produced influences the level of treatment and nature of additives). Previous studies have also highlighted that variations in sludge properties can arise even when comparing sludges from different mills that employ similar processes and/or produce similar products (Scott and Smith, 1995). Therefore, individual characterisation of sludges is crucial if informed decisions are to be made about their suitability for land application and are currently already conducted in many countries. Bulk density and water content are important characteristics of PPMS that are dependent upon the pulping mechanisms utilised at the mill, the waste treatment processes employed, and the level of dewatering (which is achieved through various means, most commonly via vacuum filtration, centrifugation or mechanical pressing using a screw or belt press system: (Amberg, 1984; Meyer et al., 2018)). Dewatering is particularly influential as PPMS in its initial state often has only 0.5 – 2 % solids content (Bajpai, 2015), but the end result of dewatering can be variable. For example, a study by de Alda (2008), which analysed 20 sludges from various paper and pulp mills, found the final water content to be 65 ± 17% (w/w), while Meyer et al. (2018) and Bajpai (2015) reported values across the range of 60 -75% water content, however the European Commission reports that up to 50% solids can be achieved by employing a screw press (Suhr et al., 2015).

Table 3-1. The reported physicochemical properties of primary, secondary and mixed PPMS (Faubert et al. (2016) and references therein; Simão et al. (2018); Faubert et al. (2017); Negi and Suthar (2013); Ganguly and Chakraborty (2018)).

Parameter	Primary PPMS	Secondary PPMS	Mixed PPMS
Dry matter (% w/w)	15-57	1-47	19-60
Ash content (% solids)	10-15	10-20	20
Nitrogen (ppm)	450-2800	11000-77000	7000-36000
Phosphorus (ppm)	100-600	2500-28000	2200-7400
Potassium (ppm)	200-900	780-7000	300-3300
C:N ratio	111:1-943:1	8:1-139:1	13:1-257:1
pH	5.0-11.0	6.0-8.5	3.8-8.5

Previous studies have reported that the bulk density of PPMS varies between 0.419 and 0.598 g cm^{-3} (Jackson and Line, 1997c, 1998; Jain et al., 2018; Rios et al., 2012), while the cation exchange capacity (CEC) of PPMS can also vary widely (e.g. 5.3 cmol (+) kg^{-1} to 297 cmol (+) kg^{-1}) depending on clay and organic matter content (Camberato et al., 2006). The work by de Alda (2008) also reported a typical pH near neutral at 7.6 ± 1.3, which was similar to the values later reported by Veluchamy and Kalamdhad (2017) who found the pH of PPMS samples to be 7.39 ±0.004. These values, in turn, are within the range of pH values (6.6 - 8.2) noted by Simão et al. (2018) for primary and secondary PPMS, which indicates that PPMS are typically in the pH neutral to mildly alkaline range. This neutral to alkaline pH is derived from their high $CaCO_3$ content that originates from paper coating materials or from causticizing which occurs during pulping when sodium carbonate reacts with calcium hydroxide to form $CaCO_3$ (Camberato et al., 2006; Norris and Titshall, 2011; Nunes et al., 2008; Vasconcelos and Cabral, 1993). This in turn imparts PPMS with a liming capacity, with the $CaCO_3$ equivalence of PPMS having been reported to be between 12.7% and 50% (Camberato et al., 2006). In keeping with this, they are also known to have a high buffering capacity (5 mol H^+ kg^{-1} sludge/pH) (Calace et al., 2003). In fact, PPMS are often applied as a liming agent when their $CaCO_3$ equivalence in above 30%, with organic and nutrient additions considered to be a secondary benefit.

Jackson and Line (1997b) determined the proportions of lignin, holocellulose, cellulose, and hemicellulose in primary pulp and paper mill sludge (via a modified Klason method) to be: isoluable lignin) 27.42 soluble lignin 2.87 holocellulose 72.65 cellulose 57.08 hemicellulose 11.23 (dry w/w %). Typically, paper mill sludges have high mineral contents, with primary sludge has a 40:60 ratio of organic material: mineral matter, while secondary sludge has a 50:50 ratio of the two (Bajpai, 2015). Primary sludge has lower N and P content than secondary sludge (0.045-0.28 % N and 0.01–0.06 % P versus 1.1-7.7 % N and 0.25–2.8 % P respectively) due to the addition of nutrients used to stimulate microbial activity during secondary treatment and the microbial biomass itself (Faubert et al., 2016). And while the C:N ratios of PPMS may vary, the C:N ratios of primary sludges are much higher than those of natural soils, up to 943:1, while those of secondary sludges may be similar to soils (~70:1), if not lower, with those reported in the literature ranging from 8:1 to 50:1 (Faubert et al., 2016).

3.4. Historic disposal methods for paper and pulp mill wastes

Although the focus of this article is on sludges from pulp and paper production, historic disposal of liquid wastes does warrant a mention also. Discharging of liquid effluents into waterways without primary or secondary treatment was historically common practice (Dolar et al., 1972). However, as regulations and environmental policy have become more stringent this practice has all but been abandoned mainly due to the high chemical oxygen demand and biochemical oxygen demand of the effluents (as high as 1100 and 550 mg l^{-1} in chemical treatment mills and 1160 and 500 mg l^{-1} in mechanical treatment mills respectively) (IPCC, 2001; Möbius, 2006). Disposal alongside municipal waste (sewage) treatment was also commonplace for smaller mills as the material is compatible with the infrastructure available at wastewater treatment plants, although this was not feasible for larger scale operations due to the quantities of pulp wastes produced (Scott and Smith, 1995). Therefore, treatment of waste to generate the semi-solids material we now refer to as PPMS became common practice to reduce the overall quantities of waste and recover water. Presently in the UK, any remaining effluent or liquid waste produced is removed from site under a consent from the regulator. Either via a discharge consent, if the waste meets the specified parameters, treated on site prior to discharge or treated in an appropriately permitted independent facility.

For the semi-solid sludge materials produced currently, a popular method of disposal is incineration; this is more feasible in pulp and paper mills where other waste materials such as debarking material are already incinerated for energy production. The high organic content of PPMS makes them potentially combustible and so suitable for incineration, with primary and secondary PPMS having modest energy contents of 2690 and 4000-5000 MJ per wet tonne of material respectively (Bajpai, 2015). Initially, PPMS contain around 0.5–2 % solids content but are generally dewatered (often by belt pressing) to 25–40 % solid content on site (Bajpai, 2015). However, their high-water content typically necessitates further dewatering before incineration, decreasing the net energy yield. A further complication is that paper sludges have high ash contents which require more specialised equipment to incinerate and therefore greater initial capital investment. Eikelboom et al. (2018) estimated the costs related to incineration, including labour, transport and quality control, at US$332–441 t^{-1}, while the products produced (energy and ash) are worth US$ 91.83. Therefore, incineration as a disposal option can only offer partial cost recovery. The impacts of incineration on the environment must also be considered. Incineration of PPMS can release NO_x and SO_2 (which are definitively linked to acid rain generation), as well as particulates, with one estimate of total emissions from paper and pulp mill waste incineration for the USA alone in the year 2005 comprising 40000 t SO_2 and 59000 t NO_x (Pinkerton, 2007). It is also possible that incineration can release potentially harmful chlorinated compounds from any plastic contaminated PPMS or those including residual cleaning agents (Simão et al., 2018). Therefore, infrastructure to treat emissions, such as gas scrubbers, is required, with the associated expense. However, on the other hand, if the mill relies on a mainly fossil fuel derived power supply, sludge incineration could be a way to offset its carbon footprint. Furthermore, paper sludge ash can also be spread to land for its liming benefits, although this will not be further discussed (Tony Marsland, 2015).

Landfilling has been a common disposal method (Scott and Smith, 1995; VEN, 1997) and remains dominant today in some countries (Simão et al. 2018), however, the cost incurred by landfilling is increasing in most countries and new legislation is constantly being developed to reduce landfill waste. An example of such legislation from within the European Union is the landfill limitations Directive 99/31/EC, which states that "biodegradable municipal waste going to landfills must be reduced to 35 % of the total amount (by weight) of biodegradable municipal waste produced in 1995" by the year of 2016 and this was followed by the promotion of the waste hierarchy through Directive 2008/98/EC (waste framework directive) which emphasised that disposal of waste, e.g. via landfilling, should be considered a final resort only. Nevertheless, landfilling remains a final alternative after reuse and recycling options are exhausted.

3.5. Land application

Driven by the financial burden and potentially negative environmental impacts of landfilling or incinerating PPMS, they are increasingly being disposed of in more environmentally positive ways while landfilling itself has decreased (Camberato et al., 2006; Gibbs et al., 2005; Scott, 2019; VEN, 1997). As the chemical composition of paper sludges shares some properties with livestock manure (Bellamy et al., 1995), landspreading of the material emerged as a clear solution to the disposal problem several years ago. In fact, the spreading of paper mill sludge to land has been recorded since the 1950s and has, for example, been considered a common practice for more than 30 years in the UK (CPI, 2015; Norrie and Fierro, 1998). Although transport costs must be taken into account, similar transport costs are incurred by any offsite movement (e.g. for landfilling or incineration). Land spreading consists of either simply spreading a layer on top of the soil (mulching), or application followed by incorporation into the soil through ploughing or other means. The aims of mulching also differ from those of incorporation; mulching is employed to help maintain heat in soil, reduce evaporation, prevent weed germination and reduce nutrient loss by runoff while incorporation aims to increase the nutrient availability of soils, alter the physical properties of the soils (i.e. bulk density and hydraulic conductivity) or to add organic matter back into the soil.

The use of paper based mulches can also play a key part in the replacing of plastic mulches which can contribute to microplastic pollution of soils (Bandopadhyay et al., 2018). However, a practical consideration is that, as it is only beneficial for land managers to spread PPMS during drier seasons of the year and before or after crop production on arable land, sufficient storage is required during wetter periods if land spreading is to be undertaken as a major recycling route. A third option when applying PPMS in an agricultural setting is to initially use them as animal bedding followed by spreading the used bedding material to land. However, this method requires further drying beforehand. Nevertheless, this method is likely to be favoured in some regions, for example in England and Wales where it allows for the material to be used on farm and subsequently spread to land without deployment of a mobile plant permit (i.e. there would be no need to wait for approval from the environmental regulator, as registration of the exemption is completed immediately online).

Possible hazards of spreading PPMS to land can be mitigated by proper regulation which includes taking into consideration factors such as site-specific conditions, soil caracteristics, and crop requirements on the site. These precautions and regulatory measures will vary from country to country, for example some members of the EU do not permit paper sludge spreading to agricultural land at all while in others there are specific legal requirements for land spreading (Suhr et al., 2015). In Europe (within the European Union countries) the use of PPMS in land spreading is regulated by two directives, Directives 86/278/EEC (the sewage sludge directive) and 91/692/EEC (standardizing and rationalizing reports on the implementation of certain Directives relating to the environment). The spreading of primary, secondary or deinking sludge from paper making is covered by European Waste Catalogue (EWC) Codes 03 03 05, 03 03 10 & 03 03 11 (CPI, 2015).

European Directives are regulated differently in each of the Member States, and still underpin a lot of the UK based regulations at time of writing despite the UK leaving the EU. In England and Wales those who landspread PPMS are regulated via the Environmental Permitting Regulations (specifically Standard rules SR2010No4 Mobile plant for landspreading, (Environment Agency, 2012)), whilst in Scotland and Northern Ireland, applications of wastes for agricultural benefit are still regulated under the Waste Management Licensing Regulations. On farm, additional regulations and codes of practice also cover landspreading and waste storage activities, such as the Reduction and Prevention of Agricultural Diffuse Pollution Regulations (Agriculture England, 2018) which underpins the Farming Rules for Water (Department for Environment Food & Rural Affairs, 2018). Many countries have further governmental advice and best practise codes (e.g. Gibbs et al. 2005).

While research has been conducted into the use of PPMS in construction (Andreola et al., 2005; Balwaik and Raut, 2011; Naik et al., 2004; Thomas et al., 1987), combustion (Coimbra et al., 2015) and ceramics (Asquini et al., 2008), application to land remains the most common beneficial recycling pathway. Land spreading is briefly touched upon in a review by Monte et al. (2009), in which a short overview is presented on some of the logistics, advantages and limitations, while Simão et al. (2018) also included information about land spreading of PPMS within a wider review of paper mill related wastes. Camberato et al. (2006) reviewed the subject of paper mill sludges including land application in North America, however deinking sludges were also discussed interchangeably and, as mentioned in section 2.2., these have very different characteristics leading to differing conclusions being derived than to those that would come about from only studying primary and secondary PPMS. Faubert et al. (2016) reported the greenhouse gas (GHG) impacts of PPMS management, concluding that further research is required into the GHG emissions of PPMS used in silviculture, land reclamation and composting.

3.6. Composting of sludges

The composting of PPMS before land application can help improve chemical characteristics, reduce pathogenic organism content and reduce the overall volume of waste while increasing its bulk density (Hazarika et al., 2017; Jackson and Line, 1998). Particular interest has been placed on decreasing sludge C:N ratios through composting, with multiple studies having explored this (Table 3-2). It is especially effective when co-composting with N rich wastes

(Camberato et al., 2006; Campbell et al., 1995; Gagnon et al., 2001; Jackson and Line, 1997a), as this not only alters the ratio by virtue of the N additions but also through stimulating enhanced microbial activity that reduces the C content. Most studies agree that composting directly leads to a decrease in C:N ratio (Table 3-2) because the organic matter present is consumed by microorganisms which release carbon to the atmosphere as carbon dioxide. An additional benefit of composting is that soluble N and P can be converted into organic forms by microbial processes which can reduce leaching and therefore extend their availability to crops (Bajpai, 2015). It can also lead to a threefold increase in CEC (Camberato et al., 2006) and an increase in the nutrient content, including P, K, Ca, Mg, Na, Fe and Mn, as organic matter is reduced (Campbell et al., 1995; Gagnon et al., 2001). In terms of organic matter composition, composting was found to lead to an increase in the concentrations of carbohydrates (61 – 105 ppm increase over 6-17 days) and lignins, whereas those of sterols, lipids and proteinaceous compounds decreased (Marche et al., 2003).

The composting conditions have a significant effect on the end product. For example, the composting period must be sufficiently long to enable this process to lead to a notable change, as highlighted by a short term (17 day) trial by Marche et al. (2003) which found the overall C:N ratio was maintained in the PPMS+woodshavings compost investigated. Two other condition which are essential for effective composting include substrate moisture content and temperature. Hubbe et al. (2010) reviewed the effect of compost condition on the composting of different lignocellulosic materials and found that relative low temperatures (35-50 ^{o}C) were required to effectively decompose pulp mill fibers and suggested compost moisture content varied from 40-65 % (Hubbe et al., 2010).

Composting of PPMS can be conducted *in situ* at the paper/pulp mill or can be conducted by the party who is responsible for land spreading the PPMS however in many countries the operator would require additional permitting to compost on site. Commercial scale composting of PPMS by paper mills dates back to the 1990s although the process has not been adopted by many mills globally (Bajpai, 2015), most likely because of space limitations at mills and additional permitting requirements. In the UK composting is conducted at composting facilities rather than on site, mainly due to space and permit requirements.

Table 3-2. A selection of pulp and paper mill sludge composting experiments and their impacts on the materials C:N ratio.

Sludge origin	Duration	Additional materials	C:N before	C:N after	Citation
Paper mill	2 years	Ramial wood, urea and fly ash	109:1	42:1	(Gagnon et al., 2001)
Pulp and paper	18 weeks	Tailings, wood ash and cattle paunch	270:1	14-67:1	(Campbell et al., 1995)
Pulp and paper	121-169 days	Mineral nitrogen, phosphorus and potassium	218:1	35-54:1	(Jackson and Line, 1997a)
Paper mill	129 days	None	41.5:1	21.1:1	(Evanylo and Daniels, 1999)
Paper mill	17 days	Hardwood sawdust	21.5:1	21.9:1	(Marche et al., 2003)
Pulp and paper	34 weeks	Fly ash	70.1:1	40-46:1	(Hackett et al., 1999)
Paper mill and deinking	28 days	Pinewood bark	42.7:1	28.1:1	(Jokela et al., 1997)
Paper mill primary	60 days	Vermicomposting with Eisenia fetida	138.92:1	13.35:1	(Ganguly and Chakraborty, 2018)
Paper mill secondary			86.21:1	6.61:1	
Paper mill mixed	150 days	Vermicomposting Eisenia fetida	85:1	44:1	(Kaur et al., 2010)
Paper mill mixed	56 days	Vermicomposting Eisenia fetida	257:1	72:1	(Negi and Suthar, 2013)

Vermicomposting (earthworm enhanced composting) has also been investigated as a method of pre-treatment before land spreading and was found to reduce the overall volume of material, increase the concentration of nutrients and lower the C:N ratio (Table 2), similar to other composting techniques (Butt, 1993; Elvira et al., 1996; Elvira et al., 1998; Elvira et al., 1997; Ganguly and Chakraborty, 2018; Kaur et al., 2010; Negi and Suthar, 2013; Sonowal et al., 2014). Ganguly and Chakraborty (2018) explored the roles played by microbes during vermicomposting. The results indicate that the activity microbial enzymes arylamidase and β-glucosidase play significant roles in altering C:N ratios.

3.7. Effects of land spreading on the physical and chemical properties of soils and plant growth

Land application of sludges benefits soils in multiple ways, most of which are derived from their high organic content (this can be as high as 94% in primary sludges) and their liming effect (CPI, 2015; Méndez et al., 2009). These benefits include improving aeration and drainage, improved nutrient cycling, the stimulation of microbial activity, increasing of microbial and fauna populations and immobilising potentially toxic elements (PTEs) (Battaglia et al., 2007; CPI, 2015; Levy and Taylor, 2003; Norris and Titshall, 2011). There are also known potential risks of N immobilisation in the soil due to the high C:N ratio of the materials, particularly primary sludge, which sometimes requires management to prevent impact on the growing crop. The level of interest in the effects that land spreading of PPMS has on soils and the plants and agro-ecosystems they support has been steadily growing over recent decades, with a major upswing in research publications on the topic since the 1980s (Figure 3-3). The principal observations from this research and the remaining knowledge gaps are discussed in the following sections.

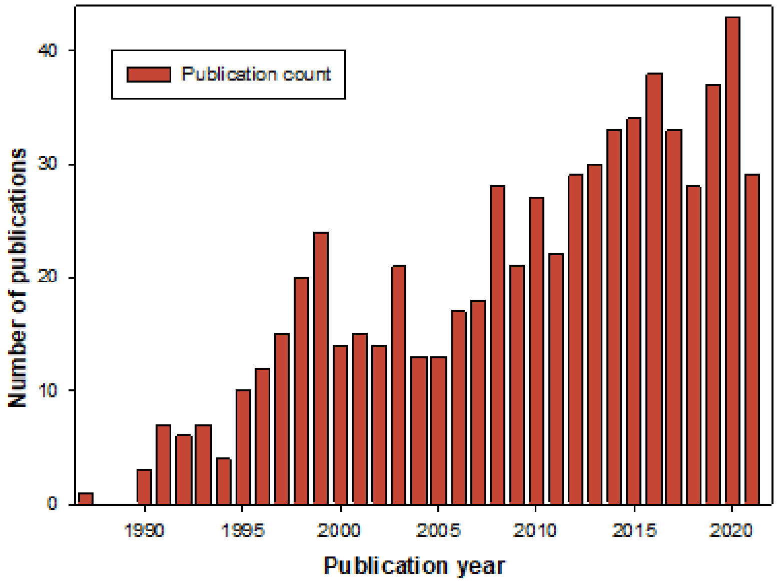

Figure 3-3. Number of publications, by year, that feature 'Paper pulp' AND 'Soil' in their topic. Data compiled from Web of Science^{TM} (https://www.webofscience.com).

3.7.1. Effects on the physicochemical properties of soils

Nunes et al. (2008) applied 40 to 120 g kg^{-1} of PPMS that led to an increase in cation exchange capacity (CEC) from 4.26 to 5.00 cmol$_c$/kg and 7.32 to 8.53 in control and 120 g kg^{-1} PPMS application plots of a Cambic arenosol and a Cromic Cambisol respectively after 188 days. Camberato et al. (2006) also reported that PPMS addition typically raises soil CEC substantially, citing examples where heavy applications increased values from 4 to 12 cmol (+) kg^{-1}. Zibilske et al. (2000) found that a single application, biennial applications and annual application of 135 and 225 Mg ha^{-1} PPMS to field plots in Maine (USA) all led to a general decrease in soil bulk density over a 4-year period (from ~1 Mg m^{-3} in the first year to 0.8-0.9 Mg m^{-3} in the fourth year). More pronounced effects on bulk density were observed (as low as 0.6 with 225 Mg ha^{-1}) after 5 years, but only under annual application. Chow et al. (2003) found that additions of 20, 40, 80 and 160 Mg ha^{-1} of air-dried pulp mill sludge led to increased hydraulic conductivity and macroaggregate formation (and therefore increasing the ratio of macropores to micropores), thus improving infiltration and water storage in soil with up to 2.1 fold increase in delay in runoff initiation and 23 % reduction in total runoff and 71 % less soil loss via erosion. Improved soil aggregation and soil stability in a sandy loam following PPMS applications were also reported by Gagnon et al. (2001). Similarly, Zibilske et al. (2000) also reported an increase in soil aggregation after 3 years of PPMS application. However, due to potentially high Na concentrations in some PPMS (e.g. 25000 mg/kg), it is possible that repeated application of such materials could lead to increased sodicity and salinity in soils found in warm and dry climates that could potentially cause aggregate instability and slaking and so also inhibit plant development (Abdullah et al., 2015; Cabral and Vasconcelos, 1993). More research on the possibility of this should be conducted, particularly in areas of Africa, Australia, and South America that have sodic soils and low annual rainfall.

Application of PPMS to land is also recognised as having a substantial liming potential, thanks to its neutral to alkaline pH and carbonate. Nunes et al. (2008) for example, found that 80 Mg ha^{-1} of secondary paper mill sludge raised soil (Cromic Cambisol) pH from 6.1 to 7.2. Other studies have reported similar capacity of PPMS addition to raise soil pH (Environment Agency, 2015; Méndez et al., 2009; Shipitalo and Bonta, 2008). Such a liming effect can be beneficial to agricultural landowners and managers as it offsets the cost of purchasing liming agents, thus providing a further incentive to facilitate land-based use of PPMS.

3.7.2. Organic matter

PPMS are rich in organic matter in the form of lignin and short cellulose fibres, while secondary sludge is further enriched with organic matter in the form of dead microbial biomass. Their applications have repeatedly been proven to increase soil C stores (Gallardo et al., 2012) which is of particular interest in the current agricultural and sustainability markets. At a field scale, Zibilske et al. (2000) showed that single applications of 180 and 225 Mg ha^{-1} of paper mill sludge (dry weight basis) on land used for corn growth led to a significant increase in soil C, and this increase was still significant after five years without further application, although initial effects were not seen during the first year following application, likely due to the time taken for soil PPMS decomposition. However, lower single application rates of 45, 90 and 135 Mg ha^{-1} had no significant impact on soil organic C% over three years. In contrast, annual or biannual applications of this paper mill sludge at 45 Mg ha^{-1} maintained C stores, which might otherwise have been lost through decomposition, within the soil while similar repeat applications of ≥ 90 Mg ha^{-1} increased soil C over the five years. Nunes et al. (2008) found application rates of greater than 120 Mg ha^{-1} increased soil C significantly by 1.3 g and 1.8 g kg^{-1}. The benefits of a single application of composted paper mill sludge (tested at rates of 45 and 90 Mg ha^{-1}) on soil organic matter content, macroaggregation, and microbial growth and activity have been found to persist even after 3 years of continuous cropping (Gagnon et al., 2001).

3.7.3. Greenhouse gas emissions

Due to their high organic content, the release of greenhouse gasses (GHG) during the decomposition of PPMS has been explored (Baggs et al., 2002; Faubert et al., 2019; Faubert et al., 2017). Land applied PPMS tend to release a peak of N_2O soon after incorporation which is related to the C additions stimulating microbial activity. This N_2O peak was found to last for 3 weeks by Baggs et al. (2002). However, over longer periods Faubert et al. (2017) found that the area-based N_2O emissions produced from PPMS application were similar or only slightly higher than those from commonly used urea fertilizer (fertilizer-induced N_2O emission factors −0.3 and 4.5% vs 0.8 to 3.1% for urea and PPMS) over two snow-free seasons (Jun-Nov 2013 and 2014). Faubert et al. (2019) compared the GHG emissions of landfilling and land spreading PPMS (equally mixed PPMS and a predominantly primary PPMS mix) and combined it with longer-term (0-100 years) modelling. Their findings indicated that land spreading could reduce GHG emissions by two thirds when compared to landfilling over the longer term. Therefore, while the GHG emissions of land spreading PPMS deserves further exploration, current evidence suggests that it offers a greener alternative to landfilling in terms of emissions.

3.7.4. Nutrient availability and plant growth

Numerous studies have investigated land spreading of primary and secondary PPMS on agricultural soils and the subsequent effects on crops and plant health. A summary of the reported observations is presented in Table 3-3 and indicates that, while there are differing results, immobilisation of N or other mechanism leading to N availability limitation is a common occurrence that requires monitoring and/or management. This can occur due to the large C pool provided by PPMS that can stimulate microbial activity and growth which in turn requires the consumption of N, and if there is not enough readily available from the PPMS itself the microbes will utilise (i.e. immobilise) N from the soil (Chen et al., 2014). Such effects were likely involved in a study by Norris and Titshall (2011) where applying as little as 10 Mg ha^{-1} of paper mill sludge reduced N uptake by ryegrass (*Lolium perenne*) significantly. Other studies similarly found that reduced nutrient uptake by common crops can often be a side effect of applying PPMS to soil above certain rates (Bellamy et al., 1995; Camberato et al., 2006; Carpenter and Fernandez, 2000; Norris and Titshall, 2011; Nunes et al., 2008) and this is managed through controlled applications in the UK that take account of site-specific factors. To counteract or mitigate against this, the benefits of co-application of nutrients have been widely reported in the literature (Bellamy et al., 1995; Camberato et al., 2006; Nunes et al., 2008; O'Brien et al., 2002), while it would also be possible to positively exploit N immobilising effects by applying PPMS in situations where there may be excessively high levels of available N (i.e. as a positive land management approach in nitrate vulnerable zones).

However, there are conflicting reports in the literature with some having concluded variously that additions of PPMS to agricultural soils can either lead to an increase or decrease in availability of nutrients, including N (Table 3-3). Also, any effects may vary with time. For example, the immobilising effects of PPMS on available N were shown to be maintained by recurring application by Gagnon et al. (2012), however once application is concluded the effects appear to be transient (Aitken et al., 1998; Douglas et al., 2003). Both studies reported that N which was initially immobilised became readily available again after two years of cropping, and in the case of Aitken et al. (1998) the overall N availability increased compared to pre-application levels. In a similar outcome, Vasconcelos and Cabral (1993) found that, after producing significant negative impacts on yellow lupin (*Lupinus luteus*) growth during the first year of cropping when PPMS were added at 50 Mg ha^{-1} and above, the negative effects were not observed in the second year. They also noted that Mn, Zn and P uptake by yellow lupin decreased almost linearly under applications of 10-130 Mg ha^{-1}, which contrasts sharply with increases in the availability and/or plant uptake of these and other nutrients reported elsewhere (Table 3-3). For example, application of secondary PPMS resulted in a linear increase in available P content (Egner-Riehm method) when applied at rates of 40 to 120 Mg ha^{-1}, rising from 88 to 196 mg P kg^{-1} and from 120 to 206 mg P kg^{-1} at the highest treatment in a cromic arenesol and cromic cambisol soil, respectively (Nunes et al., 2008), with similar increases recorded for available K. Elsewhere, combined primary and secondary PPMS applied at 34 Mg Ha^{-1} significantly increased soil available N, P and Mn in a low nutrient sandy soil (Gagnon et al., 2003).

The method of PPMS application also seems to have an influence on its effects on plant growth, as while the studies above mainly involved PPMS that underwent incorporation into soil, using PPMS as a mulch has been reported to increase the available N, K, Ca, and Mg (as determined by KCl and 1 N NH_4OAc extractants for the N and K respectively, and in soil-saturated paste extract for the latter two) as well as increase wheat (*Triticum aestivum*) N and K uptake (Amini et al., 2012). That study compared mulching versus incorporation and found that, when compared to soil incorporation, un-amended controls and separate treatments where only N, P and K fertilisers were applied (at up to 92, 50 and 83 kg ha^{-1} respectively), mulching at 100 Mg ha^{-1} produced the greatest yield of wheat (*Triticum aestivum* Var. Tajan) save for one of the fertiliser treatments while the PPMS incorporated treatments (applied at 50 and 100 Mg ha^{-1}) produced the lowest yields. Relatedly, when PPMS were trialled as a mulch for turfgrass growth, Karcher and Baser (2001) found that PPMS provided a viable, cheap alternative to the commercial mulching product hydromulch (wood fibre based) with no significant drawbacks in terms of grass height, turf cover, and soil water infiltration.

Not all plants are affected in the same manner by PPMS application. For example, Harrington and DeBell (1984) found the same treatments increased the yield of black cottonwood (*Populus trichocarpa*) while decreasing the yield of Alder (*Alnus rubra*). This was observed at application rates of 225 and 450 Mg ha^{-1} of PPMs that had been corrected to a C:N ratio of 100:1 by N fertiliser additions. Yield impacts have also been shown to depend on the soil type involved (Nunes et al., 2008).

The use of PPMS as a media for seeding and germination has also been explored (Bellamy et al., 1995; Levy and Taylor, 2003). Through a series of nursery container studies Bellamy et al. (1995) confirmed that paper sludge amendments were suited to use in nursery container cultures for a range of species (tomato (Lycopersicon esculentum L.), cucumbers (Cucumis sativus L.), and peppers (Capsicum annuum L.)), sufficing that the low N content of sludges is corrected for (to approximately 1.2% dry w/w). When germinating radish in pulp mill sludge alone, Levy and Taylor (2003) found that radish (*Raphanus sativus* L.) and cress (*Lapidium sativum* L.) germination rates were not reduced when compared to control soil, but seedlings were smaller, lighter coloured, misshapen and displayed necrotic patches (Levy and Taylor, 2003), indicating that raw or unamended PPMS is not universally suitable as a germination medium.

Table 3-3. A summary of studies examining the impacts of incorporating paper and pulp mill sludge on plant growth.

Sludge type	Application rate(s) & method	Co-applications	Crop	Soil type	Main outcomes	Reference
Paper mill sludge	8.5, 17, 34 Mg ha^{-1}	None	Blueberry (*Vaccinium angustifolium*)	Sandy soil (l'Afrique sand); pH 5.0	8.5 and 17 Mg ha^{-1} treatments increased fruit yield by more than double, while 34 Mg ha^{-1} treatment was equivalent to control.	(Gagnon et al., 2003)
Paper mill sludge (70% primary: 30% secondary)	0, 20, 100 and 200 Mg ha^{-1} applied as mulch	None	*Brassica rapa* (mustard plant)	Commercial topsoil OM 10%; clay + silt fraction =55%	20 Mg ha^{-1} enhanced plant survival (142%), flower (116%) and seedpod (125%) development, and a root-length index (111%). Extreme application rates (100 and 200 Mg ha^{-1}) adversely affected plant growth and development.	(Bostan et al., 2005)
Paper mill sludge (with ~2% w/w sewage sludge)	0, 50, 100, 150, 200, 250 g kg^{-1} (simulating 0, 112, 224, 336, 448, 560 Mg ha^{1})	0 or 200 kg N ha^{-1}	Corn (*Zea mays* L.)	Sandy loam; pH 7.8	● Delaying sowing until 21 days after sludge incorporation increased mean germination from 88% to 100% in treated soils. ● With co-applied N, sludge additions at 112 Mg ha^{1} equivalent increased crop growth (plant dry mass), while higher application rates decreased it. ● At 336 Mg ha^{1} and above, sludge applications increased plant P levels.	(O'Brien et al., 2002)
Primary pulp mill sludge	0, 10, 30, 50, 70, 90, 110 and 130 Mg ha^{-1}	0.44 g P, 0.22 g K and 0.05 g Mg per 6kg soil	Yellow lupin plants (*Lupinus luteus* L.),	Cambic Arenosol	● Applications above 50 Mg ha^{-1} depressed yield in the first year (linked to nutrient immobilisation) but not in the second year.	(Vasconcelos and Cabral, 1993)
Pulp and paper mill sludge	0, 225, 450 Mg ha^{-1}	50 kg P ha^{-1} and 100 kg of K ha^{-1} or 100 kg P ha^{-1} and 200 kg K ha^{-1}, depending on sludge application	Black cottonwood (*Populus trichocarpa* Torr. and Gray) and red alder (Alnus mbra Bong.)	Silty clay loam of alluvial origin	● Increased the yield of black cottonwood ● Decreased the yields of Alder ● Irrigation was highly significant in its effects on yield of both species	(Harrington and DeBell, 1984)
Secondary pulp mill sludge	0, 40, 80 and 120 Mg ha^{-1} (equivalent 0–40 g kg^{-1})	140 mg N kg^{-1}, 93 mg K kg^{-1} and 3 mg P kg^{-1}	Wheat (*Triticum aestivum* L.)	Cambic Arenosol (pH 6.4) and a sandy loam Cromic Cambisol (pH 5.0)	● Applications linearly increased wheat grain nitrogen levels (both soils) ● Wheat grain P levels unaffected ● Marginal effect on grain K on one soil ● Grain Ca increased. ● Variable effects on grain Cu, Mn and Zn ● 40 and 80 Mg ha^{-1} rates increased grain yield on Cromic Cambisol soil led to mild decreases on other soil (more notable decreases at 120 Mg ha^{-1}). ● Fertiliser supplements recommended when applying sludge	(Nunes et al., 2008)
Primary and secondary pulp sludge	5.1, 8.8, 9.6, 10.9, or 13.8% dry weight	0, 8.4, or 20.7% flume grit on a dry weight basis; with varying combinations of fertiliser comprising triple superphosphate + potash, and N as either 728 kg^{-1} urea or 8.6 Mg ha^{-1} chicken manure.	Grass mix (34% *Lolium perenne* L., 34% *Festuca rubra* L. subsp, rubra, 13% *Poa pratensis* L.,9% *Loliurn rnultiflorum* Lam., and 6% *Trifoliurn repens* L.), or Hybrid poplars (*Populus* spp.).	Artificial soil comprising quarry pit bank sand and the blended additives. Compared with a natural sandy soil control.	● Grass yield (all species) and tree growth were enhanced by sludge applications ● Plant tissue P and N concentrations significantly enhanced by sludge treatment	(Carpenter and Fernandez, 2000)
Primary and secondary paper mill sludge	9:1 soil-sludge weight ratio	None	Barley (*Hordeum distichum*)	Pb and Zn polluted Sandy loam; pH 7.6	● Sludge application improved plant root development and reduced chlorosis symptoms ● Decreased plant uptake of toxic levels of metals (Zn and Pb) by >50% ● ● Reduced peroxidase activity (stress response) in plants by ~50%	(Battaglia et al., 2007)

Paper mill sludge	10, 20 and 40 Mg ha^{-1} dry basis	Basal fertiliser added according to unspecified 'fertility recommendations for each soil'	Perennial ryegrass (*Lolium perenne*)	Clay, silty clay and a sandy loam; pH 5.3-6.0	● 10 Mg ha^{-1} rate increased yield in two out of three soils, while higher rates decreased yields ● 40 Mg ha^{-1} rate decreased plant N to below critical levels in all soils ● Plant P remained in the adequate range at all application rates and was enhanced in plants from two of the soils	(Norris and Titshall, 2011)
Pulp mill sludge	0, 12, and 24 dry Mg ha^{-1}	NH_4NO_3 at rates of 0, 100, and 200 kg-N ha^{-1}.	Corn (*Zea mays* L.)	Clay-silt loam; pH 5.5-6.6	● Sludge applications with and without added N increased plant emergence ● Grain yield increased with sludge application when N was co-applied (yield decreases were observed without N supplement) ● Recommended paper sludge application rate of 12 dry Mg ha^{-1} plus 100 kg N ha^{-1}	(Bellamy et al., 1995)
Primary sludge	Blends of sludge: organic soil (terre noir) in ratios 0:50, 10:40, 30:20, and 50:0 were mixed with sand (50%) and applied at rates equivalent to 0, 23, 68, and 113 Mg ha^{-1} sludge	N at 4.5 to 5.5 Mg ha^{-1}, P at 1.18 to 1.26 Mg ha^{-1}, and K at 1.34 to 1.46 Mg ha^{-1}. Increasing with sludge content	Kentucky bluegrass (*Poa pratensis* L. 'Georgetown') and perennial ryegrass (*Lolium perenne* L. 'Prelude')	Schist-Loam; pH 6.1	● With nutrient addition, ground cover, turf colour, and stand quality were maintained across all application rates ● Without nutrient addition, ground cover and stand quality decreased for some grass species at 68 or 113 Mg ha^{-1}.	(Norrie and Gosselin, 1996)
Mixed pulp mill sludge-Composted or lime-stabilised	17.7 to 25.3 dry Mg ha^{-1}	250 kg ha−1 additional mineral fertiliser (50 kg N ha-1) in 2016. 400 kg ha−1 in spring 2017 (80 kg N ha-1)	Wheat (Triticum aestivum) and oat (Avena sativa)	Sandy Clay; pH 6.2	● Higher yields than an untreated control plot. ● Yields were comparable to those of a mineral fertiliser only plot. ● Three out of four sludge treatments did not reduce nitrogen uptake compared to the mineral fertiliser treatment.	(Kinnula et al., 2020)

3.7.5. Potentially toxic elements

The concentration of potentially toxic elements (PTEs) in any type of land amendment, be it PPMS, sewage biosolids, or mineral fertiliser, is always an issue that may pose concern. However, while paper sludges may contain traces of PTEs such as cadmium, copper, lead, and nickel, with primary paper mill sludges tending to have higher levels than secondary sludges (IPCC, 2001), the concentrations are typically low and similar to those found in commonly land-spread livestock manure (Bellamy et al., 1995; CPI, 2015) (

Table 3-4). Indeed, concentrations in paper sludges remain lower than those typically found in sewage sludge which is applied to land as common practice (Deviatkin et al., 2015; Epstein, 2002). de Azevedo et al. (2019) found that As, Pb, Cr, Fe, Al, Mn, Zn and Cu in leachate and solubilised extracts (Brazilian standard for leaching (NBR 10005, 2004)) from paper sludge fell within the concentration limits for non-inert non-harmful wastes as set by Brazilian standard and the US EPA – Code of Federal Regulations. PTEs can be a concern linked to deinking sludge which may have higher concentrations of certain PTEs (e.g. Cu, Zn and Cr) due to the processes employed to remove the ink, however these concentrations have reduced in recent years to comply with packaging regulations (Environment Agency, 2013; Scott and Smith, 1995). Therefore, land application of de-inking sludge (alone or in combination with primary and/or secondary sludge) is monitored closely in most developed countries. In general, therefore, it has been concluded that paper sludge application to land, particularly at the rates relevant to agricultural practises, will present little risk of metals accumulation problems.

Table 3-4. Typical concentration (µg/kg) ranges reported for potentially toxic elements (PTEs) in primary, secondary and deinking sludge on dry matter basis (compiled from [a]Cabral et al. (1998); (Ribeiro et al., 2010) [b]; (Deviatkin et al., 2015) [c].

Element	Primary sludge[a]	Secondary sludge[a]	Primary sludge[b]	Secondary sludge[b]	Deinking sludge[c]
Cu	7-58	25-77	13	2.8	64 -345
Zn	30-40	40-130	83	12.9	34 – 1320
Cd	0.3-3	1-9	1.4	0.34	0.02 -1.54
Cr	3.6-10	12-38	19	1.9	4.8 – 96.6
Ni	5-75	10-26	10.5	1.5	<10 – 31
Pb	7.4-74	20-100	13.2	1.1	9.5 – 79.4
Hg	0.8-1.2	0.5-3	-	-	0.1 – 0.9

As opposed to posing a metals risk, retention of PTEs by paper sludges can help reduce their availability to crops and other ecological receptors and thereby decrease or even neutralise toxicity. Most early studies with this perspective focussed on the use of paper mill sludge for removing PTEs from solution, which they proved to be highly capable of even at low pH (Baek et al., 2014; Calace et al., 2002; Calace et al., 2003). Later soil studies demonstrated that the paper sludges were also able to immobilise PTEs in the soil, for example Battaglia et al. (2007) found that the addition of paper mill sludge (1:9 sludge to soil) to Pb and Zn contaminated soils reduced the availability of both elements when measured by a five step sequential extraction with increases in the i) sulphide/bound to organic matter but not soluble in NaOH fraction and ii) the non-extractable metals fraction (overall increase in these non-labile fractions of 11% and

8% for Pb and Zn respectively). These sorption effects are likely caused by the sorption of elements onto clay and organic matter within the sludge in addition to the immobilisation brought about by the increase of pH caused by sludge application. In fact, Calace et al. (2003) found that due to these properties of paper mill sludges (i.e. high lignin and clay content) they have similar metal ion sorption trends to clay and organic matter rich soils.

Battaglia et al. (2003) showed that paper mill sludge amended soil (9:1 9:2, 9:4 and 9:8 soil:PPMS w/w) has a greater Pb sorption than the original soil (Dystric xerocrept), but the Cd sorption differences were negligible during batch sorption testing. In a column leaching test by Calace et al. (2005) it was also found that Cd leaching was decreased to a lesser extent than that of Pb (viz. Cd leaching was reduced by ~30%) when applying sludge at a 1:9 PPMS:soil w/w ratio. In another study, up to 115 µg l^{-1} of Zn was leached from a 10 g sample of primary paper mill sludge during column leaching experiments when placed on top of 20 g of soil and leached with 60 mL of water (1.7 pore volume), while this concentration gradually decreased in subsequent leachings until it was below the limits of detection after six leachings (Xiao et al., 1999). However, leaching of Zn from the soil could have also contributed to this figure. In the case of Cd and Pb, no appreciable amounts of Pb or Cd were found to leach (Xiao et al., 1999). Following these experiments, the leaching columns were deconstructed, and remaining sludge or sludge-soil mixture were analysed. Leaching from the PPMS caused soils to be enriched in Zn, Pb and Ni by 0.01, 0.01-0.04 and 0.01-0.003 mg kg^{-1} respectively of when compared to control soils.

Battaglia et al. (2003) modelled sorption isotherms of PPMS for Pb and Cd and found them to be best modelled using Langmuir isotherms. This was in agreement with the findings of Calace et al. (2002) who also found that Ag(I) and Cr(VI) were best fit by Langmuir isotherms while Cu (II) sorption was best fit by Freundlich. However, Yoon et al. (2017) found that As and Cd sorption by PPMS were better fit by the Redlich-Peterson isotherm, which combines elements of the Langmuir and Freundlich isotherms.

3.7.6. Organic pollutants

Industrial plants that employ chlorine dioxide during the bleaching process can lead to the presence of organochlorine compounds in pulp and paper sludge (Simão et al., 2018). For example, Koistinen et al. (1994) found that sludge from pulp mill discharge where chlorine is employed as a bleaching agent contains trace amounts of methylfluorenes and dimethylfluorenes (0.5 and 0.06 ng L^{-1} respectively). Similarly, 2,3,7,8-tetrachlorodibenzo-p-dioxin was found at trace levels in effluents from pulp and paper mills in India (Thacker et al., 2007). However, these issues are not prevalent in developed countries today because legislation limiting the use of elemental chlorine in paper making has led to its replacement, mainly by hydrogen peroxide or ozone (Wolf et al., 2011). Nevertheless, other organic contaminants can still be present. For example, Rigby et al. (2021) estimated that the toxic equivalents (TEQs) for dioxins/furans, dioxin-like biphenyls and polychlorinated naphthalenes were approximately 10 ng/kg (dry mass) in three paper sludges from the UK, but these concentrations in the paper sludges were much lower than those determined in sewage biosolids and some poultry litter ash.

Per- and polyfluoroalkyl substances (PFAS) are a group of contaminants receiving increasing attention. They have been used in the coating of paper food packaging and can therefore be incorporated into PPMS where recycled paper products are used as a feedstock (Wiegand, 2021). PFAS are a family of > 4700 persistent chemicals which are a growing human health concern because of their accumulation in plant and human tissue and evidence of their possible links with multiple health effects including hypercholesterolemia, hyperuricemia, decreased glomerular filtration rate (GFR) in kidneys, increased chronic kidney disease, kidney cancer, and testicular cancer (Costello and Lee, 2020; Kirk et al., 2018; Pelch et al., 2019). D'eon et al. (2009) collected samples of paper fibres from paper mills in Ontario in 2002, 2003 and 2008 and detected polyfluoroalkyl phosphate diesters at up to 2200 ± 400 ng g^{-1}. Furthermore, Bugsel and

Zwiener (2020) analysed soils from agricultural sites where PPMS had been applied, and fully or tentatively identified 61 PFAS, and many of their transformation products. They estimated soil concentrations of PFOS to be up to 100 µg/kg and those of PFOA to be up to 250 µg/kg which, while being much lower than currently understood ecological effect thresholds (i.e. 20000 µg/kg for earthworm growth and 3910 µg/kg for plant growth; Jensen et al. (2012)), does indicate that some regulation and monitoring is warranted. Therefore, although studies have shown PFAS levels to be lower in PPMS than in sewage biosolids and compost from municipal wastes (Rigby et al., 2021), PFAS in PPMS does need to be considered. As is the case with sewage biosolids and animal manures applied to land, PPMS are also known to potentially contain the endocrine disruptors bisphenol A, nonylphenol ethoxycarboxylates and 17α-Ethinylestradiol (Dsikowitzky and Schwarzbauer, 2014; Fernandez et al., 2007). Research is therefore warranted to determine the extent to which these chemicals persist in soils treated with all such amendments and also into whether treatment steps can be introduced in their production lines to remove or reduce them.

3.7.7. Odour

Similar to other land spread materials such as anaerobic digestate, odour can be a concern when spreading PPMS near residential areas, particularly during the initial 30 days following spreading during which the material still has a high water content and thus can create anaerobic conditions and associated smells (Amberg, 1984; CPI, 2015; Frechen and Köster, 1998). Historically, when PPMS were discharged to river systems, there were observed impacts on the odour of water downriver of the discharging point (Kenefick et al., 1995). Odour in pulp mill treatment ponds was attributed mainly to the release of Geosmin ($C_{12}H_{22}O$) at 2000–9000 times the odour threshold (i.e. the concentration at which it is perceivable by the human sense of smell), likely released due to the breakdown of organic matter by microbial activity (Watson et al., 2003). Indeed, odour associated with PPMS is typically associated with material that has been in prolonged storage. The spreading of PPMS may be limited based on their odour emissions, as is the case in Quebec where all landspread materials are categorised by human-perceivable odour, and PPMS are considered to be amongst the most odorous materials and hence classified as "strongly malodorous" as measured by olfactometery (Camberato et al., 2006; Environnement Québec, 2004). This classification categorises the smell as being stronger than that of solid dairy cattle manure, but not as strong as hog slurry. The odour released by PPMS can reportedly be reduced by composting, although no study has quantified this (Bajpai, 2015). In the UK, PPMS are not typically malodorous, although risks from odour can occur through poor management of materials. Furthermore, risks associated with odour are managed through incorporation into soil promptly after spreading, and risk assessment prior to spreading to take account sensitive receptors and prevailing winds.

3.8. The impacts on soil ecology

3.8.1. Effects on fauna

Historically, cases of fish having their flesh tainted and obtaining off flavours down river of discharging of paper and pulp mill effluents have been documented (Kenefick et al., 1995; Norton, 1992). While these impacts are unlikely to occur with modern regulations it is worthwhile to explore the historic potential effects of landspreading PPMS on terrestrial fauna. Keenan et al. (1990) explored the potential risks of surface applying PPMS to woodland American woodcock (*Philohela or Scolopax minor*) and to humans that consume them due to the presence of 2,3,7,8-Tetrachlorodibonzo-p-dioxin (TCDD) within PPMS. It was found that the highest reported concentrations of TCDD in PPMS treated woodland soils in the literature (50 ppt) was not high enough to cause any risk to woodcock health or reproduction, nor to humans who consume them. While there are no further studies on effects on avian species, as mentioned when discussing organic compounds in section 7.6., restrictions on elemental Cl in paper making are likely to reduce the risks of similar organic contamination from land application of PPMS.

There are very few studies in the literature investigating the potential effects of PPMS land application on earthworms, or on any other soil invertebrates vital to soil ecosystems. Nevertheless, a few do exist. Piearce and Boone (1998) conducted a series of investigations and experiments on this topic, in which the first examined the earthworm population in a sandy soil in north west England (pH 5.77) that had been treated with PPMS 4 years earlier at a rate of 200 Mg ha^{-1} and had been used to grow flax (*Linum usitatissimum*) but received no further nutrient addition. Across ten x 25 cm soil cubes excavated from the treated area they found 38 earthworms from two species (*Aporrectodea caliginosa* and *Octolasion cyaneum*), compared with just one individual *A. caliginosa* from across the equivalent untreated control samples, indicating enhanced earthworm presence in PPMS treated areas. In subsequent laboratory tests on various soils the authors observed no behavioural avoidance by *Aporrectodea rosea* earthworms of soil amended with 20% (w/w) PPMS, and indeed they even noted a selection preference by the earthworms for treated soil in one case where the unamended soil had a pH of 3.9 (the treated soil had a pH >6.6). Similar behaviour was recorded in tests with the garden snail (*Helix aspersa / Cornu aspersum*), indicating no adverse effects (Piearce and Boone, 1998). When 20 Mg ha^{-1} PPMS were applied to a commercial topsoil in a laboratory test, no adverse effects on survival or cocoon production of earthworms (*Lumbricus terrestris*) were observed, while the number of juveniles present was higher in treated soils. In another study, at a restored landfill site which had been amended with a 50:50 PPMS:soil mixture to 40 cm depth and then surfaced mulched with PPMS and inoculated with earthworms, a successful earthworm community spanning 12 species across a range of ecology types (anecic, endogeic and epigeic) had established over a six year period following PPMS addition (Piearce et al., 2003). Moreover, δ^{13}C isotopic analysis revealed that the earthworms were consuming the PPMS as a food source.

Butt (1993), examined the growth of earthworms (*Lumbricus terrestris* and *Octolasion cyaneum*) when allowed to mature in loamy soil (steam-sterilised) with surface applied (i.e. mulching) paper mill sludge (50 g:150 g sludge:soil w/w) in a pot trial. Three different yeast extracts (0.75 g) were added as an additional N source to selected pots (corrected to a C:N ratio of 25:1 from 93:1). After 120 days the development of earthworms was severely hindered by the PPMS only treatment (i.e. without supplemental yeast), to the extent that none of the earthworms had sexually matured and mean masses were lower, viz 0.7 g and 0.4 g were achieved versus > 3 g and > 1 g in all supplemented treatments for *L. terrestris* and *O. Cyaneum* respectively. However, mortality of *L. terrestris* was lower in the PPMS only (no yeast) treatment after 120 days (20 % vs 40-50%) , while for *O. Cyaneum* the mortality was ~20% in three treatments but 80% for one of the PPMS plus yeast treatments. The results highlight that PPMS addition, and that of any supplementary N sources, can have varying influence on growth and development of different earthworm species and so warrants further research. However, it should also be noted that the application rate in that particular study was very high, beyond what would be used in a typical setting of application to agricultural land and was more in keeping with land restoration application scenarios in which the existing substrate at the sites is likely to be very hostile to soil biota and thus in need of improvement that could be brought about by PPMS addition. Indeed, a Department of Environment, Food and Rural Affairs (DEFRA, UK) funded study (Environment Agency, 2015) found that when applied to an agricultural plot at 75t/ha paper mill sludge had no negative effects on soil mesofauna and actually increased soil biomass N and potentially mineralisable N.

Although not specifically or directly focused on effects in a landspreading scenario, there have been studies that investigated the relationship of PPMS and earthworms during vermicomposting of PPMS and/or the use of PPMS as an earthworm growth medium that do give some further insight into earthworm related issues. Elvira et al. (1996) explored the conversion efficiency of pulp mill sludge into vermicompost and the impacts on earthworm (*Eisenia andrei*) growth and reproduction. When mixed in a 3:1 ratio of pulp mill sludge:sewage sludge, the mixture was found to be an ideal medium for composting earthworm growth and reproduction. In a follow-up growth and mortality study using *Eisenia Andrei,* Elvira et al. (1997) confirmed that the 3:1 ratio of pulp mill sludge:sewage sludge performed the best as a habitat for earthworm development when compared with mixtures of 1:1, 2:1 and 3:1 of paper pulp mill sludge with sewage sludge, pig slurry or poultry slurry. In a later study, (Elvira et al., 1998) compared earthworm growth medium potential of paper mill sludge to that of cattle manure, dairy sludge and combinations of the three and found that while PPMS led to the greatest increase in mean earthworm mass after 70 days, it also led to the greatest inhibition of cocoon production. However, beneficial effects on both earthworm growth and cocoon production can be achieved by combining it with cattle manure and dairy manure (1:4 PPMS:cattle manure or 1:1:3 PPMS:dairy manure:cattle manure). Similarly Kaur et al. (2010) found that co-composting with cattle dung increased the materials acceptability for earthworm (*E. fetida*) reproduction. Therefore, having been found to be suitable for vermicomposting and as an earthworm growth medium (with minor amendments), as well as having no notable negative impacts reported in the few land application studies that have examined earthworms, it seems unlikely that PPMS would pose an ecotoxicological threat to earthworms when applied to soils. Nevertheless, the longer-term influence of PPMS application on earthworms is a potential future research topic.

3.8.2. The soil microbiome

Gagnon et al. (2001) explored the microbial biomass (chloroform fumigation-extraction method) and enzyme activity of soils treated with raw and composted PPMS (combined with ramial wood, urea and fly ash). Both treatments led to increased microbial biomass relative to a control plot in the two years following application, however the magnitude of increased microbial biomass decreased with time. However, a longer term study in which PPMS had been applied to agricultural soil annually for 6-9 years showed a 1.5 to 2 fold increase in topsoil microbial biomass (Environment Agency, 2015).

Gallardo et al. (2012) found that soil incorporation of up to 30 Mg ha^{-1} of PPMS had very limited effects on overall structure of fungi and bacterial communities (as determined by denaturing gradient gel electrophoresis), but that a small number of new strains of fungi were introduced by the sludge and some of the bacterial strains native to the soil were reduced in abundance or disappeared from the soil. An aragose gel electrophoresis study into *E. coli* and other possible pathogens in PPMS found that the E. coli isolates found in PPMS were not pathogenic and were likely of environmental origin, while no other potential pathogens were identified (Croteau et al., 2007).

Flemming et al. (2017) conducted a series of plate experiments to assess the microbiological quality of PPMS in Ontario. It was found that pathogens, *Salmonella, Cryptosporidium* and *Shigella*, appeared in 6-8% of samples (n = 93) and at low concentration (2 MPN g^{-1} dry wt., 9 oocysts g^{-1} dry wt., and 7 cells g^{-1} dry wt. respectively). However, *E. Coli* exceeded limits set by the regional branch of the Canadian Fertilizers Regulations which uses a different unit of measurement (1000 colony forming units g^{-1} dry wt.) in a third of the samples, most of which were fresh samples as opposed to lagoon or stored samples. Additionally, *Giardia,* a microscopic parasite that causes diarrhoea, was present in 19% of samples at a mean concentration of 30 cysts g^{-1} dry wt. Overall, mills fed by recycled material contained more Bacterial contaminants, while those fed by virgin fibre were more commonly found to contain *Giardia* which is likely to persist until land spreading (Flemming et al., 2017).

3.8.3. Run-off ecotoxicological impacts

To assess any impacts on waters receiving run-off from treated fields, a series of aquatic ecotoxicological bioassays were conducted by Bostan et al. (2005) using runoff from a commercial topsoil treated with surface applications of 70% primary: 30% secondary PPMS at

20 Mg ha^{-1} (equivalent dry weight) collected after a one day 20 mm rainfall event equivalent (the run-off was generated in a laboratory rain simulator with a soil bed of angle 15% slope). Runoff was diluted with dechlorinated tap water to 0 % (control), 10 %, 25 %, 50 % and 100 % of the original concentration. *Daphnia magna* (waterflea), *Hyalella Azteca* (an amphipod crustacean)*, Selenastrum capricornutum* (algae)*, Lemna* minor (duckweed) and *Gambusia affinis* (mosquitofish) were chosen as bioassay species. No effects were observed in mosquitofish at any concentration, while for the other species there were generally no negative effects at the concentrations of run-off considered environmentally relevant (i.e. 25% and below). At higher, less environmentally relevant, concentrations, negative effects were observed in all species except for the mosquitofish. These effects at high concentrations may have been linked to increased chemical oxygen demand in paper mill sludge run-off (Shipitalo and Bonta, 2008) but, as the concentration of runoff in receiving waters would be extremely unlikely to ever reach such levels, these results indicate that run-off from well managed land spreading of PPMS is unlikely to have detrimental effects on the aquatic environment. Indeed, PPMS application typically increases water retention in soil (Environment Agency, 2015) and was found to reduce run-off fourfold to sixfold and to decrease soil erosion (e.g. from 47 Mg/ha to <1 Mg/ ha in a restored coal mine trial; Shipitalo and Bonta (2008)).

3.9. Summary and conclusions

As the economic costs of PPMS disposal are increasing, and with the growing desire to re-cycle and re-use resources within a more circular economy, land spreading continues to offer a suitable and potentially environmentally positive alternative to landfilling or incineration and should be encouraged where possible. While the physicochemical characteristics of PPMS can be partly predicted by a number of factors, namely the feedstock of the mill, the treatment methods and the end product of the processes employed, the variable nature of PPMS leads to the conclusion that the most robust basis upon which to verify suitability for land application is via a case-by-case chemical and physical analysis (or at least via a routine, periodic analysis of PPMS generated at a facility); such systems are already in place in many countries. Application methods appropriate for a site, i.e. mulching or incorporation, can be considered based on the land's requirements and application goals (e.g. soil enhancement, weed suppression or full scale land remediation). Both mulching and incorporation have a financial incentive as it decreases or removes the need to purchase commercial mulching products or liming/organic matter amendments, although coapplication at higher application rates may be required. While all PPMS are comparable in many ways, the content of primary and secondary sludge is highly important and so must be borne in mind when considering land application, i.e. primary PPMS tends to have a less favourable C:N ratio and often lower nutrient levels overall compared with secondary PPMS while secondary PPMS may be more difficult to dewater and handle.

Secondary PPMS is often more in demand for agriculture due to its increased liming capabilities and lower C:N ratio, whereas co-application of a nitrogen source is beneficial when mixed or exclusively primary PPMS are land spread. Alternatively, composting can be employed to produce a material with a more ideal C:N ratio for land application. Therefore, where composting opportunities may be available it should be considered, especially since land spreading is limited by seasonality and storage is part of typical practise. While this study did not consider deinking sludge, Camberato et al. (2006) came to similar conclusions as to land application of that material.

Long term studies into the impacts and benefits of PPMS on agricultural fields are rare, however those that do exist suggest that any benefits to soil C are long lasting, while N immobilisation issues are alleviated by nutrient co-application and/or by natural processes occurring in the year after spreading. Leaching of pollutants has been shown to be an unlikely problem under most conditions, while sorption of pollutants is an added benefit of PPMS application. Furthermore, when applied at typical field concentrations the ecological impacts on terrestrial ecology and from runoff are likely to be insignificant, especially when coapplied along with other materials. The benefits and limitations of land application of PPMS, including points specific to primary and secondary types, are summarised in Table 3-5.

Table 3-5. The benefits and limitations of land spreading primary and secondary paper and pulp mill sludges.

	Benefits	Limitations/ usage notes
PPMS in general	• Increased soil organic matter • Increased soil cation exchange capacity • Improved soil water holding capacity • Liming effect (soil pH enhancement) • Increased soil stability/ macro-aggregation • Enhanced soil microbiological activity • Weed suppression • Improved plant health and growth possible	• Nutrient addition (particularly N) along with PPMS application is sometimes recommended because PPMS can temporarily decrease nutrient availability to some crop or pasture types. Alternatively, composting of PPMS first may also address this issue. • Odour potential needs consideration
Primary PPMS	● More widely available than secondary PPMS	● High C:N ratio, may lead to N immobilisation initially (increasing the need for nutrient supplements) ● Lower micro-nutrient content than secondary PPMS ● Higher PTE content
Secondary PPMS	● More desirable C:N ratio ● Can contain appreciable amounts of P	● More difficult to dewater ● High biological activity can make handling more difficult ● Not produced at all mills

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Chapter 4 - An assessment of the impacts of spreading WTRs on earthworm populations in an active agricultural setting

Abstract: The land spreading of water treatment residuals, a by-product of the drinking water clarification process, is common practice. The effects of WTRs on earthworms in a field setting has not been widely explored in the literature. Measurements of earthworm abundance and species diversity were conducted during visits to three farm sites in Wales between May 2018 and August 2019, each having a treated and a non-treated area. Site 1 was visited on three occasions and additional CO_2 efflux measurements were taken to explore soil ecological health more broadly. Earthworms were collected at all sites via hand sorting and at site 1 also using the electrical octet extraction method. Seventeen species of earthworms spanning all three principal ecological subgroups were collected from site 1 (across both treated and non-treated areas), while only two species were extracted at each of sites 2 and 3. The low abundance of adult earthworms at site 2 and 3 was believed to be caused by the extended hot and dry weather conditions before and during sampling (June and May 2018). The only significant variation in earthworm abundance, in terms of treated versus non-treated areas, was in juvenile earthworms in the untreated section of the field at site 3 when using the octet extraction method, which had a significantly greater density (t-test p=0.01, average of untreated=18.75 treated=2). However, there was no significant difference in density identified in the hand sorting method results at that site (or at the other sites). The calculated species richness and diversity of samples from site 1 did not differ between the treated and untreated sections of the field site. Calculations could not be made for the other sites due to species number limitations. The CO_2 flux of the untreated and treated sections of field site 1 did not significantly differ. Furthermore, when comparing the two earthworm collection methods within the same site type (i.e. treated or untreated), the earthworms collected via electrical octet and hand sorting did not differ significantly in adult count, species diversity or richness.

Keywords: Water treatment residuals, Electrical octet, hand sorting

4.1. Introduction

During the drinking water treatment process, flocculants are added to the raw water to remove turbidity and suspended sediment. The most common flocculants are Alum (aluminium sulfate ($Al_2(SO_4)_3{\cdot}14H_2O$)), ferric chloride ($FeCl_3$) and ferric sulfate ($Fe_2(SO_4)_3{\cdot}9H_2O$). The resulting reaction causes the production of over 10,000 t d^{-1} globally of a by-product known as water treatment residuals (WTRs) (Turner *et al.*, 2019). These WTRs are Fe- or Al-hydroxide based materials that vary in chemical and physical composition depending on many variables, including the raw water source, flocculant type and application rate, and the treatment processes employed at the water treatment plant. This leads to high variability in the chemical components of WTRs (Table 2-2). The land spreading of these materials is becoming more common as it is an environmentally positive alternative to sending the material to landfill; furthermore, WTRs can benefit soil structure and act as a nutrient source.

Earthworms are ideal ecological receptors to monitor soil quality due to their sensitivity to chemical and physical soil properties (Paoletti *et al.*, 1998; Roubalová *et al.*, 2015). Additionally, the activity of microbial populations in soils is known to be a key indicator of soil health. There are multiple potential methods available for estimating soil microbial activity. One example is the hydrolysis of fluorescein diacetate (FDA), which can be used as a proxy for microbial activity because many common enzymes incuding lipase, protease, and esterase enzymes, which are released by a very broad range of microbes and so are ubiquitous in active soil bioecosystems, are able to catalyse the process (Green et al., 2006). Another method of estimating soil microbial activity is the use of cotton strip assays, in which strips of cotton are buried within the soil column and left for a predetermined length of time. The loss in tensile strength of these cotton strips is then quantified, or alternatively the intensity of staining by microbes can be measured (Nachimuthu et al., 2007). However, in this study the use of a chamber method for measuring soil CO_2 efflux (soil respiration) as a proxy for microbial activity was chosen over alternative methods due to its non-invasive, non-destructive nature and its ability to produce relatively quick results in the field over large sample and area sizes. The efficacy of this method is discussed further in sections 2.18.1 and 2.18.4 of this thesis, however it must be noted that while CO_2 efflux is a measure of soil respiration, which microbial respiration plays a key role in, it is not a direct measurement of total microbial activity. The same issue occurs for the other options for measuring microbial activity (i.e. FDA), in that none capture all aspects of microbial activity including more specialist processes such as nitrogen-fixation, phosphorus solubilisation, and decomposition of specific components. Therefore, selection of method always involves a trade-off. Based on the conceptual model set out in figure 2-16 the addition of WTRs has the potential to:

1. Increase the organic content of soils which could alter the size of or stimulate both microbial and earthworm populations.
2. Alter the available amount of contaminants and nutrients within soils through sorption and leaching, which may stimulate or inhibit biological responses.
3. Change the soil structure, particularly aggregate size which may be preferable for certain species of earthworms.
4. Alter the surface area of soil particles in turn altering the bioaccessibility of soil contituents such as organic matter and nutrients.

This study, for the first time, explores the effects of land spreading water treatment residuals on earthworm populations and diversity in treated agricultural fields. In order to achieve this, seasonal sampling of earthworms was conducted using two methods, the commonly employed hand sorting approach and the more specialised electrical octet method. Furthermore, complementary soil CO_2 flux measurements were conducted to explore the impacts of WTR applications on microbial activity. A more extensive field sampling campaign was originally planned but the ban on field and laboratory work in response to the covid-19 pandemic severely curtailed the extent of the work completed.

4.2. Methodology

4.2.1. Field sites

The main site chosen for this study was a field near Dolgellau, North Wales (fig. 4-1) that covers an area of 2.7 ha. This site was chosen as it exemplifies the standard model for the application of WTRs to land in the UK and had only recently (~3 years) began applying WTRs prior to the first sampling period. Furthermore, WTRs were only applied to ~40 % of the field's surface, allowing for comparative measurements and samples to be collected from both treated and untreated sections of the site. Only 40 % of the field was treated due to the available amount of WTRs. The land is used as pasture and is grazed by livestock during a majority of the year, however during the summer months it is used for the production of silage. Both sides of the field were confirmed to have similar soil type (silty clay loam) and topographically the field's elevation varied by 2m at a gradient of < 2 %. A combination of Fe and Al WTRs have been applied to the land for past 36 months prior to the first sampling date, at an application rate of up to 250 t ha^{-1} of liquid material. In addition to WTR application, the site has 10t ha^{-1} slurry spread on it annually over the entire field. Additional liming was conducted in May/June of 2019 with 350kg/hectare of Calcifert lime prills being applied. Earthworm samples were collected, and associated field measurements conducted, seasonally, in May 2018 and subsequently in March 2019 and August 2019. A second site belonging to the same landowner was visited once during May 2018; this site had undergone similar land applications and management to site 1, however the site had a gradient of ~26%. Finally, a third site in South Wales (Figure 4-1) was visited once during June 2018 that had undergone WTR application for over 20 years.

Comparison of any WTR effects at this third site was made between two separate fields, one being used as pasture which had received no applied WTRs (or potentially only a minor amount via runoff from an adjacent field, although the region bordering this adjacent field was not sampled to avoid this potential issue) and the other was used as arable land (with application most recently of 135 t ha^{-1} in 2015 and 92 t ha^{-1} in 2016). Each field at this site having differing land use adds an extra variable that needs to be considered. This different land treatment could lead to differences in soil structure due to ploughing and soil compaction after harvesting. It could also affect soil OM due to differing uptake by crops and grass. Arable fields also have more exposed soils allowing for easier loss of soil moisture during dry periods. CO_2 flux could also be affected by this change in land cover and the amount of CO_2 released from soils from plant and root interactions with soils. Although the land use of these fields differed, they were found to have similar OM contents, WHC and the same soil type, however the pH of these soils did differ most likely on account of the liming effect of WTRs (see Table 4-2).

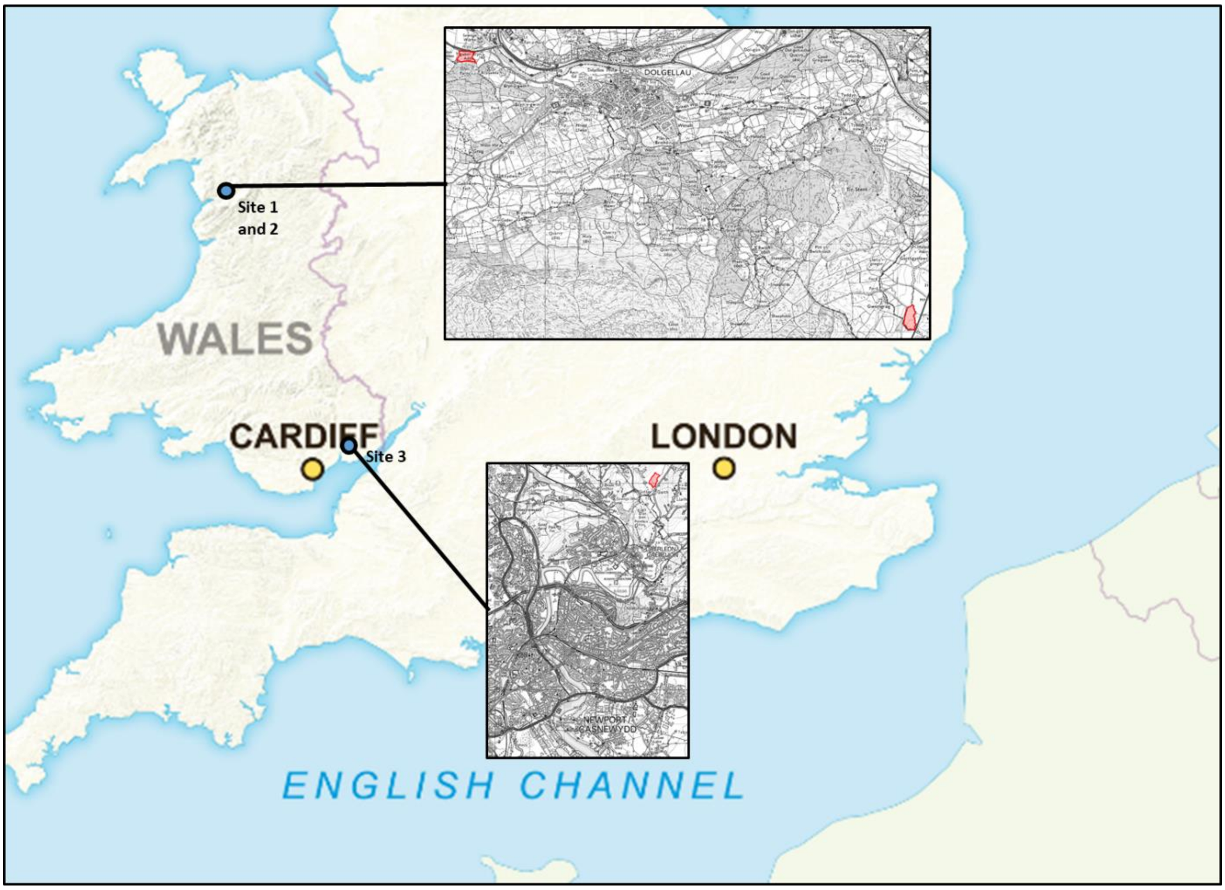

Figure 4-1. A map of the field sites in relation to the UK, where samples were collected and measured. The towns/cities of Dolgellau and Newport are used as reference points.

4.2.2. Sample collection

Earthworm samples were collected via hand sorting of soil monoliths and electrical octet expulsion (Schmidt, 2001). The May 2018 sampling at site 1 and 2 and the June 2018 to site 3 were preliminary visits intended to inform future sampling occasions and were limited due to time and weather constraints, hence gas flux and electrical octet measurements were not taken and there were fewer earthworm samples collected during the site 3 visit. . It was planned to conduct further sampling visits across 2020, but these had to be abandoned following the ban on research fieldwork brought in as part of the UK’s response to the covid-19 pandemic. When sampling a 10 m buffer zone around the edge of field was not sampled to avoid edge effects and the 5 m buffer zone used when spreading WTRs. Furthermore samples were taken at least 10 m inside the respective treated or untreated areas to ensure they were or were not treated.

Sampling at site 1 followed a triangular formation during the first sampling season, this was then expanded in the latter two field visits to include two extra sampling locations (Figure 4-2 A and B). At site 2 and 3 samples were collected from transects (Figure 4-2 C and D). At site 2 sampling transects were perpendicular to the slope of the field in an attempt to capture any variability across the slope. At site 3 the transect in the treated section of the site followed a row between crops to minimize site disturbance.

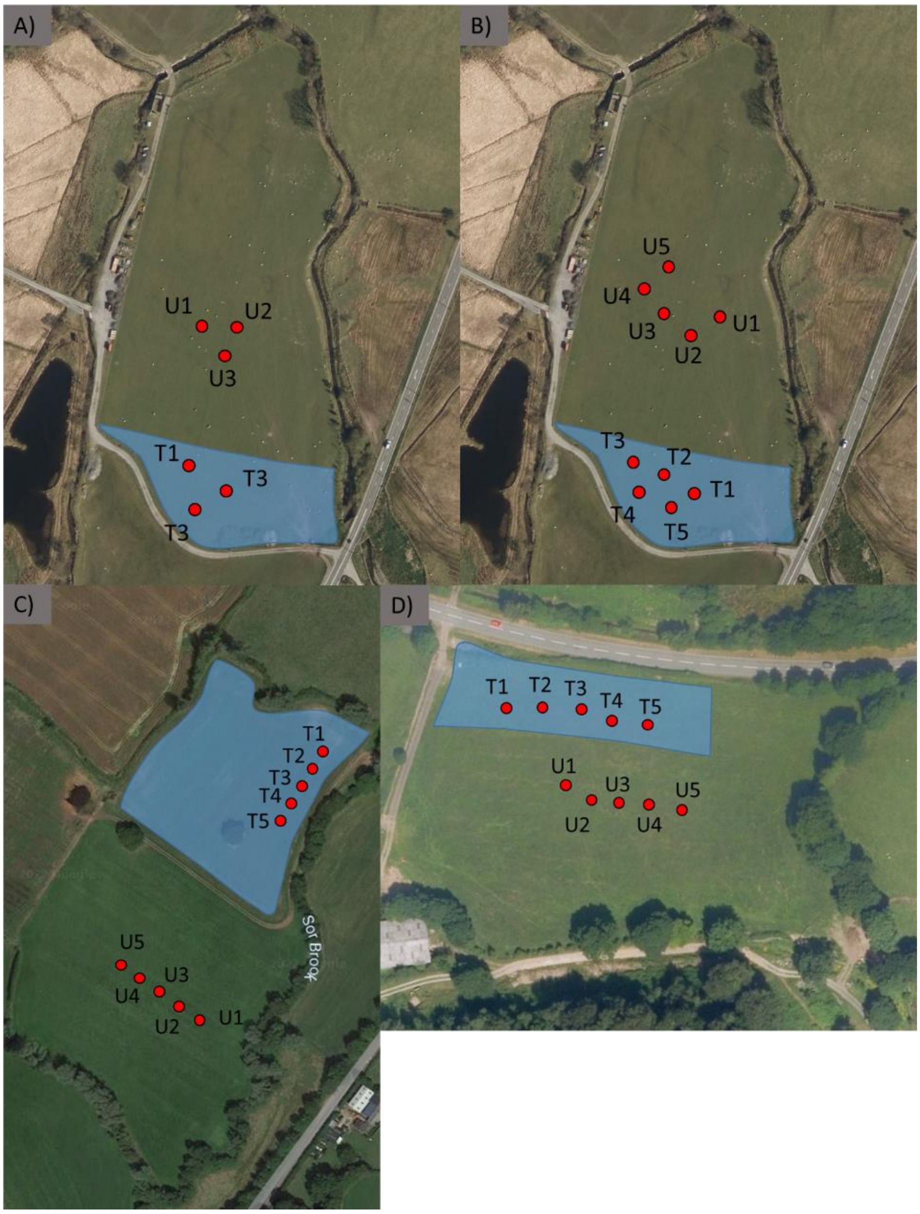

Figure 4-2. Maps of sampling sites used in this study. A) site one during May 2018. B) site one during March and August 2019. C) Site 3. D) Site 2. Red dots indicate sampling points with corresponding numbers, T=Treated U=Untreated. Areas highlighted in blue were treated with WTRs.

Pit (hand sorting) methodology

Square soil pits measuring 25 x 25 cm were dug with a spade to a depth of 25 cm or the maximum depth of soil if <25 cm (recording the depth in such instances). The soil monolith was then placed on a sorting mat to stop earthworms escaping. Hand sorting was conducted thoroughly to remove and count all earthworms (usually taking ~40-60 minutes per monolith). On each visit, approximately five pits were dug in each of the treated and untreated areas.

Octet method

The electric octet method, originally suggested by Thielemann (1986) and Thielemann (1989), employs eight electrode prongs (600 mm long, 6 mm diameter) which are inserted into the ground surrounding a circular ring that outlines a 0.125 m^2 sampling area (

Figure 4-3). Electrical impulses (voltage of 200-500) are then sequentially supplied to two of the opposing prongs at intervals. Initially the supplied voltage was set to 250 V, and then increased every 5 minutes through 50- 100 V increments (i.e. 200, 250, 300, 350, 400 V) before being maintained at 500 V for 20 minutes, leading to a total collection time of 45 minutes. Vegetation was trimmed at the operator’s discretion prior to collection, to an extent that would allow the sighting of surfacing earthworms. Earthworms that surfaced within the apparatus’ sampling area were collected while those surfacing outside the area were discarded so that they would not enter the sampling area over the surface. Five octet sampling points were conducted in the treated area and the untreated area.

Figure 4-3. The electrical octet method as used in this study.

Earthworm measurement and preservation

The collected earthworms were sorted into adults and juveniles and counted, with maturation being determined by the presence of a clitellum. Juveniles were counted and then released, as their identification to species level is difficult without DNA analysis, while adult earthworms were collected for later species identification and stored in 80% ethanol. Subsequently these adult earthworm samples were transferred into fresh 80% ethanol solution as suggested by Sherlock (2012) after gastric emptying had occurred and stored this way until identification.

Gas flux measurements

Gas flux measurements were taken to evaluate the overall net microbial activity of soils. Measurements were collected using a PP Systems- EGM-5 Portable CO_2 analyser. This system is a variation of the 'chamber method' in which a chamber that covers a surface area of 78.5 cm is placed on the soil and produces an airtight seal, air is then pumped through the system and the difference in CO_2 concentration between the inflowing and outflowing air is measured. Measurements (n=3) were collected at each pit over 60-s with an initial 15-s purge time and a 12-s equilibration time. CO_2 efflux measurements were taken in triplicate, forming a triangle 1 m around the soil hand sorting pit.

Soil sample collection and analysis

Soil samples were collected from the hand sorted monoliths, ~ 3kg subsamples were collected from each soil pit. Soil samples were stored in polyethylene bags for transport. The pH and electrical conductivity of samples were determined using a Jenway 3510 tabletop pH meter and handheld Hannah instruments EC meter in supernatant solutions from 1:5 soil:water suspensions. Soil samples were dried in an oven at 105 ^{0}C for 24 hours to determine the water content. Organic matter content was determined by loss on ignition at 550 ^{0}C over 4 hours. Soil particle size distribution was determined by analysis by Coulter LS230 particle size analyser following suspension and dispersion in calgon solution. This was conducted on soil samples which had undergone the previously described drying and loss on ignition steps. Due to the preliminary nature of the visits to site 2 and 3 soil samples were not collected.

4.2.3. Earthworm identification and data analysis

Earthworms were identified to species level using a light microscope (4x-10x magnification, dissecting microscope) and an identification key (Sherlock, 2012). Species richness was calculated using the Menhinick's index (equation 4.1).

$$R = \frac{s}{\sqrt{N}} \qquad (Eq. 4.1)$$

Where R is the species richness, s is the number of species and N is the total number of earthworms.

The diversity of earthworm populations was determined using the Simpson’s diversity index, a method developed for the description of species diversity rather than simple abundance or richness. It yields a probability (between zero and 1) that two individuals drawn randomly from the population will be of different species (Simpson, 1949). It thus indicates the evenness or spread (or dominance) of species within the population. The Simpsons diversity index was calculated using equation 4.2. Which expresses species richness considering the overall sample size (thus facilitating comparison between sites with differing total numbers of earthworms collected).

$$D = 1 - \left(\frac{\Sigma\, \mathrm{n}(\mathrm{n}-1)}{N(N-1)}\right) \qquad (Eq. 4.2)$$

Where D is the diversity index, n is the number of individuals of a species and N is the total number of earthworms.

Both of these indices were calculated for each individual pit before being averaged to provide the indices for the treatment area. The same was done for the data from octet sampling points.

4.2.4. Data analysis

Data processing was conducted in Microsoft excel and data was analysed using t-tests and ANOVAs in SPSS following appropriate checks for adherence to normality (Kolmogorov-Smirnov) and associated underlying assumptions (e.g. Leven's tests). Juvenile count data from site 1 was Log 10 transformed to achieve normality. Where assumptions were not satisfied after transformation Mann-Whitney tests were applied in place of parametric tests.

4.3. Results

4.3.1. Soil

The soils from both sections of site 1 were characterised as a silty clay loam (clay 14.7-15.6 %, silt 70.2-84.4 %, sand 0-15.1 %) while other properties are reported in Table 4-1. The pH of soils increased between March 2019 and August 2019 in the treated and untreated sections of the field site (from 5.57 ± 0.18 and 5.3 ± 0.05 to 7.82 ± 0.13 and 6.76 ± 0.10 respectively) due to liming of the field between these visits (350kg/hectare of Calcifert lime prills at the end of May/early June). However, the EC decreased (from 187 ± 13 and 456 ± 16 to 75 ± 6 and 13 ± 3 respectively). The WTR-treated area of the site had a higher water content at 77.5 and 54.6 compared to 35.4 and 37.3 in March 2019 and August 2019 respectively. The treated soil also had a higher organic content of 28.34 % compared to 11.43 %. Site 3's soil properties can be seen in table 4-2, both fields were found to be sandy clays, similar organic matter (~12% for both), but the treated area had a higher pH (7.05 vs 5.79). Soils at site 2 were not analysed but the soils in site 2 are similar to those of site 1 (based upon the BGS mySoil app; available at https://www.bgs.ac.uk/technologies/apps/mysoil-app/).

Table 4-1. The physical properties of soils at site 1 that have been treated with WTRs and untreated soils from the same field. Measurements were not taken in May-19.

Season	Soil	Water content (%)	pH†	EC (µS/cm)	Average organic matter % (w/w)
Mar-19	Treated	35.4 ± 3.1 *	5.57 ± 0.4†	187 ± 9†	28.34 ± 2.6*
	Untreated	77.5 ± 2.5†*	5.3 ± 0.3†	456 ± 12†	11.43 ± 1.0*
Aug-19	Treated	37.3 ± 1.6*	7.82 ± 0.3†*	75 ± 6†	-
	Untreated	54.6 ± 2.3†*	6.76 ± 0.2†*	13 ± 1†	-

† *The pH differed between sampling times because of a lime treatment carried out by the farmer.* * indicates a significant difference between treated and non-treated areas.

Table 4-2. The physical properties of soils at site 3, collected in June 2018.

Sample	pH	Organic matter (%)
Farm treated	7.05 ± 0.0*	12.09 ± 0.07
Farm untreated	5.79 ± 0.01*	12.05 ± 0.26

* Indicates a significant difference.

4.3.2. Earthworm data

No significant differences between treated and untreated areas using either sampling method were observed at site 1 Figure 4-4. Based on the results of independent sample t-tests within sampling seasons the number of adult and juvenile earthworms collected via octet and hand sorting site did not significantly differ when comparing the untreated versus treated areas of the field ($p>0.05$). Furthermore, repeated measures ANOVA analysis of the data from all of the seasons found that season was the only controlling variable that significantly effected adult and/or juvenile counts ($p < 0.001$ for both). There were not any significant differences in earthworm abundances at site 2 either (fig. 4). However, there were significantly more juveniles at site 3 in the untreated section of the field (Mann-Whitney p=0.02, median of untreated=19.0; treated=0.0) (Figure 4-5). While the data suggest some potential differences in other aspects if the means are compared (Figure 4-4 and Figure 4-5), these were found to not be statistically significant due to the high variability of samples.

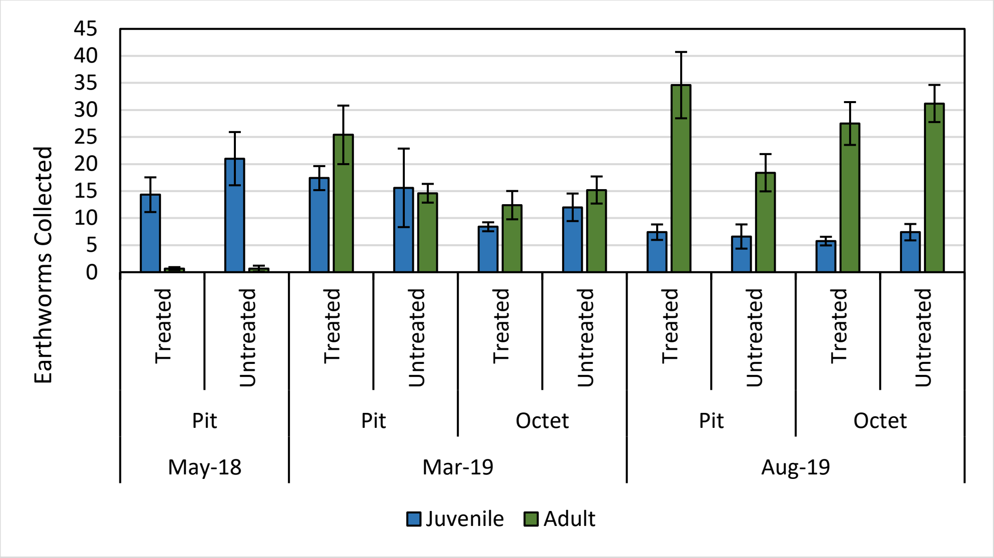

Figure 4-4. Average quantity of adult and juvenile earthworms in each pit at site 1. Error bars show one standard error.

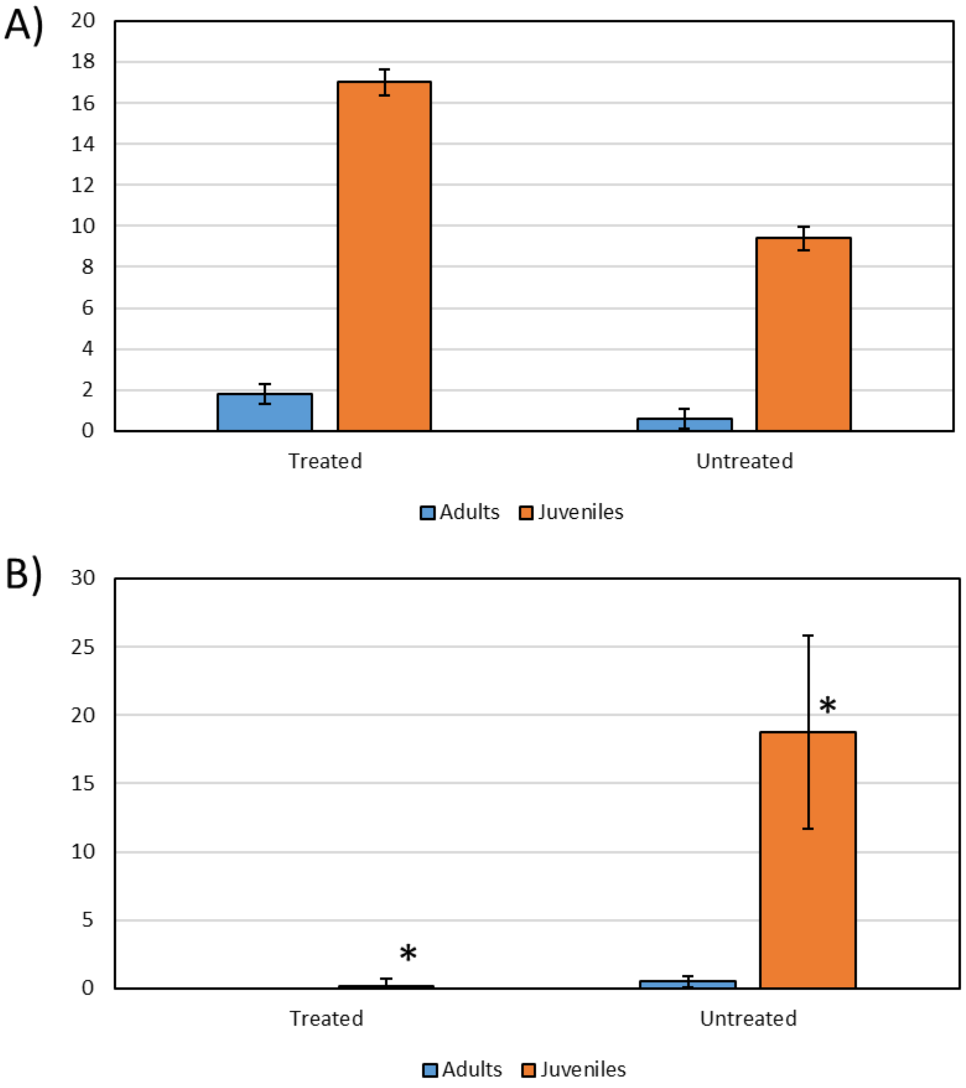

*Figure 4-5. Quantity of adult and juvenile earthworms collected from pits at site 2 (A) and site 3 (B). Errors bars show one standard error. Low treatment at site 3 is due to potential runoff from an adjacent field. Symbol * indicates significant differences*

Species and ecological groups

Seventeen species of earthworms were found at site 1, spanning all three broad ecological subgroups (i.e. anecic, epigeic and endogeic; Bouché (1972)) (Figure 4-6, Figure 4-7 and Figure 4-8). The most commonly occurring species were *Allolobophora Chloritica* with 143 individuals identified, followed by *Aporrectodea Caliginosa* and *Lumbricus Festivus* with 87 and 35 identified individuals respectively. Of the ecological subgroups, endogeic earthworms were most abundant followed by epigeics, with anecic species having the lowest abundance. *Octolasion Lacteum* was only present in untreated samples (20 found in total; Figure 4-6). Two species were found at site 2, *Aporrectodea Rosea* and *A. Icteria*, both of which are endogeic (Figure 4-7). At site 3 there were also two endogeic species collected, *L. Rubellus* and *A. Rosea* (Figure 4-8).

Looking at mean numbers observed at site 1, pit samples in treated areas yielded more adult earthworms than pits in untreated areas, while the octet method produced the opposite result (i.e. more adults in the untreated areas) (Figure 4-6); however, none of these differences were statistically significant (t-test $p > 0.05$). There were no significant differences in ecological subgroups abundance between octet and hand sorting samples within any of the sampling periods when comparing the overall earthworms collected in untreated and treated areas (Figure 4-9).

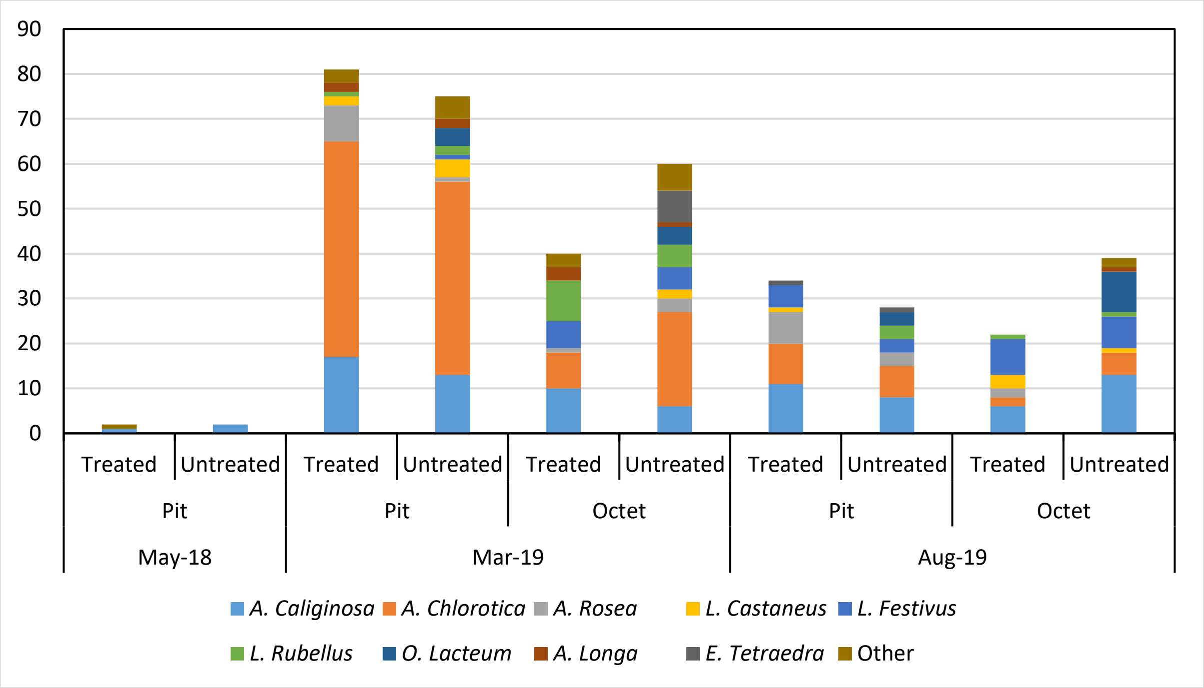

Figure 4-6. Total species breakdown of adult earthworms collected from WTR treated and untreated farm soils at site 1.

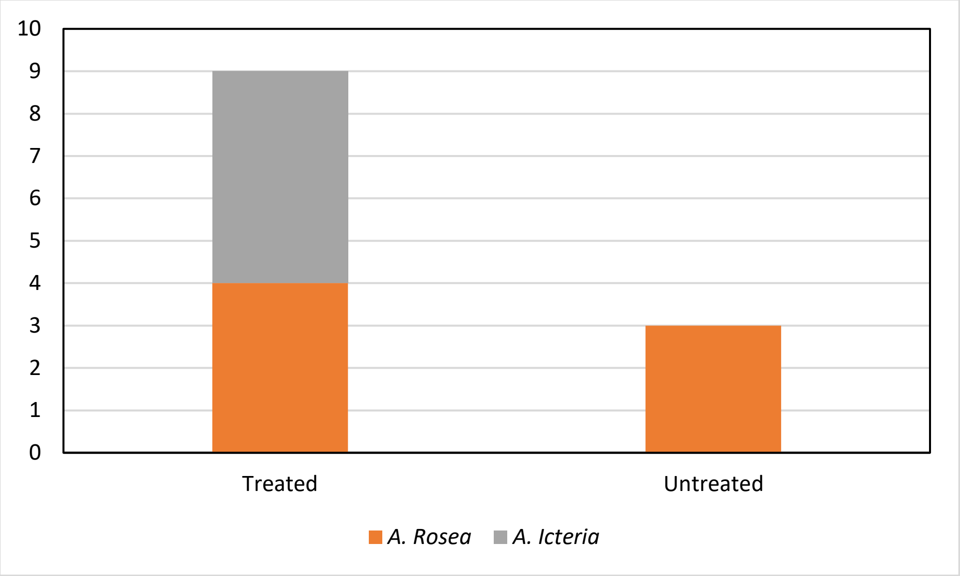

Figure 4-7. Total species breakdown of adult earthworms collected from WTR treated and untreated soils at site 2.

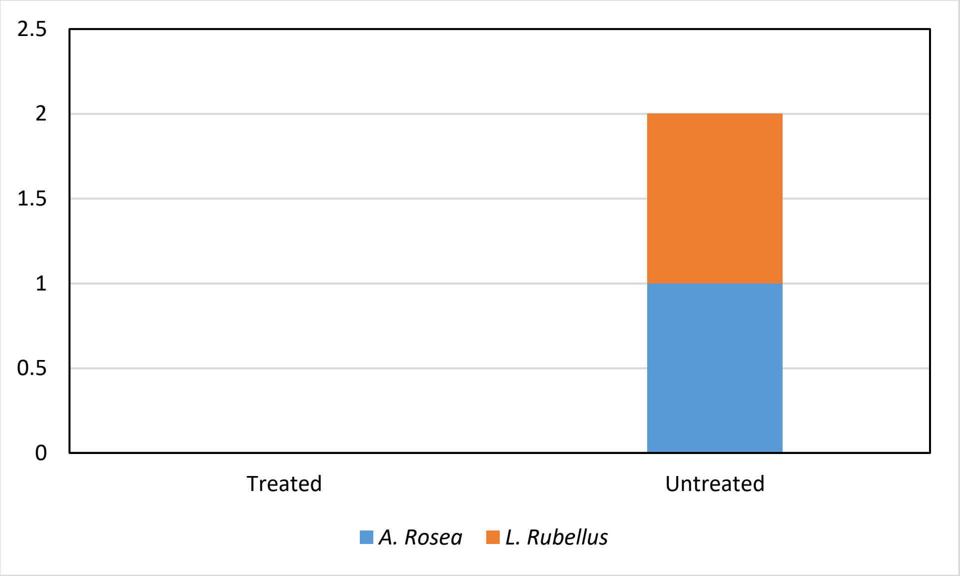

Figure 4-8. Total species breakdown of adult earthworms collected from WTR treated and untreated soils at site 3.

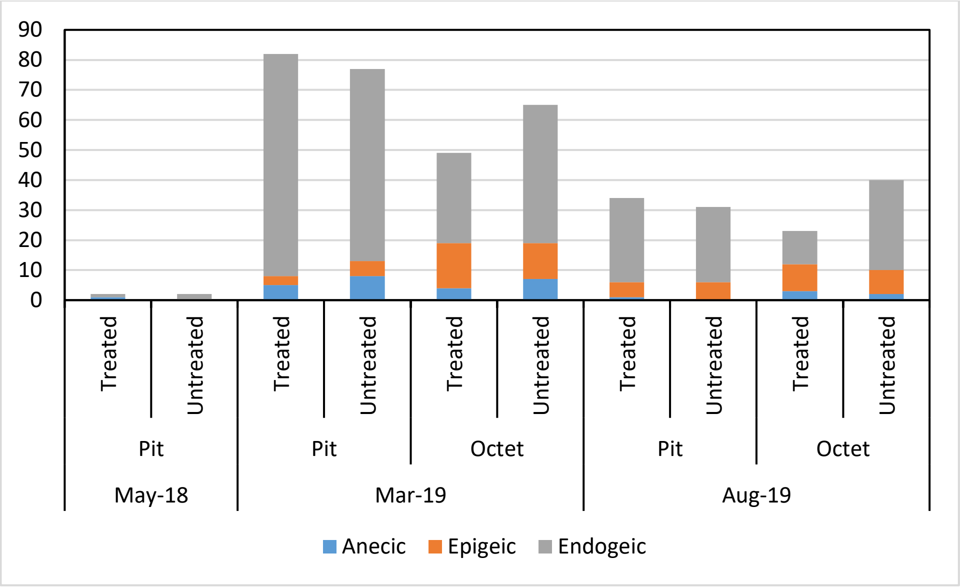

Figure 4-9. A breakdown of the earthworms collected from WTR treated and untreated farm soils into their ecological subgroupings.

Species richness and diversity

Of the 17 species found at site 1, only *A. Caliginosa* was found on all three sampling occasions. Interestingly, only 12 species were found in both sampling occasions in 2019 when additional electrical octet sampling was conducted. Due to their only being 1-2 adult species identified in each pit sample at sites 2 and 3 their Simpson’s index and Menhinick's index values were not truly representative due to the method of calculation. At site 1 the Menhinick's index of untreated and treated soils did not differ significantly when using either sampling method (t-test > 0.05, and Mann-Whitney Test > 0.05 for octet samples week 3), neither did the Menhinick's index when comparing different sampling methods or seasonal values (Figure 4-10). Similarly, the Simpson’s index results did not have any significant differences between treated and untreated areas when using either sampling method, or between samples collected in different seasons, or using different sampling methods (Figure 4-11). Repeated measures ANOVA conducted on the whole data set and it was found that none of the controlling variables significantly affected the Menhinick or Simpson’s index (p > 0.05).

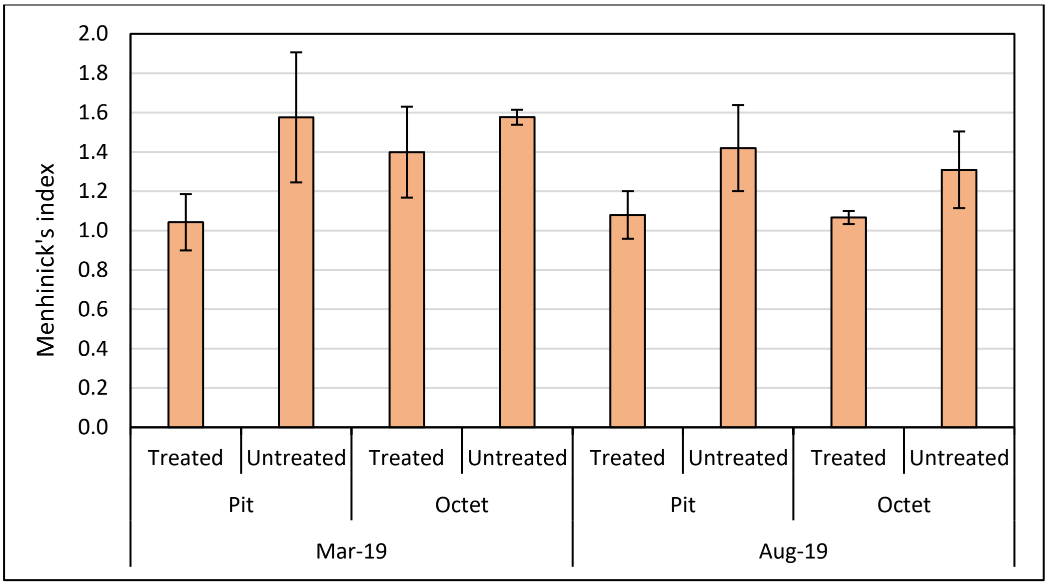

Figure 4-10. Species richness determined by the Menhinick's index for WTR treated and untreated farm soils at site 1, error bars indicate one standard error.

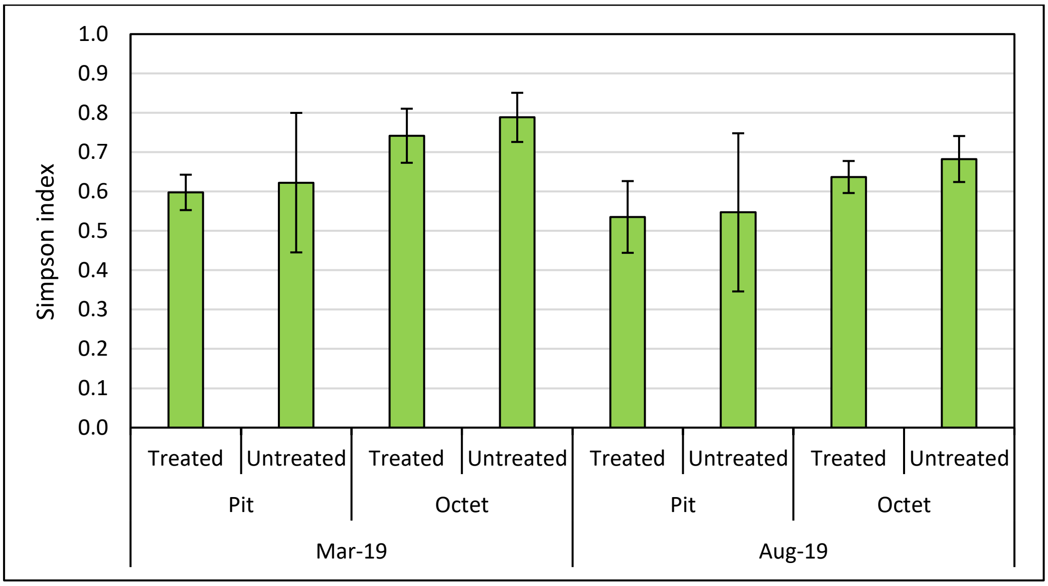

Figure 4-11. Simpson's index by season for WTR treated and untreated farm soils at site 1, error bars indicate one standard error.

4.3.3. CO_2 flux

T-test results show that gas flux in the untreated and treated sections of field site 1 did not differ in March nor August ($p>0.05$) (Figure 4-12). CO_2 efflux at the site did not vary significantly between seasons either ($p > 0.05$).

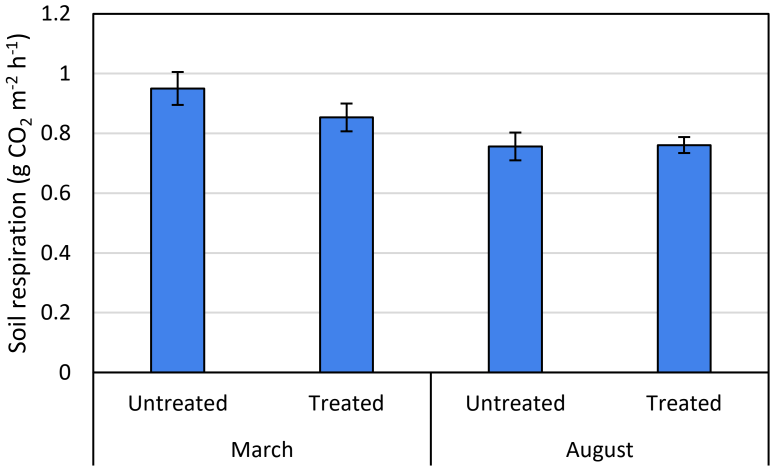

Figure 4-12. The CO_2 efflux of WTR treated and untreated farm soils at site 1, error bars indicate one standard error.

4.4. Discussion

The higher organic content in the treated area of site 1 is to be expected due to the high organic content of WTRs, which is often highlighted as a benefit of WTR land application (Turner *et al.*, 2019). The fact that the microbial activity did not differ significantly (as measured by the gas flux differences) is evidence that WTR additions are not having a negative (or positive) impact on overall microbial activity within the soils at the site. The higher water content of untreated areas was more likely due to topography rather than WTR application; the untreated area of the field site has a slightly lower elevation to the treated area and the untreated side of the field borders a river. Based on the conceptual model in figure 2-16 an increase in soil organic matter was expected to lead to an increase in the CO_2 efflux from the soil as it would increase microbial activity by providing more utilisable substrate, however this trend was not seen when comparing the treated and untreated areas of the site. Furthermore, as temperature plays a key role in microbial activity and CO_2 flux (Fang and Moncrieff, 2001) so it is surprising that CO_2 efflux results did not differ between the sampling seasons. The temperatures at the times of sampling were highs of 12°C in March 2019 and highs of 21°C in August 2019 (Thorsen 2021). While soil temperature was not measured it can be assumed that this increased air temperature would lead to increased soil temperature. Soil pH and moisture have been shown to influence CO_2

efflux and may have played a role in limiting the effects of temperature change between sampling seasons (Reth et al., 2005). Soil moisture only differed in the untreated area during sampling periods (77.5% vs 54.6% in March and August), whereas pH increased significantly during August sampling in both treated and untreated areas (5.57 and 5.3 vs 7.82 and 6.76 in treated and untreated areas during March and August, respectively) likely due to lime additions in June 2018 (table 4-1). Furthermore, liming of acidic soils can released CO2 (Goulding, 2016), however Lochon et al. (2019) found that application of lime (Calcimer T400 at a rate of 1200 kg ha^{-1}) did not lead to any change in chamber measured soil respiration in an French grassland system with similar initial pH (~pH 5). Therefore, with so many variables at play, it is difficult to determine which factors may have cause CO_2 efflux to remain similar between sampling seasons.

The only significant difference found between untreated and treated sections of a site/field was between the numbers of juveniles at site 3, however this is most likely attributable to the differing land usage (i.e. pasture in untreated area, arable crops in the treated area) highlighting a limitation of using this site for a control-impacted experiment design. For example, Haynes *et al.* (2003) found the number, and biomass, of earthworms in native grassland pasture (*Pennisetum clandestinum*) and annual ryegrass (*Lolium multiflorum*) enhanced pasture were higher than under a variety of arable crops (*Zea mays* and *Saccharum spp.*). The different tillage of the arable land could also be a contributing factor, Briones and Schmidt (2017) conducted a global meta-analysis of available data and concluded that conventional tillage decreases the abundance and biomass of earthworms and alters their community structure.

There are 27-29 earthworm species found in the UK and Ireland (Natural-England, 2014) , and typically earthworm communities normally consist of 8-12 species under favourable conditions (Lavelle *et al.*, 2004). The number of species found at site 1 in this study (i.e. 17) higher than this when considering the latter two visits to this site, and are therefore indicative of normal, healthy earthworm communities. Additionally, *A. Chloritica* and *A. Caliginosa*, the two most commonly occurring earthworms in site 1 sampling, are the two most abundant earthworm species in the UK, therefore their high abundance is in keeping with this (Jones and Eggleton, 2014).

The results make for interesting comparison with studies in which other waste or recycled materials have been land applied. For example, Nicholson *et al.* (2018) explored the long term (20 years) effects of field biosolids applications (approximately 3 tonnes dry solids [tds] ha^{-1} y^{-1}) on earthworm population size and biomass, as measured by a hot mustard vermifuge application method. None of the biosolids treatments negatively impacted earthworm populations compared to an inorganic fertilizer-only control treatment, while treatment with a low metal content biosolid approximately doubled earthworm numbers and biomass relative to the control. Earthworms were not identified in that study so community structure comparison cannot be made to the current study. In another study, Whalen and Benslim (2021) explored the impacts of applying biochar to agricultural land over 5 months in Bromptonville, Quebec, Canada. Three types of biochar (two from different hardwood feedstocks and one from softwood feedstock) were applied at two application rates (5 and 10 t ha^{-1} biochar). Only three species of earthworm were identified in all plots; *Aporrectodea turgida*, *Aporrectodea tuberculata*, and *Lumbricus rubellus*, however this is typical for agroecosystems in North America (Whalen and Benslim, 2021). There were no effects on earthworm populations by the end of the study under biochar application or biochar+dairy cattle slurry application (the latter being an additional treatment in separate plots). Therefore, as the application rate of WTRs at site 1 is higher than those used in Nicholson *et al.* (2018) and Whalen and Benslim (2021), it can be inferred that the impacts of WTR on earthworm ecology are negligible and so their use is comparable to those of biochar and biosolids in that regard (i.e. no observable negative impacts on earthworms at typical agricultural application rates).

The overall number of adult earthworms and species at sites 2 and 3 were far below the numbers considered typical (Lavelle *et al.*, 2004). This low number of adult earthworms collected during the preliminary visit to site 1 (May 2018) and at site 2 and 3 can be attributed to the unusually high temperatures for the time of year (Highs of 22 °C at site 1&2, and 26 °C at site 3 (Thorsen, 2021)). This led to low soil moisture (this was unmeasured but based on anecdotal evidence). Earthworm populations are known to show seasonal variation in density and biomass (Lavelle, 1983). This is in part due to the adverse effect of overly dry soils, with many species favouring soils of 14-25% moisture content (Lowe and Butt, 2005), furthermore they are also known to migrate deeper into the soil column during dry, and cold or hot temperature extremes (Reddy and Pasha, 1993). Due to the sampling depth limitations of the highly clastic deeper soil horizons these deeper migrating earthworms may not have been picked up in this study. While there was still a consistent quantity of juveniles, this can be attributed to juveniles generally living closer to the soil surface (Gerard, 1967; Rundgren, 1975) and the lower burrowing force of smaller earthworm individuals (Quillin, 2000) which would make the deeper clastic soils harder to penetrate. Earthworms can remain burrowed for extended periods through a process known as aestivation, during which earthworms tie themselves into a knot, form a protective mucus barrier, and slow their metabolic rate. This is in line with a study by Reddy and Pasha (1993) which conducted a monthly study of earthworm population in grassland and found that while there were often no (or very few) adult earthworms (*Octochaetona phillotti and Barogaster annandalei*) collected via hand sorting in certain months of the year, juvenile and pre-adult populations remained present in all but one monthly sample (July 1985 - July 1987).

4.4.1. Method comparison

As both hand sorting and electrical octet extraction were conducted, the effectiveness of these extraction methods can be compared. The lack of significant differences between the quantity of earthworms extracted and ecological subgroups abundance produced from octet and hand sorting samples found in this study is in agreement with some past studies. For example, Schmidt (2001) established that the electrical octet method could extract earthworm communities which were similar in size and species composition to that of hand sorting, aside from in recently ploughed land. Similarly, Weyers *et al.* (2008) found that electrical extraction expelled approximately 90% of the number of earthworms that hand sorting collected.

The main restriction of the octet method is weather conditions. For example, during the August sampling session only four octet measurements could be collected in the treated area, as the use of the electrical method is limited during rainfall unless a mobile shelter can be erected. Nevertheless, the non-intrusiveness of the method is a great benefit for sites where the operator does not want (or is not permitted) to disturb the soil.

4.5. Conclusions

The relatively low number of adult earthworms found at site 2 and 3 and during the first sampling period at site 1 compared to the number of juveniles is indicative of deeper burrowing of adult earthworms due to hot and dry weather conditions at the time of sampling.

Earthworm populations found at site 1 were indicative of normal and healthy earthworm communities; seventeen species of earthworms were present spanning all three of the ecological subgroups but endogeic earthworms were most abundant with dominant species, similar to the rest of the UK (*A. chloritica* and *A. caliginosa*). Earthworm abundance remained similar between untreated and treated sections of all three sites, suggesting that overall earthworm populations are not affected by WTR application to soils at rates typically used. However, while this study explored three sites with control and treated soils, sampling at site 1 was limited by Covid-19 restrictions and the robustness of results from site 2 and 3 was limited by either land usage or topography. Furthermore, there were no detectable influences of WTR application on soil respiration at the field site, which is suggestive of no impact on the overall net activity of the soil microbiome. The results from comparing both earthworm collection methods used in this study align with those of past publications in the fact that hand sorting and electrical octet methods produced similar quantities of earthworms of all ecological groups.

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Chapter 5 - Determination of the leachable fraction of elements in water treatment residuals through batch extraction and column leaching

Abstract:

Water treatment residuals are a waste product produced during the treatment of drinking water that are often applied to agricultural land. WTRs vary physically and chemically depending on water source, changes in weather, and the coagulant used during their formation. Samples of 17 different WTRs from water treatment plants in Wales, England and Scotland were collected and subjected to leaching tests with 0.01M $CaCl_2$ in a batch extraction study (2 g solid: 20 g solution). Samples of soil from a previous WTR treated vs untreated farm soil experiment were also subjected to the batch extraction procedure for comparison. Extract solutions were analysed for element contents via ICP-OES. Separately, a column leaching experiment was also conducted using one of the WTRs, chosen as it was representative (middle range of numerous parameters) of most of the WTRs from the batch extractions, and a previously well characterised soil (Kettering loam). The column leaching procedure examined WTRs applied in two separate ways; incorporated into soil (simulating ploughing in) and surface applied. The WTR application levels were at 0, 5 and 10% w/w of total soil+WTR mass and the leaching solution employed was 0.001M $CaCl_2$. The column leachates were analysed for element content via ICP-OES and also assessed for total organic carbon content to determine any C release from treated soils. Batch extraction experiments highlighted a high variability in the mobility (leachability) of some elements across WTRs from different sources. For example, the concentration of Fe in leachates from a majority of samples were below detection limits whilst two samples leached over 239

mg kg^{-1} of WTR (both Fe-salt based WTRs from plants that treated raw water from reservoirs). Column leachate Fe, Co and Mn concentrations were significantly lower in the 5% incorporated treatments when compared to the control, and Fe was also significantly lower in the 10% treatment when compared to the control. When comparing incorporated and surface applied columns it was found that the 5% incorporated treatment leached significantly less Cr, Co and Ni than the surface applied columns relative to the control (0.7139, 0.6201, 0.7909 vs 1.02108, 1.01435, 1.02583 µg kg^{-1} respectively). Total organic carbon in column leachate was significantly reduced by incorporation of 5 and 10% WTRs (w/w), but not by surface applications of the same rate. This study highlights the importance incorporation of WTRs into the soil column via methods such as ploughing.

5.1. Introduction

Water treatment residuals (WTRs) are produced as a by-product of the drinking water clarification process (see chapter 2). They can vary highly, both chemically and physically, depending on multiple factors. The most major of these factors are the source raw water, the coagulant used, and the employed dewatering process. The most common components of WTRs are Al and Fe hydroxides, however the concentrations of major elements, organic matter content and pH of dried WTRs vary highly (Table 2-2). Land spreading of water treatment residuals is commonplace as it reduces the associated disposal costs and is considered to be environmentally beneficial to agriculture. The impacts of land application of WTRs to were discussed in section 2.5.4.. Past studies have reported increased soil aeration, saturated hydraulic conductivity, N content and cation–exchange capacity following WTR application (Bugbee and Frink (1985), Geertsema *et al.* (1994), Ahmed *et al.* (1998), Park *et al* (2010)). Furthermore, WTRs application can change soil and porewater chemistry through their sorption and release of elements, for example Elkhatib and Moharem (2015) found that Cu, Pb and Ni in extractant from calcerous soil was decreased after WTR application. It is generally acknowledged that the largest concern when applying WTRs to land is the possibility of Al leaching (Babatunde and Zhao, 2007; Turner *et al.*, 2019). In the UK, the issue of potential leaching of Al and Fe has been addressed in regulations by limiting application of Al WTRs to only those soils with pH above 6 and Fe WTRs to soils above a pH of 5, due to their increased mobility below these points (Environment Agency, 2013).

Multiple past studies have explored the extractability and leachability of elements and ions from WTRs (Table 2-3). Elliott *et al.* (1990) conducted sequential extractions (modified Tessier *et al.* (1979) sequential extraction procedure) of eight $FeCl_3$ and alum based WTRs from Pennsylvania, U.S.A. They found that most of the measured metals were present in a weakly mobile, non-leachable form and therefore not of environmental concern. Similar findings were produced from the chemical analysis of WTRs in China where the six collected WTRs were found to be able to pass the US Environmental Protection Agency's (USEPA) Toxicity Characteristic Leaching Procedure (TCLP) (Wang *et al.*, 2014). In a recent study, Howells *et al.* (2018) conducted sequential extraction (Community Bureau of Reference sequential extraction scheme) of WTRs alongside 0.001 M $CaCl_2$ extraction, and found that the leachable fraction of elements from WTRs was very low, however these amounts could be increased if the pH of samples was forced low enough (i.e. up to 382 mg/kg of Al from the Al-WTRs at pH 4.4). However, while these studies exist, they each cover only a handful of WTRs from specific locations and treatments and thus cannot fully capture or reveal the variability possible in leachable components of WTRs from different locations. More studies are therefore needed to further examine leachable components of WTRs and to assess their environmental implications, if any. When considering the conceptual framework in figure 2-16 it is expected that the WTRs addition to column studies effects will vary between elements as existing literature has suggested the sorption and release mechanics of WTRs vary as discussed in section 2.5.1 and 2.5.4. This effects on soil organic carbon retention are of particular interest as it is unclear whether WTRs will retain carbon or release it under the conditions present in a leaching experiment.

In the current study, a series of batch extraction and column leaching experiments were conducted with the following main aims:

1. To assess the mobility of elements in 17 WTRs from around the UK via a batch extraction approach using 0.01M $CaCl_2$.
2. To similarly compare the mobility of elements in one WTR treated pasture soil and a corresponding non-treated control arable soil from a farm site in Wales, while considering the potential limitations of using soils with differing land usage.

3. To determine, via a column leaching procedure, the mobility of elements in soils treated with WTRs at various treatment rates via either surface application or surface application followed by incorporation into the top soil layers.
4. To determine how WTR treatments influence carbon export from soils in a column study.

5.2. Methodology

5.2.1. Materials for batch extraction

Water treatment residuals were sourced from water treatment plants in England, Wales and Scotland (some were collected by the author, while others were kindly supplied directly from the plant operators or via a waste recycling firm, 4R, which handles the material on behalf of the plants). A total of 17 WTR samples were collected (Table 5-1). Additionally, farm soils (sandy clay; pH 7.05 and 5.79 for treated and untreated soils respectively) were collected from two adjacent fields from a farm in South Wales. One field had WTRs (WTR 2 in Table 5-1) applied for many years (most recently 135 t ha^{-1} in 2015 and 92 t ha^{-1} in 2016), whilst the second has had little or only incidental WTR application (i.e. it is a control soil). All samples were dried at 105 ^{o}C for 24 hours, ground using a pestle a mortar and sieved to 2 mm or 4 mm, for WTRs and soils respectively, in order to homogenise them. $CaCl_2$ solutions were made using analytical grade $CaCl_2·2H2O$.

Table 5-1. Sample selection for batch extraction experiments.

Sample	Sample media	Coagulant base	Water source	Country of origin
Treated farm soil	Soil	n/a	n/a	Wales
Untreated farm soil		n/a	n/a	Wales
WTR 1	WTR	Al	Reservoir	Wales
WTR 2		Fe	Reservoir	Wales
WTR 3		Fe	-*	England
WTR 4		Fe	Reservoir	Wales
WTR 5		Al	Reservoir	Wales
WTR 6		Al	-*	England

WTR 7		Fe	Reservoir	Wales
WTR 8		Fe	Reservoir	Wales
WTR 9		Al	River/reservoir	Wales
WTR 10		Al	Reservoir	Wales
WTR 11		Al	Reservoir	Wales
WTR 12		Fe	River	England
WTR 13		Al	River	Wales
WTR 14		Fe	Reservoir	Wales
WTR 15		Al	Reservoir	Scotland
WTR 16		Al	Reservoir	Scotland
WTR 17		Fe	Reservoir	Wales

n/a = not applicable; * Information unavailable

5.2.2. Batch extraction method

Batch extraction experiments were conducted using 0.01 M $CaCl_2$ as a leachate, following an approach employed previously with soils (Houba *et al.*, 2000). An aliquot of 20 ml of $CaCl_2$ (pH ~3.3) was added to 2 g of each soil/WTR sample. Samples were then mixed for 2 hours in an end over end shaker at 35 rpm. Following this, the samples were centrifuged for 15 mins at 855 RCF (Relative Centrifugal Force) to separate the liquid and solid fractions. From this sample, 13 ml of this supernatant liquid was then removed via syringe and filtered to 0.45 µm using a polyethersulfone filter. Samples were acidified with 0.1 ml of high purity concentrated HNO_3 and stored at 4 ^{0}C until further analysis.

5.2.3. Column leaching

Column leaching experiment adopted a methodology that was modified from the OECD Leaching in Soil Columns guidelines. The chosen soil for this study was Kettering loam (Kettering, Northamptonshire, UK, supplied by Boughton Ltd, *www.boughton.co.uk*), which has been used in previous studies (Butt, 2002; Rajapaksha *et al.*, 2014; Brami *et al.*, 2017). The WTR material used for the column leaching study was that designated '7' in Table 3, which was collected from the storage lagoon of a site in Wales and stored in a 20 l polypropylene tub until being dried, ground and homogenised for use in the leaching experiment. This WTR was chosen as it was representative of a majority of the samples in this study, i.e. a reservoir sourced sample from

Wales, and was not one of the outlier samples found to contain a large leachable fraction of Fe (see section 5.3.1.). It was planned to conduct followup column leachings with a similar Al WTR, however lab access restrictions stopped this experiment being conducted.

Column leaching apparatus

Columns were produced from 200 mm lengths of PVC guttering pipe with a diameter of 68 mm. The base of the pipes was covered with a fine polyester mesh supported by a 1.5 mm mesh made of PVC plastic-coated nylon, both of which were taped in place. These were held in place by PVC guttering brackets attached to a length of wood which in turn was held up by clamps attached to retort stands. Whatman number 5 filter paper was placed at the base and on top of the soil column to allow for even distribution of water during leaching (OECD, 2004).

Column fill

Two column experiments were conducted, one with surface applied WTRs, and the second with incorporated WTRs (the latter simulating WTRs having been ploughed into soil; Figure 5-1; Table 5-2). Each column was filled with 423 g of substrate. Initially columns were filled with 423, 401.85 or 380.7 g of Kettering loam (for controls, 5% treatments and 10% treatments respectively) and then wetted to their water holding capacity (previously determined as ~60 mL/ 100 g). WTRs were then added to columns at 0, 21.15 and 42.3 g in a wet state to create the control, 5% treatment and 10% treatment columns respectively (n=3 per treatment). In the incorporated study WTRs were then mixed into the soil inside the column using a stainless-steel laboratory spoon. The resulting bulk density of the wet column substrate was ~1.24 g cm^{-3}. These amendments are equivalent to 120-240 t ha^{-1} (considering the established bulk density and a column/soil depth of 20 cm). For context, in England and Wales the application of WTRs is limited to 250 t ha^{-1} (wet mass) per annum (DEFRA, 2011; Environment Agency, 2013).

Table 5-2. Column treatments (n=3)

Sample	Application rate (% w/w)	Equivalent application rate (t ha^{-1})*
Kettering loam (control)	0	0
Incorporated WTR 5%	5	120
Incorporated WTR 10%	10	240
Surface WTR 5%	5	120
Surface WTR 10%	10	240

*Calculated based upon a soil profile with a density 1.2 g cm^{-3} and depth of 20 cm.

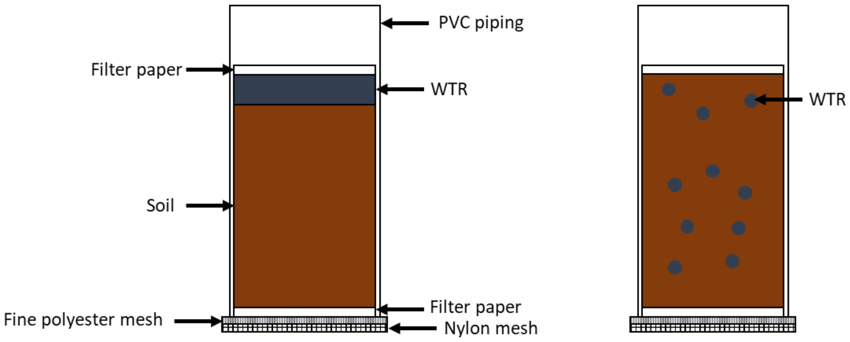

Figure 5-1. A diagram of the column setup used in this study, the left column shows the experimental set up in which WTRs were applied as liquid to the soil surface to simulate surface applications, while the right column illustrates the experimental set up in which WTRs were first mixed through the soil to simulate ploughing in. Column height was 200 mm and diameter 68 mm.

Leaching procedure

The combined substrates were first leached on the day they were combined. A 200 mm equivalent of rainfall is suggested by the OECD in their Leaching in Soil Columns guidelines (OECD, 2004), this equates to 407 ml based on the diameter of the column used in this experiment. This equates to approximately 1.5 times the water holding capacity of the Kettering loam (water holding capacity of 0.64 mg/g). Leachings were conducted every 2-3 days (allowing for weekends). The 407 ml of 0.001M $CaCl_2$ (pH ~5.6) leaching solution was all added at once and allowed to accumulate on the surface of the columns. Columns were allowed to drain for 30 minutes which allowed for the columns to completely drain. Leachate was collected in PET containers during leaching. Two types of subsamples were then collected from the PET containers for analysis. Firstly, subsamples for chemical analysis were filtered to 0.45 µm using polyethersulfone filters and stored in 15 ml centrifuge tubes (n=2 per column per leaching). These samples were acidified with 0.1 ml of high purity concentrated HNO_3 and stored at 4 ^{o}C until further analysis. Secondly, 50 ml unfiltered samples were collected for TOC determination; these were frozen after collection. Columns were leached a total of 6 times per experiment over a 2-3 week period.

5.2.4. Total organic carbon measurement

Frozen leachate samples were thawed at 4 ^{o}C for 24 hours. TOC was calculated by analysis of total carbon and inorganic carbon using a Shimadzu TOC-L with a Shimadzu ASI auto sampler. Results were corrected for background atmospheric carbon drift by running a deionised water blank every 9 samples. Data was analysed using t-tests and ANOVAs in SPSS.

5.2.5. Data analysis and management

Chemical data was obtained for a general suite of metal and nutrient analytes (Al, Cr, Cu, Fe, Pb, K, Mg, Mn, Na and Zn) through analysis by ICP-OES (Varian Vista Pro, with an APEX-E sample introduction system) and ICP-MS (Agilent 7500ce) along with certified solution standards at Edinburgh University. Batch extraction results were standardised to the leachable element concentration per kg of solid material. Because the leaching column study was accomplished in two stages (firstly controls and the surface applied WTR columns, then subsequently controls and the incorporated WTR columns) and there was a difference in leachable amounts of ICP-analysed elements from the controls between the two stages, ICP element data from column studies was normalised by setting the control to 1 when comparing the incorporated study to the surface applied study. Data was analysed through the use of t-tests and ANOVAs in SPSS following checks for normal distributions and meeting of other assumptions for parametric tests. Specifically, the batch leachings were statistically evaluated using ANOVAs comparing the amounts leached per treatment type, while the leaching column data were assessed via ANOVAs on the cumulative (total) amount of each element leached across the total period of the leaching study for each treatment type and repeated measure ANOVAs to explore the interaction between treatment and number of leaching events on the leached amount of each element. T-tests were used to compare each treatment (5% and 10%) between the incorporated and surface applied treatments.

5.3. Results

5.3.1. Batch extraction

Batch extraction solutions from WTRs had Pb concentrations below detection limits, hence the extractable Pb was <0.149 µg/kg. Furthermore, a majority of the WTRs tested produced leachate with concentrations of Fe below detection limits. However, a small number of samples leached relatively higher quantities of Fe, i.e. 239±19, 339±11 and 70.9±3.5 mg kg^{-1} from WTRs 4, 8 and 14 respectively (Table 5-3). Concentrations of leached Al remained low (<0.6 mg kg^{-1}) throughout (Table 5-3). Extractable amounts of K, Mg and Mn varied, with some WTRs releasing amounts that may be significant in terms of plant nutrient supply (e.g. WTRs 3, 7 and 16 released >40 mg/kg). Extractable Na also varied, with the maximum amount observed having been 358±16 mg/kg in WTR 7 (Table 5). However, while a statistically significant difference was detectable (t test $p<0.05$), the extractable Na did not differ substantially enough to impact soil ecology between treated and untreated farm soils (i.e. 94.7±1.2vs 84.1±0.69 mg/kg; Table 5). Statistical analysis (T-test) results indicate that treated soils were also significantly enriched in leachable K ($p < 0.01$) compared to untreated soils. Treated soils were significantly depleted in leachable Al, Mg and Mn ($p < 0.01$), indicating that the WTRs were able to retain mobile metals otherwise released from the soil. Differences between Zn and Cr were not significant ($p > 0.05$) for treated vs untreated soils. Measured concentrations of Pb and Fe in leachates from the farm soils were below detection limits in all batch extraction experiments.

Table 5-3. Selected element concentrations in leachates from soils and water treatment residuals, as determined by ICP-OES, errors display standard deviation. N/A indicates samples below the detectable concentration of the instrument.

Sample	Concentration (mg kg^{-1} of dry material)								
	Al	Cr	Cu	Fe	K	Mg	Mn	Na	Zn
Treated farm soil	0.528±0.027	0.0376±0.0031	0.921±0.0083	N/A	27.2±1.0	109±3.1	11.1±0.74	94.7±1.2	23.3±0.11
Untreated farm soil	1.85±0.032	0.0465±0.0014	0.860±0.0053	N/A	17.0±0.16	151±2.7	30.3±1.2	84.1±0.69	23.3±0.093
WTR 1	0.708±0.036	0.0984±0.020	2.54±0.49	N/A	22.6±0.30	19.9±0.48	103±2.5	109±1.1	23.9±1.1
WTR 2	0.389±0.047	0.0319±0.0020	1.96±0.22	N/A	43.6±3.9	62.5±10.0	0.757±0.085	163±11	23.4±0.23
WTR 3	0.531±0.052	0.0276±0.0040	1.20±0.046	N/A	170±10	189±12	0.407±0.040	248±12	23.0±0.089
WTR 4	1.94±0.11	0.0267±0.00069	0.963±0.032	239±19	4.05±0.041	12.3±0.77	10.1±0.70	86.8±1.1	24.0±0.19
WTR 5	0.674±0.042	0.0319±0.0026	1.04±0.047	N/A	28.6±1.1	7.26±0.17	18.4±0.50	141±5.8	23.4±0.16
WTR 6	2.47±0.15	0.0343±0.0031	1.50±0.011	N/A	67.6±2.8	76.1±3.4	0.173±0.013	273±12	23.5±0.11
WTR 7	1.49±0.56	0.0372±0.0086	2.31±0.39	N/A	210±6.4	43.7±2.4	195±7.9	358±16	17.2±0.076
WTR 8	2.12±0.25	N/A	1.48±0.12	339±11	21.8±2.3	11.6±3.3	14.4±3.6	116±6.6	17.9±0.25
WTR 9	1.77±0.41	N/A	0.883±0.18	N/A	55.3±4.2	52.4±7.2	146±2.8	152±12	16.3±0.31
WTR 10	1.92±0.32	0.0297±0.0042	0.371±0.0071	N/A	44.2±0.55	45.9±0.97	460±6.7	172±3.2	16.6±0.20
WTR 11	2.46±0.22	0.0372±0.0045	0.579±0.027	N/A	47.7±2.3	49.5±3.5	478±22	181±9.6	15.9±0.15
WTR 12	1.54±0.00049	N/A	0.601±0.026	N/A	47.9±0.34	112±1.5	-8.80±0.23	169±0.82	16.3±0.26
WTR 13	1.87±0.086	N/A	0.424±0.013	N/A	48.2±2.3	67.5±3.1	38.7±0.78	274±9.5	16.7±0.084
WTR 14	2.08±0.043	N/A	0.316±0.033	70.9±3.5	26.5±0.61	25.2±0.88	13.3±0.76	173±5.2	17.4±0.13
WTR 15	3.40±0.000048	0.00161±0.000000087	0.0334±0.000017	10.9±0.00030	35.1±0.0020	33.2±0.0027	58.9±0.0039	34.5±0.0028	0.352±0.000022
WTR 16	2.66±0.00014	0.00200±0.00000022	0.0470±0.0000026	7.81±0.00015	105±0.018	44.9±0.0052	47.6±0.0019	38.0±0.0043	0.274±0.000022
WTR 17*	1.96	0.00250	0.0470	4.59	187	58.8	35.6	42.7	0.186

** only one sample was analysed of WTR 17 due to sample volume after drying.*

5.3.2. Column leaching

When comparing column leachate elemental concentrations over the course of the study there was a general trend of decreasing concentration over time for each treatment, presumably reflecting removal of a finite quantity of leachable components. Figure 5-2 shows an example of this decreasing trend with Mg in leaching columns, other elements showed similar trends under both treatments). Cd was near or below detection limits in most columns, while Pb was only above detectable limits in one column (5%, soil incorporated WTRs). Cumulative leachate was calculated based on the total concentration leached for each treatment over six leaching sessions corrected to account for the total amount of liquid leached and the mass of substrate in the column. Table 5-4 presents the cumulative amount of leached elements across the 6 leaching events. ANOVA analysis of cumulative leaching data indicated that Fe, Co and Mn were all significantly different in incorporated treatments compared to the control (p, 0.05). Tukey's post-hoc analysis was conducted, and all three elements were significantly lower in the 5% treatments, while Fe was also significantly lower in the 10% treatment (Table 5-4). There were no significant differences present in surface applied columns when compared to the control. Subsequently repeated measures ANOVAs were conducted to explore whether any trends in difference between groups were present through the leaching events. It was found that P, Cr and Cu differed significantly ($p < 0.05$) when considering treatment and time in the incorporated columns, whereas none of the elements showed this trend ($p > 0.05$) in the surface applied columns.

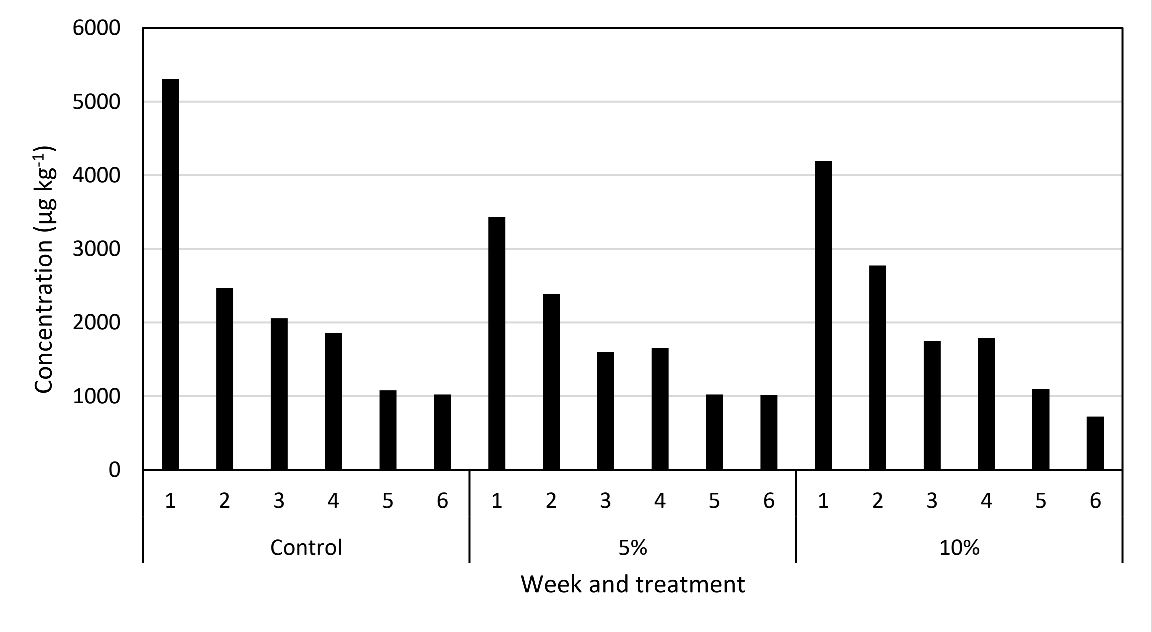

Figure 5-2. The weekly concentration of leached Mg in surface applied columns.

Table 5-4. Cumulative column leachate concentrations after 6 leaching sessions in mg per kg of wet substrate.

Sample		Cumulative leachate after 6 leaching sessions µg kg-1												
		Mg	Al	P	K	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Incorporated	Control	79622.2±418.1	426.9±18.5	12684.2±210.9†	132153.2±3221	15.8±0.4†	2896.2±150.2^{a}	904.9±51.5^{a}	43.2±1.3^{a}	210.7±4.1	528.5±8.6†	138.7±3.7	-	-
	5%	64169.1±338.4	773.4±80.6	9363.8±239.1†	124172.5±1915.4	9.3±0.4*†	1530.7±86.6^{b}	490.8±8.9^{b}	24.4±0.8*^{b}	151.3±3.8*	403±9.4†	147.8±6.3	-	27.9±7.3
	10%	71136±330.2	364.9±16.1	10102.3±315.5†	130292±1741.5	11.1±0.3†	1887±68.5^{ab}	537.6±11.1^{b}	29.8±0.8^{ab}	169.8±4.1	458.8±11.3†	122.5±5.5	-	-
Surface applied	Control	140733.5±144.7	422.2±11.6	17958.1±261.9	171332.6±591.3	37.9±0.2	11799.6±158	3931.1±211.5	116±1.7	352.9±2.9	118.7±2.7	717.9±4.1	1.9±0	1.6±0.1
	5%	151960.1±167.1	436.1±16	18549.9±123.6	179638.1±244.3	39.1±0.6*	12327.7±213.3	2924.7±101.7	115.2±1.7*	359.9±3.8*	140.3±2.5	735.9±3	2±0.1	1.7±0.1
	10%	148376±138.2	592.6±19.8	18092.8±174.7	175218.8±324.5	39±0.4	12964.8±124	4545.4±214.3	123.1±1.5	363.6±2.8	125.2±0.9	687.1±2.2	1.8±0	1.4±0.1

^{ab} indicate significance groupings based upon Tukeys post hoc for control vs 5% WTRs vs 10% WTRs treatments for elements with statistically significant differences (Co, Fe, Mn). *indicates significant difference ($p < 0.05$) between surface applied and incorporated treatments after normalising with regards to the control. †indicates elements that differed significantly ($p < 0.05$) when considering treatment (control, 5, 10%) and the number of leaching events.

Surface applied versus incorporated WTR application

T-tests revealed that all incorporated samples, including controls, leached significantly less Mg, K, Cr, Mn, Fe, Co, Ni, and significantly more Zn than their counterpart (i.e. control vs control, 5% vs 5% and 10% vs 10%) cumulatively after 6 leachings (Table 5-4). Therefore, to enable further comparisons between the application methods, the data set was normalised by setting the untreated control group in each experiment as 1 (Figure 5-3). The normalised cumulative data was compared using t-tests, and it was found that the 5% incorporated treatment leached significantly less Cr, Co and Ni than the surface applied columns relative to the control (0.7139, 0.6201, 0.7909 vs 1.02108, 1.01435, 1.02583 µg kg^{-1} respectively). Whereas there were no other significant differences discovered between the incorporated and surface applied columns in the 5% treatments or any significant differences between the incorporated and surface applied columns in the 10% treatments (Figure 5-3).

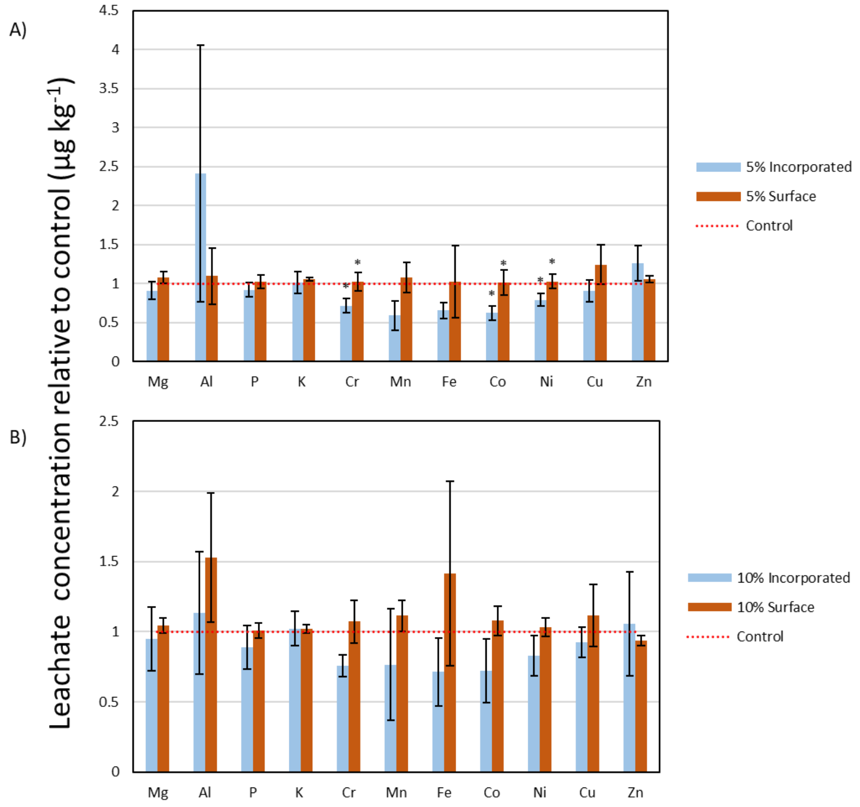

Figure 5-3. Cumulative total leached element (µg kg^{-1}) from columns of Kettering Loam soil containing incorporated or surface applied WTRs at 5 and 10% (w/w), normalised to amounts leached from respective control column. Error bars indicate one standard deviation. Symbol '' indicates significant difference between the treated and untreated ($p<0.05$).*

5.3.3. Total organic carbon

The TOC concentration of leachate samples showed a general trend of decreasing week on week (Figure 5-4). ANOVA analysis of incorporated and surface applied columns compared to their controls showed that when comparing each treatment by week, the 5% and 10 % incorporated treatments leached less TOC than the control in week 3 and the 10% column leached less than the control in week 6 (ANOVA; $p<0.05$) (Figure 5-4). No other significant differences were found between controls and treated columns. Weekly comparisons between the incorporated and surface applied columns of respective treatments (i.e. 5% vs 5% and 10% vs 10%) found significant differences with significantly less TOC leached from incorporated 5% and 10% columns in week 1, week 3, week 4, week 5 and week 6. Furthermore, comparison of the cumulative TOC leached from each treatment after 6 leachings showed that there the cumulative leachate from surface applied treatments did not differ from the control, while the incorporated treatments were both significantly lower than the control (ANOVA; $p<0.05$).

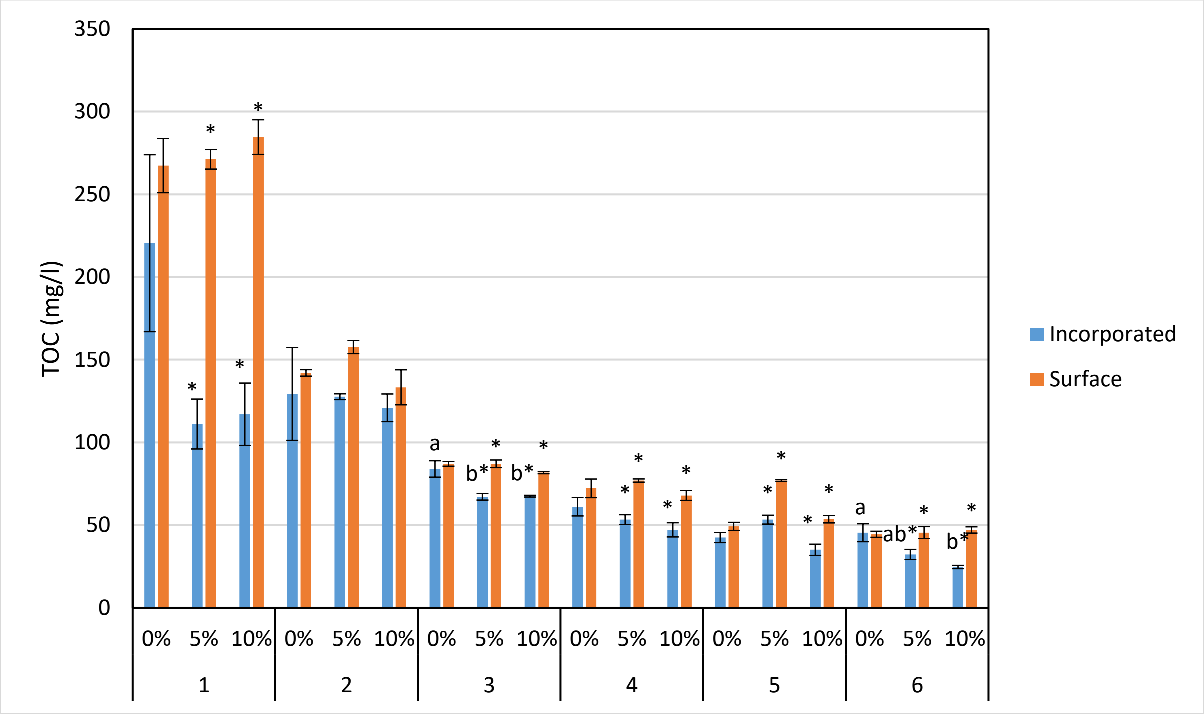

*Figure 5-4. The average (n=3) TOC of columns leached with 0.01 $CaCl_2$ over 6 weeks. Error bars indicate one standard error. Significant differences between incorporated and surface applied treatments within the same treatment rate within a given sample period are indicated by *. Where statistically significant differences were found between controls and treatments (which was only for the incorporated treatments and only on leaching occasions 3 and 6), their post hoc analysis groupings are indicated by 'a', 'b' or 'ab'.*

5.4. Discussion

5.4.1. Batch extraction

The low leachate Al concentrations observed during batch extraction experiments from such a large variety of WTRs is reassuring as Al leaching is often reported to be one of the most common concerns when spreading WTRs to land due to aluminium’s toxicity to crops and fauna including humans (Kochian, 1995; Exley, 2016). This is in line with the general consensus of previous WTR research in which Al toxicity has been proven to rarely occur after WTR application in typical agricultural and silvicultural settings (Turner *et al.*, 2019). Past leaching studies in particular have produced similar findings (Chiang *et al.*, 2012; Caniani *et al.*, 2013; Howells *et al.*, 2018).

The concentration of Fe produced by three samples appeared to be relatively high, however the concentrations of leached Fe from WTRs which were found in three of the leachates in this study were still lower than have been previously reported in the literature (i.e. 239 ± 19, 339 ± 11 and 70.9 ± 3.5 mg kg^{-1} from WTRs 4, 8 and 14 respectively, compared to 600 mg kg^{-1} in Howells *et al.* (2018)). All three of these WTR samples are Fe based WTRs. Therefore, the amount of Fe leached could be dependent on the use of Fe based salts (i.e. ferric sulphate, ferrous sulphate or ferric chloride) during coagulation. However, considering that Fe WTRs typically have ~10-20% w/w Fe by mass, the amounts released by these WTRs during batch leaching is an insignificant fraction (e.g. <0. 4% of the total Fe) and thus would contribute a very small amount of mobile Fe relative to the vast supply typical of soil.

Many of the nutrients measured in this study are applied to land in agriculture for crop benefits, including K, Mg and Mn. Some WTRs leached appreciable amounts of nutrient elements, specifically K, Mg, Mn and Na (e.g. WTR 7 had 210 ± 6.4, 43.7 ± 2.4, 195 ± 7.9 and 358 ± 16 mg kg^{-1} of K, Mg, Mn and Na respectively). Manganese for example is essential for the synthesis of proteins, while plants are known to be incapable of growing once a seed's K stores are depleted (Mozafar, 2018). The leaching of certain macronutrients and micronutrients from WTRs, particularly K which is one of the main three nutrient additions in fertilisers, could therefore reduce the costs that landowners need to spend on fertilisers (Timilsena *et al.*, 2015).

5.4.2. Column leaching

The decrease in leached elemental concentrations with each subsequent leaching was expected as the more readily available element pool became gradually exhausted. The results indicated that >65% of the cumulative leachable content of all elements aside from Mn and Fe were removed by the 4th leaching event, which gives an indication as to how long any increase in readily leachable contents would remain in treated soils. Furthermore, incorporation brought the rate at which the Fe and Mn was cumulatively leached in line with other elements. The higher concentrations of P, Cr, Ni and Cu in leachate in the control soils compared with treated soils during the incorporated leaching columns experiment can be attributed to the sorption capabilities of WTRs that would have retained mobile elements released from the soil. Leachate concentrations of these same elements were not decreased relative to untreated controls in the case of the surface applied treatments, which also supports the conclusion that WTRs were able to retain mobile elements released from the soil when the WTRs were incorporated (i.e. in the surface applied treatments the WTRs were mostly above the soil and so were not able to sorb elements released from soil in downward moving leachate solution). The sorption of Cu, Cr, Cd, Ni, Co and P by WTRs has been widely explored and reported by past studies (Turner *et al.* (2019), and table 2-5) so this result is consistant with the literature.

A comparison of the results of this study can be made with those of a pelletized biosolids column leaching experiment conducted by Yang *et al.* (2008) who used Plexiglas leaching columns with 6.6 cm inner diameter filled with ~300 g of soil and biosolids (applied at rates of 1.25, 5.0 and 10.0 g kg^{-1} of biosolids) and leached them with 258.9 mL of deionised water. Unlike the present study, Pb was detectable in the biosolids leachate. Furthermore, the highest recorded amount of Cr, Cu and Zn by Yang *et al.* (2008) were often an order(s) of magnitude higher than those leached in this study (e.g. 32900 vs 735.9±3 µg kg^{-1} of Zn leached in the highest reported concentration from treated soils by Yang *et al.* (2008) and the highest measured concentration in this study respectively). These comparisons may partly highlight the low leaching ability of $CaCl_2$, but also the low leachable fraction of WTRs when compared to biosolids, which can likely be attributed to their sorption capacity.

The data in Table 6 indicate that, for numerous elements (Co, Cr, Fe, K, Mg, Ni, P, and Zn) the control soil released more leachable elements in the surface applied experiment than in the incorporated experiment. The reason for this remains unclear. If it were the reverse, i.e. that the incorporated controls released greater amounts, one could speculate that the mixing process (i.e. stirring through to keep the procedure equivalent to the WTR incorporated treatments) may have ground up some of the soil aggregates to finer particles and thus exposed greater surface area for leaching. However, this does not explain the results as the opposite was found. The difference may therefore be linked with variation between soil batches, or to differing levels of grinding achieved in the preparation for the two experiments.

Total organic carbon results show that addition of WTRs to soil does not increase C loss via leaching under typical conditions and that, on some occasions, the incorporation of WTRs can even reduce the susceptibility of soils to leach carbon. This is an important finding because a previous study noted the potential for WTRs to release C, at up to ~550 mg/kg, when under flooded and anaerobic conditions (Oliver *et al.*, 2011). This may still be an important consideration for any land managers or agriculturalists contemplating using WTRs in soils that undergo long periods of flooding (e.g. rice paddy systems), but has been shown here in the present study to not be an issue for other farming systems. This present study has indicated that WTRs application could improve soil carbon retention, improving soil carbon stocks and therefore reducing atmospheric carbon emissions. This ties into one of the COP 21 initiatives, 4 per 1000, the goal of which is to increase soil carbon stock by 4% per year in the top 30-40cm of soil (Minasny *et al.*, 2017). Interestingly surface applied WTRs did not provide the same function, highlighting the importance of ploughing soil in an agricultural setting.

5.5. Conclusions

Similar to previous studies, the results of batch extraction experiments suggest that there is not the potential for excessive Al leaching from WTRs when land applying WTRs. There was some Fe in leachate from three WTR samples, however these leached concentrations were a tiny fraction of the total and even remained low compared to a previously reported experiment.

There were indications of reduced elemental leaching of Fe, Co and Mn, and Cr, Co, and Ni in incorporated columns when compared to the control and surface applied columns at each treatment level respectively, which emphasises the sorption capacities of WTRs in a field setting. Furthermore, WTR application did not increase TOC concentrations in the soil column leachate and even reduced the TOC in the case of incorporation of 5 and 10% WTRs (w/w) in some of the leaching events (however the reductions were not observed in any of the surface applied only columns). This emphasises the benefits of soil incorporation of WTRs (e.g. through ploughing) after application.

This research offers one of the most comprehensive studies of WTRs and their leaching characteristics. Future work could focus on the leaching of Al or Fe from WTRs when applied across a broader range of soils that vary in texture and pH. Furthermore, there is room within the literature for longer-term leaching studies with multiple WTR applications to better simulate field application. These would not necceserily need to be extended much further than the current study, as the number of annual WTR applications are often limited to between 1 and 2, and the annual rainfall in the UK is equivalent to ~5 leachings using this procedure.

Further research on the sequential extraction of a large range of WTRs would complement this study and previous sequential extraction studies with smaller sample sizes.

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Chapter 6 - Assessing the impacts of land spreading water treatment residuals on the anecic earthworm Lumbricus terrestris, soil microbial activity and porewater chemistry

Abstract: Water treatment residuals (WTRs), by-products of drinking water clarification, are increasingly recycled to land to promote circular economy and reduce disposal costs, yet there is a lack of published literature on their effects on soil ecology. In the present study, the effects of WTRs on earthworm growth, soil respiration, and soil porewater chemistry are investigated throughout a seven-week outdoor mesocosm trial. WTRs derived from both aluminium and iron coagulants were applied to a loam soil at 0-20 % (w/w). Additionally, soil from a field that had received long-term WTR applications and that of an adjacent non-treated reference field were included in the study. Earthworm mass increase was significantly higher in all but one laboratory treated soils when compared to the control. Furthermore, a linear regression model can be used to predict increases in weekly soil respiration based on the application rates of both Al and Fe WTRs. In addition, a significant increase in soil respiration was observed from the treated farm soils during the first four weeks of the trial. Measured sodium, magnesium, potassium and iron porewater concentrations were higher in the treated farm soils than reference site soil in a majority of samples, although these differences may be related to land management. Laboratory treated soils had elevated porewater arsenic concentrations (e.g. ~17 µg L-1 in controls vs ~62 µg L-1 in the 20 % w/w Al WTR treatment in week 1), while porewater nickel concentrations were respectively elevated and lowered in Al WTR and Fe WTR amended samples. Overall, observed disturbances to soil ecology were determined to be minimal.

Keywords: Earthworms, Pore water, Soil ecotoxicology, Water treatment residual, Soil respiration

6.1. Introduction

Water treatment residuals (WTRs) are a by-product generated during treatment of drinking waters. Their main component is determined by the flocculants used in the treatment process, the most common of which are Alum (aluminium sulphate), iron chloride and iron sulphate, leading to WTRs being referred to as Al WTRs or Fe WTRs respectively (Turner *et al.*, 2019). Typically, drinking water purification produces around 10-30 mL of WTRs for every litre of water clarified (Dassanayake *et al.*, 2015) and, while current global production figures of WTRs are difficult to obtain, older estimates suggest that 10000 t d^{-1} are produced globally (Waite and Dharmappa, 1993). WTRs are regularly applied to land, which is considered to have environmental benefits, including a liming effect, adding organic matter, and the immobilisation of a variety of contaminants and excess nutrients such as Cu, Ni, As, Cd, Pb, Zn and P (Garau *et al.*, 2014; Elkhatib and Moharem, 2015; Nagar *et al.*, 2015). However, while a number of studies have explored aspects of potential chemical impact of WTRs on the environment, very few have specifically explored the ecological impacts.

Earthworms are one of the most abundant terrestrial invertebrates in the temperate regions and important ‘ecosystem engineers’. They are well suited to use in monitoring potential contamination or other soil impacts, due to the constant contact between their permeable skin and the surrounding soil which makes them sensitive to changes in the chemical and physical soil properties (Paoletti *et al.*, 1998; Roubalová *et al.*, 2015). Indeed, Spurgeon and Hopkin (1999) demonstrated that earthworm abundance and biomass decreased with proximity to a Pb/Zn/Cd smelting works in the UK. However, little research has been done to date on the effects on earthworms following soil amendment with WTRs, although an initial short-term (14 day) study by Howells *et al.* (2018) found that earthworms exposed to 0-20 % w/w WTR amended soils did not have their biomass, survival or reproduction affected. However, the same study found that earthworms avoided soils amended to ≥10 % w/w of Fe WTR and to 20 % w/w

Al WTRs which, together with a lack of published data relating to potential ecological impacts warrants further investigation. Potential negative influences on soil earthworm ecology from WTR application are discussed in the conceptual framework in section 2.20 and include the release of Al, Pb, or As, immobilisation of key nutrients, however the addition of organic matter and increased moisture content associated with WTR application could positively affect earthworm communities. Overall, it is hypothesised that the influence of WTRs in a field setting will be negligible due to the low application rates relative the volume of bulk soil.

The influence of land application of WTRs on soil microbial activity is also yet to be fully understood. It is known that WTRs themselves are a source of microorganisms such a *Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia*, and *Planctomycetes* (Würzer *et al.*, 1995; Oliver *et al.*, 2011; Xu *et al.*, 2018), but the overall effect of WTRs addition is still not certain. Pecku *et al.* (2006) found that an application of 300 t ha^{-1} of WTRs had no detrimental effect on soil respiration or microbial diversity. However, mixed results were obtained by Garau *et al.* (2014) when applying Fe WTR amendments (3% w/w addition rate), with an increase in the amount of culturable heterotrophic bacteria and actinomycetes and a decrease in the number of heterotrophic fungi. They concluded that the overall microbial biomass of samples remained approximately constant although the suite of species present changed. A commonly employed method of estimating overall microbial activity is the measurement of soil CO_2 efflux. In soils, carbon dioxide is primarily released through microbial decomposition of soil organic matter (SOM), while a few percent is caused by root interactions (e.g. root respiration and rhizo-microbial respiration) and chemical oxidation of organic matter (Raich and Schlesinger, 1992; Kuzyakov, 2006; Smith *et al.*, 2008). Therefore, as discussed in the conceptual framework presented earlier in this thesis (figure 2-16), monitoring CO_2 efflux could reveal changes in microbial activity which may be increased due to increases in soil organic matter related to WTR application. The increase of soil moisture could potentially increase soil respiration through decreasing the activation energy required for respiration, although this effect is likely minor when the soils are already sufficiently moist (Fang and Moncrieff, 2001). Other than simple bulk organic matter from added WTRs stimulating microbial activity, the potentially different nature of organic matter in the WTRs compared with that naturally occurring in these soils may also stimulate a change in microbial growth and population dynamics; e.g. differences in proportions of readily decomposable (usable) organic components, such as sugars and low molecular weight organic components, and differences in carbon-nitrogen ratio, could accelerate growth of certain bacterial strains. Alternatively, release of metals from WTRs after their addition to soil may favour or disadvantage different microbial groups, as many have been found to be sensitive to increased soluble metals added to soil (Smolders et al. 2004).

The potential for WTRs to leach constituent elements, particularly Al, into the surrounding environment is often considered to be the greatest concern in relation to land application of WTRs. In some countries this is accounted for in legislation, for example in England and Wales the application of Al WTRs and Fe WTRs are limited to soils above a pH of 6.0 and 5.0 respectively, due to the increased mobility of Al and Fe in soils at low pH (Environment Agency, 2013). The importance of these restrictions was highlighted by Howells *et al.* (2018), who found that the amount of leachable (0.001 M $CaCl_2$) Al from the Al WTRs increased from 4.5 mg kg^{-1} to 382 mg kg^{-1} when decreasing the pH from 5.5 to 4.4.

The present study aims to accertain whether the expected changes in porewater chemistry discussed in this thesis' conceptual model (section 2.20) are substantial (in terms of metal and metalloid concentrations) enough to inhibit plant or soil biota health, and that no potential negative effects on earthworm growth occurs. Furthermore, we aimed to test for the first time how WTRs addition affected overall microbial activity measured by microbial respiration (measured using a chamber method). This method measures soil CO_2 efflux, microbial respiration is generally the largest component of soil CO_2 efflux, however it is not a direct measurement of microbial activity and does not quantify other components of microbial activity such as decomposition, nitrogen-fixation and phosphorus solubilisation.

6.2. Materials and methods

6.2.1. Soils and Water Treatment Residuals

Soil treatments were prepared in the laboratory by adding Al WTRs or Fe WTRs to a commercially supplied natural soil, Kettering Loam (Kettering, Northamptonshire, UK, supplied by Boughton Ltd, *www.boughton.co.uk*). This soil was steam sterilised before purchase (~4 years prior to use) and then stored under cover outdoors. The effects of this sterelisation were believed to be important due to these 4 years of storage allowing for microbial populations to restablish. Kettering loam was selected because it has been previously used in earthworm studies and is known to be suitable for a range of soil dwelling species (Butt, 2002; Rajapaksha *et al.*, 2014; Brami *et al.*, 2017). In addition, sandy clay soils were collected from two adjacent agricultural fields in South Wales to compare the impacts of long-term WTR application in a parallel study. One field had received heavy applications of WTR solids for many years (most recently 135 t ha^{-1} in 2015 and 92 t ha^{-1} in 2016) while the other acted as a control and had received no (or only incidentally through runoff from an adjacent field) applied WTRs (henceforth referred to as ‘Farm treated’ and ‘Farm reference’ soils respectively). It is worth noting that the treated area had more commonly been used as arable land while the reference area had more commonly been used for pasture and therefore the soils may have differing properties in terms of compaction and available nutrient levels which have the potential to create differences in microbial activity levels and community composition and must be considered when evaluating the results. However, these soils were all dried at 105 ^{o}C and sieved to 4 mm to improve sample structural and microbial homogeneity. The WTRs used in the present study were sourced from treatment plants in the southwest of the UK (The Al and Fe

WTRs were WTR1 and WTR2 from chapter 5 respectively). The Fe WTRs came from the same water treatment plant as the WTRs applied to the treated farm soil. As per the soils, the WTRs were dried at 105 °C and crushed using a jaw crusher before being sieved (to 2 mm).

The water content of the WTRs, as received, were 25.85 % and 65.27 % (w/w) for the Al and Fe WTRs respectively. Soils and WTRs were characterised for pH by 1:5 solid: deionised water suspensions, organic matter by loss on ignition at 550°C, and water holding capacity (WHC) by saturation and drainage (Table 6-1).

Table 6-1.Properties of the soils and water-treatment residuals used inthe present study

Sample	pH	Organic matter (%)	Water holding capacity (mg g^{-1})
Farm treated	7.05 ± 0.0	12.09 ± 0.07	0.76
Farm untreated	5.79 ± 0.01	12.05 ± 0.26	0.6
Loam	7.65 ± 0.02	16.05 ± 0.11	0.64
Fe WTR	7.48 ± 0.01	24.81 ± 4.56	-
Al WTR	6.2 ± 0.04	66.93 ± 0.72	-

WTR=Water treatment residual

6.2.2. Mesocosm setup

Experiments were conducted in semi field conditions using purpose built outdoor mesocosms. These consisted of cylindrical pots with a depth of 12.5 cm and a diameter of 14.5 cm. Drainage holes were drilled in the base of the pots. Velcro was attached to the inner rims of the pots in order to discourage earthworm escape. The Kettering loam soil was hand mixed with the Al or Fe WTRs at rates of 0 %, 5 %, 10 % and 20 % by dry weight. The 5 % WTR application rate was selected as an upper level of what is ever likely to be used in land spreading practices on agricultural soil (i.e. 5 % equates to ~120 t ha^{-1}, assuming a soil density of 1.2 g cm^{-3} and depth of 20 cm), whereas the 10% and 20 % application rates were selected as extremes to determine the extent of application required to bring about ecological effects. For reference, within England and Wales, WTR application is limited to 250 t ha^{-1} per annum, although this is often further reduced for WTRs with high solids content. Each mesocosm was filled with 1.5 kg of the corresponding substrate. Farm and laboratory soils were wetted to 60 % and 50 % WHC respectively. The resulting bulk density of substrate was ~1.30 and ~1.22 gm cm^{-3} for farm and laboratory soils, respectively. Once installed outdoors the water content was allowed to fluctuate naturally and was checked weekly to ensure they did not dry out.

Ten sexually mature (visible clitellum) *Lumbricus terrestris* earthworms were rinsed with deionised water, patted dry and weighed, and then placed in each pot (earthworms were originally sourced from Yorkshire Worms, Goole, UK). On average, there was 24.0 g of earthworms per kg of substrate in each mesocosm. This density of earthworms is lower than the 50-60 g of soil per earthworm specified in ISO and OECD protocols for earthworm studies, but is in keeping with rates recommended by others for long term tests (e.g. Bart *et al.* (2018)). *L. terrestris* is a species of earthworm that falls within the anecic ecological subgroup. Anecic earthworms characteristically create and live within permanent vertical burrows. This species was chosen because they are commonly found in mineral soils, unlike other species often employed in ecotoxicology assays such as *Eisenia fetida* and *Eisenia andrei* that generally live in high organic matter substrates such as composts and litters. Rhizon samplers (Rhizosphere Research Products, The Netherlands) were installed at 5 cm depth in all mesocosms. Once prepared, mesocosms were sown with 3 g of ryegrass (*Lolium Perenne*) seeds to create an environment that reflected a pasture soil scenario and would act as a food source for the earthworms. The mesocosm treatments thus included two farm soils, six laboratory amended soils and one control soil (non-amended Kettering Loam) (Figure 6-1). Four replicates (n=4) were prepared for each, resulting in 36 mesocosms being assembled in total. For the duration of the study the mesocosms were situated in an enclosed (fenced off) outdoor site. Mesocosms were elevated off the ground on wooden frames with plastic mesh around them to prevent access to birds and other wildlife but otherwise keep conditions consistent with field conditions (i.e. natural field temperatures and rain conditions for central UK during October-November 2018) (Figure 6-2).

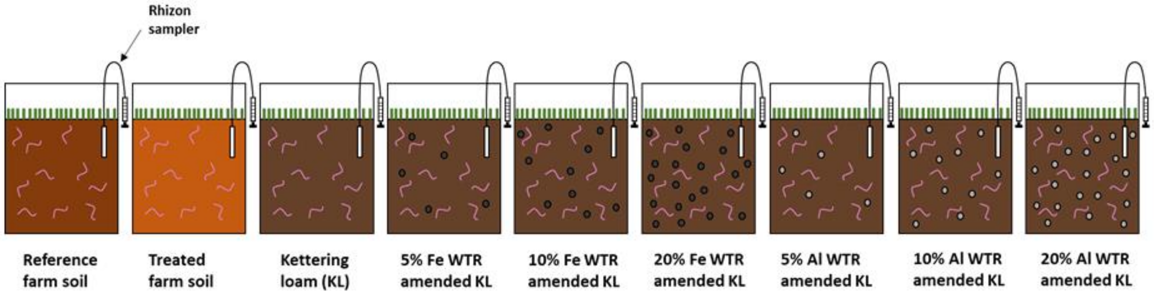

Figure 6-1. A summary of the different mesocosm substrates prepared for the present study.

Figure 6-2. A photo of the outdoor setup used for holding mesocosms during the present study.

6.2.3. Earthworm, porewater and CO_2 flux measurements

Earthworms were recovered from mesocosms at the end of the experiment (after 49 days) via hand sorting. They were washed and weighed in the field to measure their average weight for comparison to weights before the experiment. The change in average weight of earthworms was chosen as an indicator of earthworm health.

Porewater samples were collected weekly over a five-week period via the installed rhizon samplers. These samplers comprised of a porous ceramic-like filter attached to a PVC tube through which water can be extracted using a syringe under vacuum conditions. Collected samples were acidified with analytical grade HNO_3 and analysed via ICP-MS (Agilent 7500ce) along with certified solution standards. Soil porewater sampling has many benefits compared to other measures of element bioavailability (such as extraction with neutral salt solutions), because it directly samples the solution that plant roots and soil invertebrates experience, and it does not rely on an artificial reagent to displace solutes. Moreover, the rhizon sampler method allowed repeated samples to be taken in a non-destructive manner.

CO_2 flux was measured weekly over a six-week period using a PP Systems- EGM-5 Portable CO_2 analyser. This method works by placing the device's chamber (surface area of 78.5 cm^2) on the soil surface to produce an airtight seal, then air is pumped through the chamber and the difference between CO_2 concentrations in the inflowing and outflowing air streams determines the CO_2 flux from the soil. The CO_2 flux measurements were conducted over a 60-second period for each sample after a 15-second purge time and 12-second equilibration time.

6.2.4. Additional data sources and statistical methods

Elemental analysis of WTRs were also determined by a certified, commercial laboratory via USEPA Method 3050B, following standard QA/QC protocols (see supplementary material table 1). In summary, 0.5g of dried material was digested in 12 ml of aqua-regia (9 ml HCl + 3 ml HNO_3) in a hot-block digestion set at 125 °C. The digestate was then diluted to 50 ml with DI water and elemental concentrations determined by either ICP-OES or ICP-MS depending upon the concentration present. Meteorological data was collected from a weather station situated ~ 500 m from the site of the experiment, allowing highly accurate hourly weather data to be obtained. All data was processed, analysed and statistically assessed using Microsoft Excel and Minitab. Linear regressions, T-tests and ANOVAs were conducted following appropriate checks for adherence to normality (Anderson Darling test) and associated underlying assumptions (Levene's test). Mann whitney and Kruskal Walis tests were applied where data was found to be non-parametric prior to and after data transformation.

6.3. Results

6.3.1. Earthworm weight change

When assessed with a standard Kruskal-Wallis there were no significant differences found between the weight changes of earthworms in treatments and controls for any of the farm treated or laboratory amended soils (Al WTR treatments vs control Kruskal $p = 0.1004$; Fe WTR treatments vs control ANOVA $p = 0.06904$; farm treated vs reference soil t-test $p = 0.264$), however, prompted by apparent visual trends (Figure 6-3), assessment via Mann-Whitney u-tests and t-tests revealed significantly higher earthworm mass increases in the 10% Al-WTR treated soils and the 5% and 20% Fe-WTR treated soils compared with the control ($p < 0.05$), t-tests also showed that there was also no no detectable significant differences between the treated and reference farm soils ($p = 0.20$). A statistically significant positive relationship was also identified by linear regression analysis between Fe WTR addition (% w/w) in laboratory amended soils and earthworm mass increase, although only a low proportion of the variance could be accounted for by this model ($y = 0.3972 + 18.82\ x - 75.14\ x^2$; R^2 0.38, $p = 0.045$ for Al WTR application and $y = 0.5790 + 5.699\ x$; R^2 0.30, $p = 0.027$ for Fe WTR application).

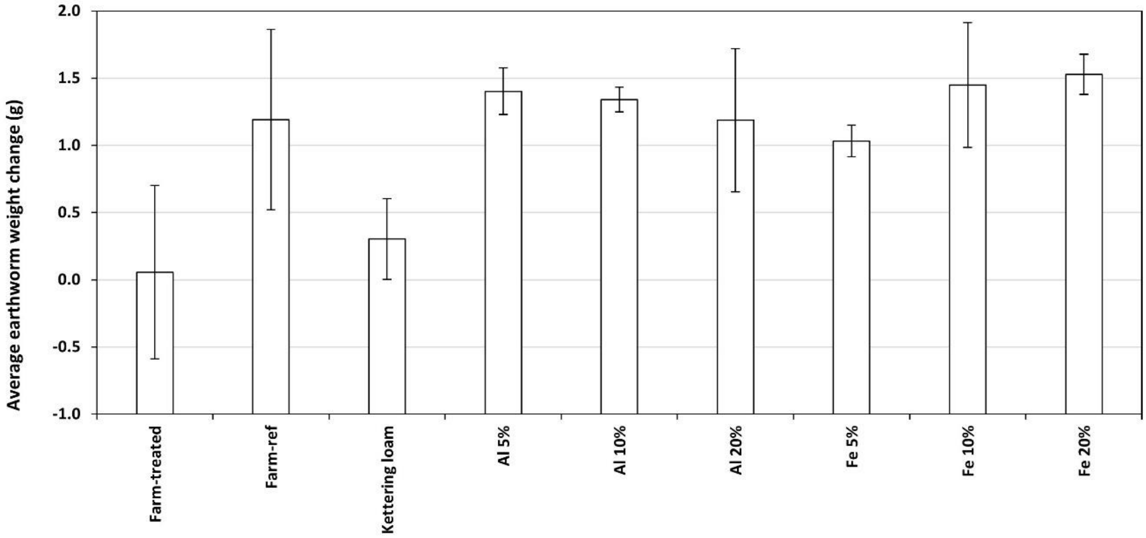

Figure 6-3. The average change in earthworm weight after 49 days, error bars display one standard error.

6.3.2. CO_2 flux (Soil respiration)

Field treated farm soils

The CO2 efflux data was log 10 transformed to achieve normality. There was a significant difference between CO_2 fluxes of the treated and reference farm soil mesocosms in weeks 1 to 4, but not weeks 5 and 6, with the treated farm soils having a higher CO_2 efflux in every case (t-tests $p < 0.05$, Figure 6-4).

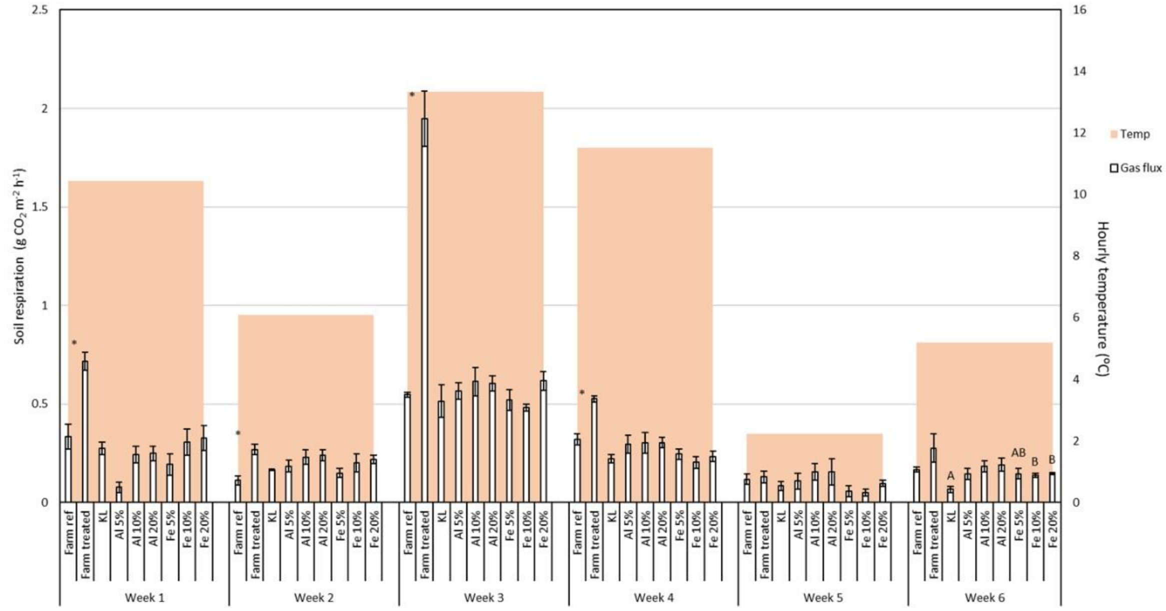

*Figure 6-4. A summary of CO_2 flux measurements and air temperature during the six weeks of sampling; error bars display one standard error, * indicates weeks where the farm treated soil significantly differed from the untreated farm soil, A and B indicate significant difference groupings during week 6, the only week in which lab treatments shown significant differences.*

Laboratory amended soils (Kettering Loam)

When examined on a weekly basis, the only significant difference in CO_2 flux observed following laboratory additions of Al or Fe WTRs to Kettering loam was recorded during week 6 under Fe WTR application (ANOVA $p > 0.05$ in every other case, figure 6-4). Multiple linear regression analysis of the overall data set indicated that the main predictor of gas flux was air temperature ($p < 0.001$, R-sq 0.60). However, when considering the whole data set on an independent weekly basis (allowing a degree of normalisation for air temperature), regression results indicated that the application rate of Al and Fe WTRs could be used to predict for the CO_2 flux of soils ($p = 0.004$ and 0.018 respectively) and accounted for a large amount of the variance ($R^2 = 0.7405$ and 0.7782 respectively). Regression equations for Al and Fe WTR amended CO_2 flux can be seen in equations 6.1 and 6.2 respectively.

$$CO_2\ flux = c + 0.00381A \tag{6.1}$$

$$CO_2\ flux = c + 0.00278A \tag{6.2}$$

Where *c* is a constant that varies from week to week and *A* is the application rate expressed as a dry weight percentage.

6.3.3. Pore waters.

Farm soils (field treated and reference)

Porewater element concentrations were assessed week by week and evaluated for differences between the field treated and reference farm soils via t-test comparisons. Interestingly the Al concentrations of porewaters were not significantly different. Concentrations of Cu, Zn, As and Pb only differed significantly (treated vs reference soil) during one week over the entire sampling period, however these were all lower in the treated soils, aside from Pb which was marginally enriched in treated soils (0.3 µg L^{-1} vs 0.07 µg L^{-1} in treated vs reference farm soils during week 3). More notably, Na, Mg, K, Mn and Fe concentrations differed significantly during at least three weeks of the study (Figure 6-5). In every case, these five elements were elevated in treated soils, for example, Fe concentrations were 339 µg L^{-1} and 8062 µg L^{-1} during week four in reference and treated farm soils respectively.

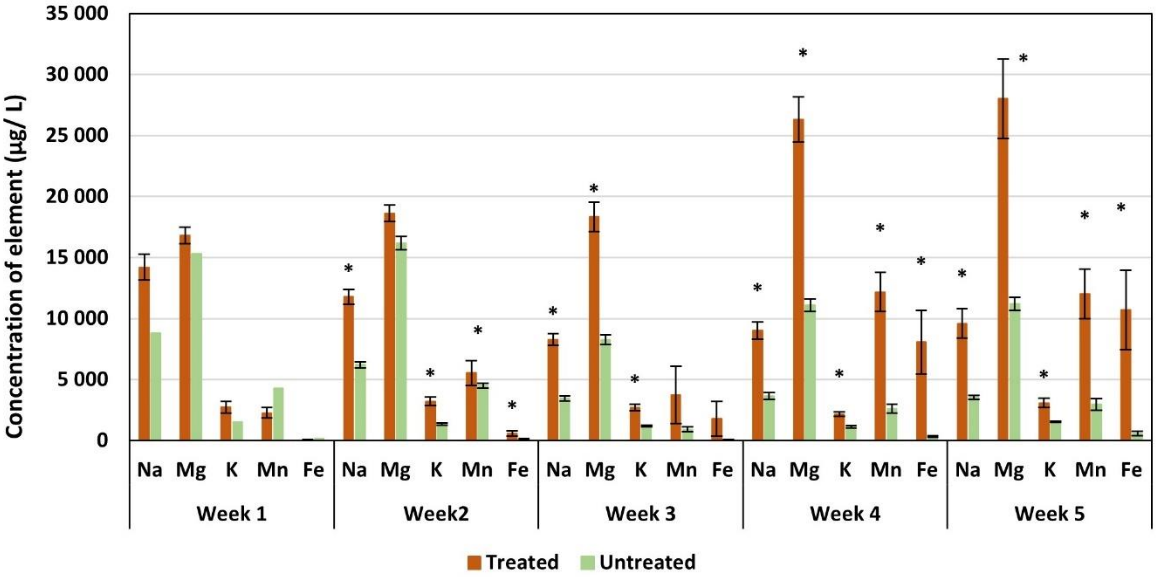

Figure 6-5.The concentrations of elements of interest in porewaters from farm soils, with significant differences noted by asterisks, error bars represent one standard error.

Laboratory amended soils (Kettering Loam).

The Al concentrations in porewaters of Al- or Fe-WTR laboratory amended soil did not differ significantly from controls (Figure 6-6), neither did the concentrations of Fe except for under Al-WTR application in week 5 (Figure 6-7). However, additions of Al WTRs significantly elevated the As concentration compared with the untreated control in every week except week 3 (Figure 6-7), with the increase typically being at least 3-fold at the highest application rate (e.g. from ~17 µg L^{-1} in the control to ~62 µg L^{-1} in the 20 % Al WTR treatment in week 1). The addition of Fe WTRs also increased porewater As concentration in three of the weeks during which porewaters were monitored, but the increases were more modest than in the case of Al WTRs (Figure 6-7). Additionally, in the case of Fe WTR treated soils, Ni concentrations in porewaters were elevated during the same weeks As enrichment was observed, by a factor of 1.3 – 2.0 (Figure 6-7). Contrastingly, Al WTR treated mesocosms had reduced Ni concentrations in every week of sampling. Other elements, particularly Cr, were also either decreased in concentration or were unaffected by Al and Fe WTRs additions. The large increases in Fe concentrations observed in the amended farm soils were not replicated in Kettering loam amended with Fe-WTRs in the laboratory.

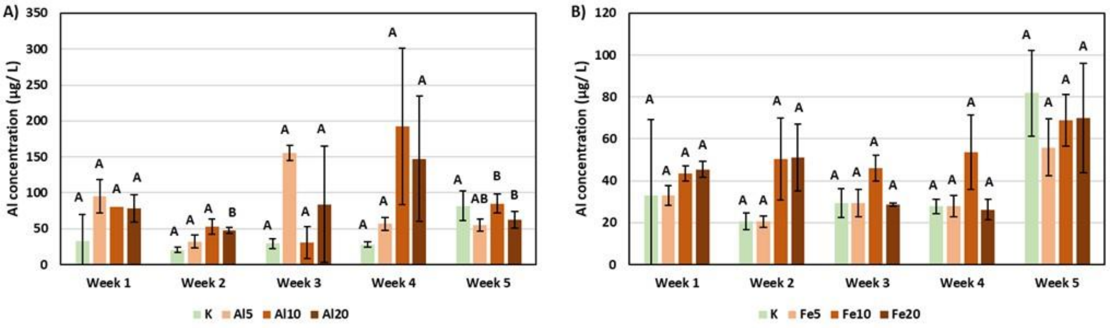

Figure 6-6. Mean concentration (μg L-1) of Al in Al WTR (A) and Fe WTR (B) amended Kettering loam soil. Error bars display one standard error.

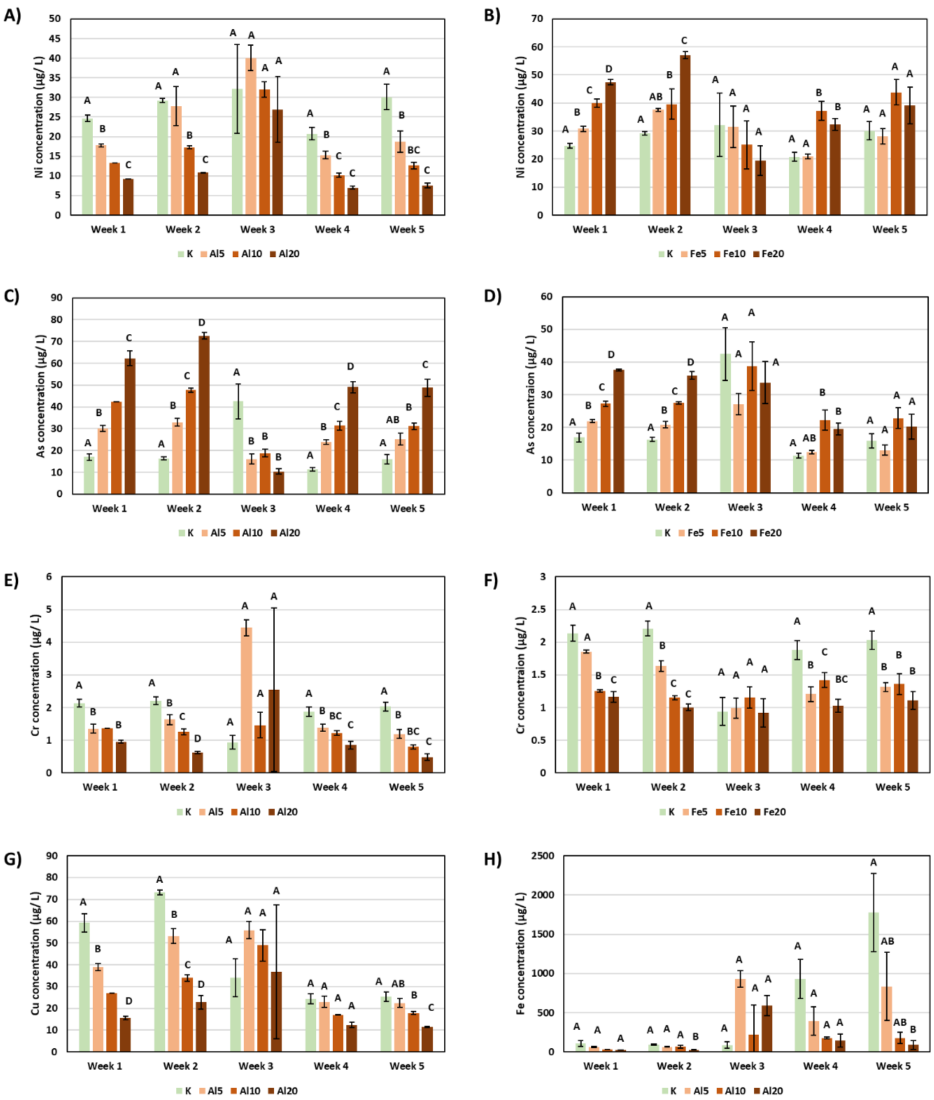

Figure 6-7. Mean concentration (µg L-1) of nickel (A and B), arsenic (C and D), chromium (E and F), copper (G) and iron (H) in porewaters of Al WTR and Fe WTR treated Kettering loam soil. ANOVA groupings indicated by letter. Error bars display one standard error.

6.4. Discussion

As hypothesied in this thesis' conceptual framework, earthworm mass increase was found to be higher in the laboratory-amended soils. However, this finding contrasts with results from laboratory experiment reported by Howells *et al.* (2018) who applied WTRs at similar application rates (0, 5, 10 and 20 % by weight), and found that there was no significant difference in the growth rate of a different earthworm species (*Eisenia fetida*). Between the present study and that of Howells *et al.* (2018) two of the three principal earthworm subgroups (anecic and epigeic) are covered and therefore, there can be a degree of confidence that WTRs application is unlikely to have any negative impacts on earthworm growth when they are applied at typical rates. The positive relationships between WTR application rate and earthworm weight increase found in the present study may be due to the organic matter additions from WTRs. The fact that the farm treated soils showed no difference in earthworm growth might indicate that any enhancement generated by WTRs addition has a time-limited effect. This should be explored further with longer term studies.

The subtler differences between laboratory amended and non-amended Kettering loam gas fluxes when compared with those between the treated and reference farm soils could indicate that previous soil conditions and management practises of the farm soils may also have played a role in the differences in flux observed or that the pre-treatment of the Kettering loam including steam sterilisation ~4 years prior to use may have influenced the microbial response observed during the experiment. The surface WTR amendments may have also led to indirect effects on CO_2 flux due to changes in water holding capacity and bulk density and albedo. However, regression analysis in the present study did indicate that Fe and Al WTRs could influence soil respiration at higher application rates. These results differ from those reported by Mukherjee and co-workers (2014a; 2014b), who found no effect on CO_2 emissions in soils amended with low rates of WTRs (0.5% w/w), but is consistent with those from Pecku *et al.* (2006) who observed a general increase in CO_2 flux after higher application rates of WTRs (up to 25% w/w) when measured in 24 h jar incubation experiments. It is possible that the increase in organic matter and/or alteration or stimulation in the microbial community introduced by the addition of WTRs may contribute to differences in gas flux (and microbial activity) over longer periods or at higher application rates, and therefore understanding the underlying mechanisms (including changes to microbial species suites) is an avenue for further research.

Difference in the porewater Al and Fe concentrations of laboratory-amended and non-amended Kettering loam soils were rarely statistically significant (the only exception is Fe in week 5; figure 6-7H), suggesting both Al and Fe from the WTRs are non-leachable under these conditions. This is likely, in part, due to the pH of the soils which were all above 5.5 (i.e. above the point where Al and Fe become more readily mobile). The As concentration, on the other hand, was notably higher in the WTR amended Kettering loam soils (in the region of ~13 – 70 µg L^{-1} at all application rates). The As concentrations in the present study were below typical toxicity thresholds reported for porewater As, however the highest level observed was comparable to the 50 % effect concentration for cucumber plants recorded in one sensitive soil from Australia (viz. 60 µg L^{-1}; Kader *et al.* (2017)). Certain regulations in some jurisdictions require WTRs to be periodically analysed for elemental content if they are to be applied to land, and such analysis can be used to set limits on how much can be applied. This is the case in the UK, where the WTRs used in the present study were obtained, and previous unpublished analysis of Al WTRs from the same water treatment plant identified that As is an element that can limit the amounts of the material that can be applied to land under those regulations. The porewater results of the present study indicate that such a limit is a prudent precaution because they demonstrate that As in these WTRs is potentially mobile to some degree. However, regional differences in WTR composition and properties must be considered, as past studies have produced mixed results. For example, a study by Chiang *et al.* (2012) found that during sorption/desorption tests of goethite and WTR mixtures the leaching of As was proportional to the WTR content of the blend (i.e. WTRs contributed leachable As). However, Al WTRs from elsewhere have previously been shown to significantly reduce As mobility in treated soils (e.g. Garau *et al.* (2014); Silvetti *et al.* (2014)). Neither the modest release of Ni by Fe WTRs nor its sorption by Al WTRs, as observed here for the laboratory amended soils, have been previously documented in the literature. In contrast to the Kettering loam laboratory amended soils, the field treated farm soils showed no changes in porewater Ni concentrations. Indeed, there were few examples of similar trends in porewater chemistry between the laboratory treated Fe WTR-Kettering loam samples and the treated farm samples. It is possible that differences in the rates of WTR application and previous soil management at the farm, in addition to any aging effects and mineralogical composition, could have given rise to the dissimilar porewater results. Aging effects have been reported by Agyin-Birikorang and O'Connor (2009), who found that Al mobility decreased in WTRs over time, and a similar process could potentially also occur for other elements. However, to our knowledge, no further investigation has been done on the subject. Reductions in other porewater elemental concentrations, particularly Cu in Al WTR amended samples and Cr in all of the amended samples, can be accounted for by the high sorption capacity of WTRs. For example, Zhou and Haynes (2011) established that, even at a low pH of 5, Al WTRs added to test solutions (10 g L^{-1}) could sorb up to 114 g kg^{-1} Cr and up to 52 g kg^{-1} Pb at the highest metal doses imposed. Similarly, Ngatenah *et al.* (2010) found that 100% of the Cu in a 65 mg L^{-1} Cu solution was removed using 2 g L^{-1} of ground WTRs. Meanwhile, Soleimanifar *et al.* (2016) found that 81 % of a 100 µg L^{-1} dose of Cu was sorbed by WTR coated mulch (≥1:3 WTR to mulch w/w) over a period of 120 minutes. It is possible that the presence of earthworm and their degradation of organic matter may have influenced mobility of some elements (Sizmur *et al.*, 2011).

6.5. Conclusions

Mean earthworm mass increase was significantly enhanced in the majority of the laboratory amended soils, while no significant differences were discernible between soils treated with WTRs previously on the farm and the non-treated farm reference soils. When examined across the whole dataset, a small positive association between fresh WTR additions and earthworm weight change was identified; investigation over a longer period may help provide a better picture of these effects.

Soil porewater Al was not appreciably affected by WTR addition in either the freshly applied or field applied and aged samples, indicating that Al leaching is not likely to be a concern with these

WTRs under normal field conditions (i.e. pH and application rates). Porewater As content was largely unaffected in the farm treated soils but was increased in the fresh laboratory amended soils when additions far above regular agricultural practises were made (up to ~70 µg L^{-1} at the highest rate of Al WTR application) and this warrants further research. Freshly applied Fe WTRs also appeared to be a minor source of soluble Ni, but this was not observed in the farm treated soils suggesting that this affect may reduce over time. Results indicate that the elemental mobility in freshly treated soils and in aged, treated soils may vary. Generally, the leachable amounts determined in the porewaters represent a tiny fraction of the total element contents, indicating low mobility of elements within the WTRs. Nevertheless, longer term and/or intensive leaching studies are warranted to confirm this remains the case over time.

At the rates that WTRs are commonly applied, and considering the bounds of normal field conditions, the application of WTRs are unlikely to have a negative impact on earthworms or soil respiration. Although, there is still scope for longer-term experiments to be conducted.

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Impacts of water treatment residuals on earthworms and microbial activity in soils: indoor mesocosm trial

Abstract: Water treatment residuals (WTRs) are produced during the clarification of drinking water. They are often applied to land for their organic and nutrient contributions, however little work has been conducted to explore their impacts on terrestrial ecology and soil microbes. To explore these questions mesocosms were established with 5, 10 or 20 % (w/w wet) treatments of aluminium or iron based WTRs. Soil respiration measurement (CO_2 flux) were taken to explore microbial activity while earthworms (L. Terrestris) were added to the mesocosms to explore ecological impacts. Additionally, porewater samples were collected for chemical analysis however this analysis was not conducted due to covid restrictions ending all laboratory work for ~1 year. Earthworm survival rates were greater than 75% under all treatments. However, earthworms lost bodyweight in all treatments including the control, but these changes did not significantly differ between groups. Soil respiration initially increased with WTR application (from 0.3584 in the control to 0.6870 and 0.6244 g CO_2 m^{-2} h^{-1} under 20 % Al and Fe WTR respectively). However, after the first week these increases were not present.

6.6. Introduction

Within the previous chapter it was concluded that the overall observed disturbances to soil ecology were minimal. Nevertheless, results indicated that initial application of WTRs led to an increase in soil respiration, likely due to the organic matter additions. Furthermore, earthworm mass increase was significantly higher in the treated soils. However, due to the outdoor nature of the previous chapter's experiments, there were uncontrollable variables, such as temperature and rainfall which can introduce uncertainties. Additionally, within the previous chapter WTRs were incorporated into the soil column to emulate the effect of ploughing. This is only one commonly employed agricultural method of application, and in fact surface application without incorporation is also common in a pasture setting or where the moisture content of WTRs is high.

Therefore, this chapter expands on the previous one by assessing ecological impacts of land spreading WTRs through within a climate-controlled chamber, allowing for additional constraint of temperature, humidity and light cycles within set ranges. The effects of WTRs on earthworm populations and soil respiration are again explored with additional geochemical data supplied from porewater extraction to help produce a better understanding of possible underlying causes for any observed impacts. In this experiment WTRs were surface applied and the mesocosms sown with grass to recreate a pasture setting.

6.7. Methods

Two water treatment residuals were sourced from water treatment plants in the south west of the UK. Samples (~20 l) were collected from storage tanks in Oct 2019 by colleagues and stored in polypropylene containers. The soil chosen for this experiment was Kettering loam (Kettering, Northamptonshire, UK, supplied by Boughton Ltd, *www.boughton.co.uk*) which has been used in many past environmental studies (Arnold and Hodson, 2007; Gomez-Eyles *et al.*, 2010).

6.7.1. Physicochemical properties of WTRs and soil

Water content was determined by drying at 105 ^{o}C for 48 hours. Organic content was determined by loss on ignition at 550 ^{o}C over four hours. The pH and EC of samples were determined by 1:5 solid: deionised water suspensions using a Jenway 310 tabletop pH meter and handheld Hanna EC meter respectively (Table 6-2).

Table 6-2. The general physicochemical properties of WTRs and Kettering loam.

Sample	Water content (% mass of material as received)	Organic content (% dry mass removed by ignition 550 ^{o}C)	pH	EC (µS/cm)
Al based WTR	79.25	62.45 ± 0.7	6.69 ± 0.3	720 ± 34
Fe based WTR	67.15	25.79 ± 0.4	7.72 ± 0.2	1550 ± 142
Kettering Loam	-	16.05 ± 0.11	7.65 ± 0.02	476 ± 20

6.7.2. Experimental setup

Mesocosms (1.5 l capacity) were filled with 800 g, 900 g, 950 g or 1000 g of Kettering loam soil, facilitating subsequent addition of WTRs that would create treatments of 0%, 5%, 10% and 20% WTRs (w/w) respectively. Substrates were wetted to 60% water holding capacity of the Kettering loam (WHC = 0.64 mg g^{-1}). Resulting bulk densite of soils was ~1.30 gm cm^{-3}. A rhizon sampler (Rhizosphere Research Products, The Netherlands) was installed in each mesocosm to allow for porewater extraction. Ryegrass (*Lolium perenne*) seeds were sown (5 g per mesocosm) and the grass was allowed to grow for 25 days. Water content was checked regularly by mass loss and replaced with deionised water as necessary. A period of 25 days was selected for ryegrass establishment as this was considered as sufficient for enough tissue mass to have developed in order to provide a food source for earthworms. Five adult earthworms (*Lumbricus Terrestris*) (Sourced from Yorkshire worms, Goole, UK) were washed, dried via paper towel, weighed and placed in each mesocosm pot. In order to deter earthworms from escaping sufficient headroom (~ 10 cm between substrate surface and mesocosm top) in the pots was established and bespoke clear acrylic lids (2 mm thickness, supplied by Cut My Plastic, West Sussex, UK, with fine holes drilled to allow air exchange) were placed on each pot and held tightly in place with large rubber bands. After allowing earthworms to form their burrows for 48 hours, WTRs were surface applied to make up the substrates to 1000 g (i.e. 800 g soil was amended with 200 g WTR, 900 g soil with 100 g WTR, 950 g soil with 50 g WTR, and the 1000 g of soil was the control). Water treatment residuals were applied in their as received state (20.75 and 32.85 solids content for Al and Fe WTRs respectively), simulating how they would be applied in agriculture. The application rates of 5, 10 and 20% by weight were equivalent to approximately 120, 240 and 480 t ha^{-1} assuming a soil and WTR density of 1.2 g cm^{-3} and soil depth of 20 cm. For context, the maximum application limit in the UK of WTRs is 250 t ha^{-1} although this can be further reduced on a case-by-case basis (Turner *et al.*, 2019). Furthermore, for comparison with past studies the dry application rate was also calculated to be 1.56 %, 3.12 % and 6.24 %, and 2.47 %, 4.94 %, 9.88 % for Al and Fe WTRs respectively.

Throughout the study, mesocosms were stored in a climate-controlled chamber (Fitotron SGC 120). A day night light cycle (16 h and 8 h respectively) was established, and the temperature was maintained at 15 ^{0}C and 10 ^{0}C during the day and night portions of the cycle respectively. The humidity was kept at 60% throughout. All of these conditions were chosen to emulate the conditions present in a field used for pasture, which is where many WTRs are land spread in the UK. To further this aim, WTRs were not incorporated as has been done to in previous studies, as pastureland is unlikely to be ploughed and only natural incorporation by leaching or bioturbation is likely to occur.

Porewater samples were collected weekly using the rhizon samplers. These instruments consist of a plastic tube with a ceramic filter at one end and a Luer lock at the other. A syringe is attached to the Luer lock end and used to produce a vacuum within the tubing, allowing for porewater to be extracted. This method was chosen due to its non-destructive nature allowing for minimal disturbance to earthworms and their burrows. The water moisture content of soils was maintained by additions of Milli-Q® water according to mass lost. Following each porewater extraction samples were weighed and water was added until the initial weight of mesocosms at the start of the experiment was achieved. By doing this a maximum amount of time was allowed between water additions and subsequent porewater sampling (ensuring that a state of equilibrium would be established by the next porewater sampling occasion). Measurements of CO_2 flux were taken every week using a PP Systems- EGM-5 Portable CO_2 analyser; a chamber-style gas flux analyser. The exact method used is described in Chapter 4, section 2.2; in summary CO_2 flux was measured over 60 second with a 15-second purge time and 12-second equilibration time. The sudden implementation of covid restrictions in March 2020, which included laboratory shutdown and access prohibition with <1 day notification, resulted in a forced ending of the experiment 50 days after WTRs addition. At that time, earthworms were hand sorted from the mesocosms, counted and weighed. Mean earthworm mass gain or loss per mesocosm pot was determined based upon initial and final average masses of earthworms, with a mean per treatment or control then calculated.

Gas flux and earthworm data was statistically analysed in Minitab. Porewater samples were abandoned because of the length of time they were stored during the covid-19 response lockdown.

6.7.3 Statistical analysis

Statistical analysis was conducted in R. To confirm appropriateness of parametric tests, data were checked for meeting the underlying assumptions, i.e. normality of data and equality of variance. For CO_2 efflux, following data appropriateness checks ANOVA tests with Tukey post-hoc analysis were conducted separately for each week of data to determine whether any treatments varied from the control during that week's measurements. Regression analyses, with checks of normal distribution of residuals and equality of variance, for WTR treatment type versus CO_2 efflux over time were also conducted. For earthworm measurements, survival data were statistically evaluated via ANOVA on controls and treatments, while regression analysis of survival rate and soil parameters were also probed. Earthworm mean mass changes were evaluated via t-tests of each treatment against the control, while mean final masses for each treatment and for the control were evaluated for significant differences via ANOVA.

6.8. Results

Grass growth was sufficient for the initial biomass to be maintained until the end of the experiment, presumably supplying sufficient foodstuff for earthworms throughout. The soils treated with Al WTRs tended to be wetter due to the higher initial water content of the WTRs.

6.8.1. Measurements of CO_2 efflux

ANOVA analysis revealed that the WTR application at the highest rate was the only amendment to cause a significant increase ($p < 0.05$) in CO_2 efflux during week one of the study, whereas during week two ANOVA analysis indicated that the 10 % Al WTR treatment led to a significant decrease in CO_2 efflux (fig. 1). During analysis of the succeeding weeks the CO_2 flux data indicates that there were no significant impacts of WTR application on measurements (Figure 6-8). Furthermore, regression analysis of the data showed that there were no trends in WTR application rate vs CO_2 efflux after the first week.

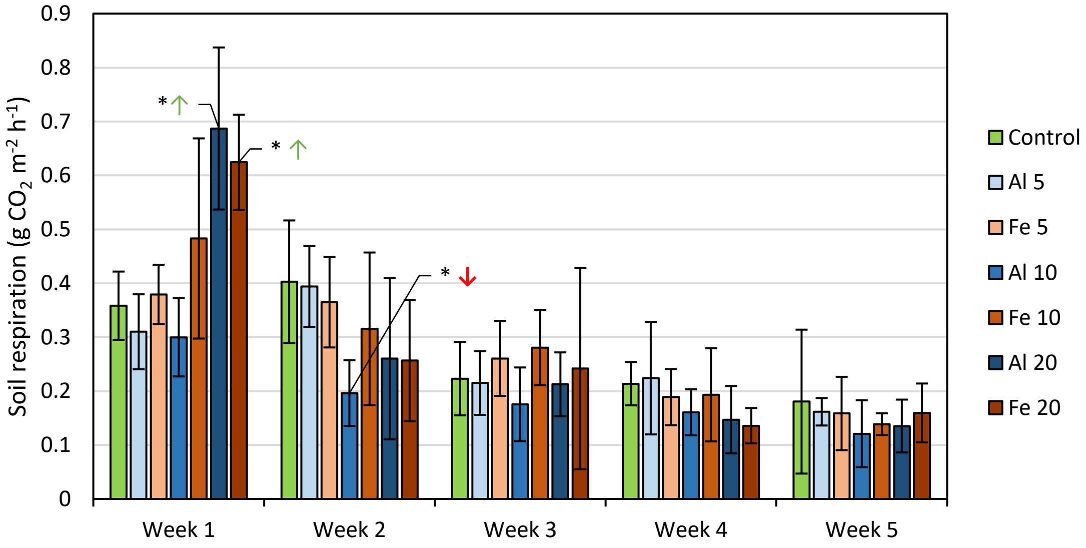

Figure 6-8. Weekly measurements of gas flux from mesocosms, as measured by an EGM-5 portable chamber system, error bars show one standard deviation. The '' symbol indicates significant difference from control, with the green arrows indicating direction of change (increase).*

6.8.2. Earthworm measurements

It must be noted that during the first week of the study two earthworms escaped their mesocosms and could not be replaced due to unknown sources of origin. Nevertheless, earthworm survival rates were still high (> 75%) in all treatments and the control. There were no significant differences (ANOVA $p > 0.05$) in survival rates amongst treatment groups and no trends were found within the data through regression analysis.

Earthworm weight loss was observed in all treatments including the control (Table 6-3) with the highest mean decrease observed in Al 5 % treatment at 0.99 ± 0.31 g per earthworm (~16% weight loss from the start of the experiment), however none of the weight changes were significant (t-test; $p > 0.05$) and no significant trends were discovered during regression analysis. Furthermore, the differences in final mean weight between treatments were not significant (ANOVA; $p > 0.05$).

Table 6-3. Earthworm weight change and survival rate before and after 50 days.

Sample	Average weight change (g)	Average survival rate (%)
Control	-0.28 ± 0.37	100 ± 0
Al 5	-0.99 ± 0.31	100 ± 0
Al 10	-0.39 ± 1.23	75 ± 32.79
Al 20	-0.81 ± 0.19	100 ± 0
Fe 5	-0.25 ± 0.18	90 ± 10
Fe 10	-0.52 ± 0.22	100 ± 0
Fe 20	-0.28 ± 0.71	95 ± 21.79

6.9. Discussion

As earthworm mass decreased in all treatments including the control, the cause for this decrease is likely due to the conditions in place during this study. It could be that the ryegrass shoots and roots were not sufficient food for earthworm growth, but this seems unlikely as the same method of feeding was used in the previous outdoor mesocosm WTR experiment (Section 6.2, Turner *et al.* (2019)). Moreover, it has been established that *L. terrestris* can use living, growing plants as food sources even if it is not optimum for them (Kirchberger *et al.*, 2015). Additionally, surface application of WTRs has previously been shown to increase earthworm growth when compared to other incorporated organic amendments (Lowe and Butt, 2002). The temperature, humidity, earthworm stocking density, soil texture and soil depth conditions set for the experiment were all within preference ranges for *L. terrestris* (Lowe and Butt, 2002), so these would not have been expected to pose limitations on growth. While it is unexplained, it has been noted previously that earthworms can, even in controls, lose body weight during ecotoxicological assays. For example, Alves *et al.* (2013) found that earthworm body mass in all treatments and controls decreased during an ecotox assay examining effects of pesticides on earthworms. Similarly, during an earthworm herbivory experiment, Kirchberger *et al.* (2015) found that earthworms in all treatments and controls had lost weight.

The soil respiration results in this study are similar to those produced by two studies by Mukherjee and co-workers (2014a; 2014b). In both of these studies it was found that there were no measurable impacts on CO_2 flux under low application rates of WTRs (0.5% w/w dry equivalent) (Mukherjee *et al.*, 2014a; Mukherjee *et al.*, 2014b). However, studies with higher application rates such as Pecku *et al.* (2006) (up to 25% w/w dry equivalent) and Turner *et al.* (2021) (up to 20% w/w dry) have produced observable positive impacts on CO_2 flux. For example, in chapter 5 this research group found that WTR additions generally had little impact on CO_2 flux in an outdoor mesocosm experiment at lower application rates (5%), but positive trends could be identified when higher application rates than those used in this study are used (i.e. 10 and 20 wt% dry) (Turner *et al.*, 2021). This supports the concepts put forward in section 2.20 of this thesis regarding WTRs increasing soil carbon stocks and providing a larger foodsource to microorganisms, which subsequently has an influence on the volume of CO_2 efflux produced by microbial respiration.

6.10. Conclusions

Overall, application of WTRs was found to have a negligible effect on soil respiration after the first two weeks. Similarly, earthworm’s survival was not significantly impacted by WTR application after a 50-day period, and while earthworm weights decreased, the decreases were not significant, nor where they significantly greater than in the control. Therefore, taken alongside results from the previous outdoor mesocosm experiments (Chapter 5) it can be inferred that microbial activity was not negatively affected either.

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Chapter 7 - Microbial genome (Illumina MiSeq) sequencing of drinking water treatment residuals and treated soils from the UK

Abstract: The clarification of drinking water leads to the production of large quantities of water treatment residuals (WTRs), a sludge like by-product. These WTRs vary highly in chemical and physical composition depending on the source water, choice of coagulant and dewatering processes. However, whether their microbiome varies widely and which, if any, of their physical and chemical attributes influences their microbiome remains unclear. DNA was extracted from six WTR samples collected from water treatment plants within the UK to compare their bacterial community and whether environmental factors such as coagulant usage (aluminium versus iron salt), the type of water source (reservoir or river), or leachable chemical composition influence these communities. Additionally, DNA was extracted from two aged farm soils, one from a field that was treated with WTRs and one from an adjacent field that was not; this was to explore the influence of applying WTRs to soil on bacterial communities. Bacterial 16S variable region 4 (V4) was amplified and sequenced using Illumina MiSeq sequencing. The most abundant phyla in WTR samples were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Firmicutes representing 92.77-97.8 % of the total bacterial sequences, collectively. Statistical (Mann-Whitney U test) analysis of the resulting microbial profiles indicated that water source played a significant role in microbial community structure, diversity and richness, however coagulant type did not. Canonical correspondence analysis (CCA) of WTR microbiomes revealed that multiple chemical environmental variables influenced the overall microbial community structure of WTRs, and a model using all of these variables could be used to explain the microbial structure of WTRs (the model accounted for the variables in a statistically meaningful manner; $p < 0.05$). Proteobacteria, Actinobacteria, and Firmicutes were the dominant phyla in soil

samples. Untreated soils had a significantly higher richness compared with treated soils (530 compared to 482 respectively). However, the relative abundance of *Massilia*, *Micromonospora* and *Devosia* were higher in treated soils: 7.47 ± 0.35 % vs 1.59 ± 0.08 %, 7.41 ± 1.15% vs 1.40 ± 0.15 %, and 1.08 ± 0.09 % vs 0.539 ± 0.04 % in treated and untreated soils respectively. No common, potentially toxic cyanobacteria or pathogens of concern were found within any of the soil or WTR samples. Analysis with PICRUSt showed that WTRs and soils all had similar predicted microbial functional profiles. Overall, results would suggest that WTRs pose no threat to soil microbial structure and that there were not any negative microbial impacts of WTRs application to farm soils. This study indicates the future research option for further analysis of seasonally collected WTR samples, and analysis of a larger range of soils that have been treated with WTRs or that have WTRs applied in a laboratory setting.

7.1. Introduction

During the treatment of drinking water, colloidal sediment and other impurities are typically removed by a process of coagulation and flocculation brought about by dosing with aluminium or iron-based salts. This produces clean water fit for human consumption but also generates large quantities of water treatment residuals (WTRs as a by-product (up to 1-3% v/v of treated water), comprised of the aluminium or iron salt derivatives and any removed impurities (Dassanayake *et al.*, 2015). Once dried WTRs are in some ways comparable to soils as they consist of iron and aluminium oxi-hydroxides, organic matter, and varying amounts of other elements including many that are important plant nutrients (Turner *et al.*, 2019). Therefore, in an effort to move toward a more circular economy and the sustainability ideal of recycling valuable nutrients, and also to avoid or reduce the escalating costs of disposal via landfill or incineration, WTRs are increasingly being applied to land for general soil improvement and nutrient additions (Turner *et al.*, 2019). It has been determined that the main contributors to WTR's initial chemical properties are the properties of the water source and the type of coagulant used during treatment (Al or Fe) (Babatunde and Zhao, 2007; Turner *et al.*, 2019). There has however been very little research conducted to date on the microbial suites within WTRs or the influence that additions of WTRs might have on native soil microbial communities.

The microbial community composition of WTRs and any influence it might have on soil microflora is still poorly understood. The overall abundances of culturable anaerobic microbes in WTRs have been reported to be similar to those found in soils (Oliver *et al.*, 2011), and in one study the application of Fe-WTRs to soils has been found to increase the total culturable heterotrophic bacteria within soils (Garau *et al.*, 2019). Studies analysing WTR treated soils (aged between 5 days and 6 months) employing gradient gel electrophoresis staining and Biolog EcoPlates (Biolog Inc., Hayward, CA) have found that the land application of WTRs at up to 25%

(w/w) led to no negative impacts in terms of microbial biomass or total heterotrophic bacteria, fungi and actinomycetes (Pecku *et al.*, 2006; Garau *et al.*, 2014).

More recently, a small number of studies have emerged that extracted and sequenced the DNA from WTRs (Xu *et al.*, 2018; Wang *et al.*, 2021a; Wang *et al.*, 2021b). For example, Xu *et al.* (2018) analysed six WTRs from China and determined that the principal phyla were Proteobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Planctomycetes and, notably, Cyanobacteria. The results highlighted possible concerns as three genera of potentially toxic cyanobacteria (*Planktothrix, Microcystis* and *Cylindrospermopsis*) and four potential pathogens (*Escherichia coli, Bacteroides ovatus, Prevotella copri* and *Rickettsia*) were identified as present. Both *B. ovatus* and *P. copri* is associated with the human gut (De Filippis *et al.*, 2019; Yang *et al.*, 2020). Ai *et al.* (2019) analysed WTRs produced from water sources affected by cyanobacterial blooms, and found that the dominant phyla were *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria* and again *Cyanobacteria.* However, both of these studies were based on treatment plants that are known or thought to be supplied by cyanobacteria-rich water sources. Therefore, the question remains as to whether these and other micro-organisms of concern are prevalent in WTRs generally and whether land application of WTRs causes a shift in the microbial community composition of treated soils that may affect soil microbial functions.

It is hypothesised in section 2.20 that the addition of WTRs could stimulate microbial activity in soils due to their high organic content, however it is unclear which microbes will be initially present in WTRs and which will be affected by this stimulation in soils. In order to explore these aspects, this study intends to achieve the following aims through the amplification and sequencing of the 16S rRNA gene within WTR and soil sample:

1. Examine the bacterial communities of six selected WTRs from across the UK (from England, Scotland and Wales) which are produced from a variety of water source types (river or reservoir), principal treatment salts (aluminium or iron), and of varying chemical

composition, enabling an assessment of the influence of these variables on bacterial composition.

2. Separately, to assess any influence of Fe based WTR application to soils on the soil bacterial microbiome through the comparison of bacterial communities of WTR treated and untreated soils.

7.2. Methods

7.2.1. Sample collection and preparation

Water treatment residual samples

Samples of WTRs were obtained from the storage tanks and lagoons of six water treatment plants located within the UK (England, Wales, and Scotland) and stored in 1 L HDPE bottles. The individual plants were selected to cover a range of geographic locations, principal coagulant used (i.e. aluminium or iron salt), and raw water source type (i.e. river or reservoir) (table 2). At the time of collection, these WTRs had been stored for a period that was typical for each plant before being transported off site (in many cases for land spreading). WTRs can be removed from a water treatment plant daily, or they may be stored for months before being removed. This storage time varies by site but also depends on the season and demand for WTRs. This makes it difficult to estimate an exact storage time for each site. WTR properties have been found to change with dewatering (Wang *et al.*, 2021b). Therefore, because of WTRs differing levels of dewatering at the different plants, the WTRs were centrifuged at 855 RCF (Relative Centrifugal Force) for 10 minutes and the supernatant removed via syringe. The remaining sediment was stored at -20 ^{o}C until further use.

Soil samples

Soil samples originated from a farm in South Wales where WTRs have been applied in the past. The first soil (henceforth referred to as ‘treated farm soil’) was collected from a field in which

WTRs had been spread (135 t ha^{-1} and 92 t ha^{-1} in the two years prior to collection, respectively), and the second soil from an adjacent non-treated field which had received no, or only incidental WTR application (henceforth referred to as 'untreated farm soil'). These soils had been exposed to differing land usage (i.e. the treated farm soil was used as arable land, while the untreated farm soil was pasture land). The soils were being used as part of an on-going outdoor mesocosm trial (See Turner *et al.* (2021)). Subsamples (0.25g, n=3) were collected directly from mesocosms for DNA extraction and other analyses (see below).

7.2.2. Organic matter, pH and extractable element concentration determination of soil and WTR samples

The pH of samples was determined using a handheld Hannah pH meter in a 1:5 solid: de-ionised (milli-q) water mixture. Organic matter content was determined by loss on ignition; samples were dried at 105 °C for 24 hours before placing 5 g subsamples (n=3) in a muffle furnace at 550 °C for 4 hours. The bioavailable fraction of elements in WTRs and soils was estimated through batch extraction experiments using 0.01M $CaCl_2$ (Houba *et al.*, 2000; Oliver *et al.*, 2005; Houben *et al.*, 2013). To accomplish this, soils and WTRs were dried at 105˚C for 24 hours before taking 2 g subsamples (n=3) of each and adding 20ml of 0.01M $CaCl_2$ solution. Samples were equilibrated in an end over end shaker for 2 hours, centrifuged for 5 minutes at 855 RCF (Relative Centrifugal Force), with the supernatants then collected, filtered (0.45 µm syringe filter), acidified with 100 µL high purity HCl and stored at 4°C until analysis by ICP-OES (Optima 5300 DV, Perkin Elmer, UK). Soils were analysed via a Coulter LS230 laser particle size analyser, after preliminary heat treatment in a muffle furnace at 550°C to remove organic matter (OM) and then dispersion in a calgon (sodium hexametaphosphate) solution; the results identified these soils as sandy clays according to the International Society of Soil Science particle size classification system. A summary of the physical and chemical properties of WTRs and soils can be seen in Table 7-1.

Table 7-1. Mean (n=3) 0.01M $CaCl_2$ extractable element concentrations (bioavailable fraction), organic matter content and pH (1:5 de-ionised water) of WTR and soil samples. Measurements below the limits of detection of the analytical instrument were expressed as N/A.

Sample	WTR 1	WTR 2	WTR 3	WTR 4	WTR 5	WTR 6	Treated farm soil	Untreated farm soil
Type of sample	Residual						Soil	
Coagulant type	Fe	Al	Fe	Fe	Al	Al	Fe	Fe
Source	Reservoir	River	River	Reservoir	Reservoir	Reservoir	N/A	N/A
Country of origin	Wales	Wales	England	Wales	Scotland	Scotland	N/A	N/A
Al (mg/kg)	2.08±0.043	1.87±0.086	1.54±0.0049	0.289*	1.30±0.14	3.40±0.048	0.528±0.027	1.85±0.032
Cr (mg/kg)	N/A	N/A	N/A	N/A	N/A	N/A	0.0376±0.0031	0.0465±0.0014
Cu (mg/kg)	0.316±0.033	0.424±0.013	0.601±0.026	0.0455*	0.0592±0.0026	0.0334±0.017	0.921±0.0083	0.860±0.0053
Fe (mg/kg)	70.9±3.5	N/A	N/A	0.108*	1.51±0.15	10.9±0.30	N/A	N/A
K (mg/kg)	26.5±0.61	48.2±2.3	47.9±0.34	14.3*	259±18	35.1±2.0	27.2±1.0	17.0±0.16
Mg (mg/kg)	25.2±0.88	67.5±3.1	112±1.5	73.1*	71.5±5.2	33.2±2.7	109±3.1	151±2.7
Mn (mg/kg)	13.3±0.76	38.7±0.78	- 8.80±0.23	7.71*	25.7±1.9	58.9±3.9	11.1±0.74	30.3±1.2
Na (mg/kg)	173±5.2	274±9.5	169±0.82	59.4*	46.9±4.3	34.5±2.8	94.7±1.2	84.1±0.69
pH	7.91 ± 0.24	7.26 ± 0.30	7.71 ± 0.18	7.85 ± 0.20	6.89 ± 0.16	7.25 ± 0.13	7.05 ± 0.0	5.79 ± 0.1
Organic matter content	33.2	38.5	19.1	27.2	35.8	44.2	12.1	12.1

* Single replicate only due to limited sample quantity

7.2.3. DNA extraction

The extraction of DNA from substrates was conducted using the Qiagen DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. DNA was extracted from 0.25 g subsamples of each dewatered WTR, and soils freshly collected from the ongoing field trials. DNA was extracted in replicate samples of each WTR and soil (n=3 generally, and n= 2 for WTR 2) to assess homogeneity of samples. Extracted DNA was stored at 4 ^{o}C until downstream use. All plasticware and extraction solutions were exposed to UV light prior to conducting the DNA extractions to reduce the risk of contamination from plasticware or the laboratory environment. Further, a control sample comprising molecular grade water (RNase-, DNase-, and protease-free) was processed through the entire DNA extraction procedure in parallel to control for this eventuality.

7.2.4. PCR amplification and sequencing

Extracted DNA was subjected to nanodrop analysis to check the purity and concentration of extracted DNA. Following this quality assurance protocol, subsamples of extracted DNA were analysed by Eurofins Genomics where the V4 region of bacterial 16S rRNA was amplified and Illumina MiSeq sequencing was conducted (Bisht et al., 2018).

Bioinformatic analysis

Soil and WTR data were analysed separately. Bioinformatic analysis was performed using QIIME implemented as part of the Nephele 16S paired-end QIIME pipeline using open reference clustering against the SILVA database for bacteria at a sequence identity of 99%. All other parameters remained as default (Weber *et al.*, 2017). This process resulted in an average 63424 and 77861 reads for WTRs and soils respectively.

Tags were clustered into operational taxonomic units (OTUs) which were assigned based on their sequence similarity to the SILVA 99 (v132) database. Analysis of core diversity was conducted considering the best taxonomic classification in each instance. Down sampling was not used due to the inclusion of a negative blank.

The functional profile of samples was determined using PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) (Douglas *et al.*, 2018). This method uses marker genes and the Kyoto Encyclopedia of Genes and Genomes (KEGG) reference genome databases to predict the metagenome functional content of samples without the need for metagenomic analysis.

7.2.5. Statistical analysis

The statistical significance of differences between samples in terms of taxonomic richness, and diversity of genera (quantified via the Shannon diversity index, Eq. 7.1) were explored using ANOVAs in SPSS following appropriate checks for adherence to normality and associated underlying assumptions. To compare the microbial community of samples and explore the influence of environmental variables and physico-chemical parameters, correspondence analysis (CA) and canonical correspondence analysis (CCA) were conducted using the vegan: Community Ecology Package in R. data was square root transformed to reduce the potentially distorting influence of large counts of certain genera on CA and CCA analysis (Lenehan et al., 2017).

$$Shannon\ index = -\sum_{i=1}^{s} p_i \, ln \, p_i \qquad (Eq. 7.1)$$

Where p is the number of individuals of one particular species found (n) divided by the total number of individuals found, and s is the number of species.

7.3. Results

Following post analysis processing, at least 33,775 and 47,242 read pairs were obtained per sample of WTRs and soils respectively and the negative blank sample produced 2039 read pairs. The negative blank contained Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes as the most dominant phyla, accounting for 95% of phyla present. All of the notable genera and families discussed herein were present in the control negative at under 0.01% relative abundance, aside from *CL500-29* marine group which had a relative abundance of 0.74 % in negative control. The reproducibility of samples was good, as can be seen in Figure 7-1 and Figure 7-5. Community composition was explored at phyla level, while analysis of taxonomic richness and diversity, and component analysis, was conducted at genus level.

7.3.1 Water treatment residuals

Variations in community composition

The most abundant phyla in WTR samples were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Firmicutes (Figure 7-1). Bray-Curtis dissimilarity coefficients showed that

WTR 2 and 3 (river samples) had more similarity with each other than any other samples (Figure 7-2). Correspondence analysis and subsequent canonical correspondence analysis was conducted on square root transformed WTR sample data to explore the effect of environmental variables on microbial community structure. Reservoir samples (WTR 1, WTR 4, WTR 5, and WTR 6) were generally clustered together while river samples (WTR 2 and WTR 3) plotted separately from this cluster (Figure 7-3). CCA analysis of WTR samples revealed that the bacterial community of samples could be explained by the supplied environmental variables (p<0.05). The first two components explained 58.6% of the variance.

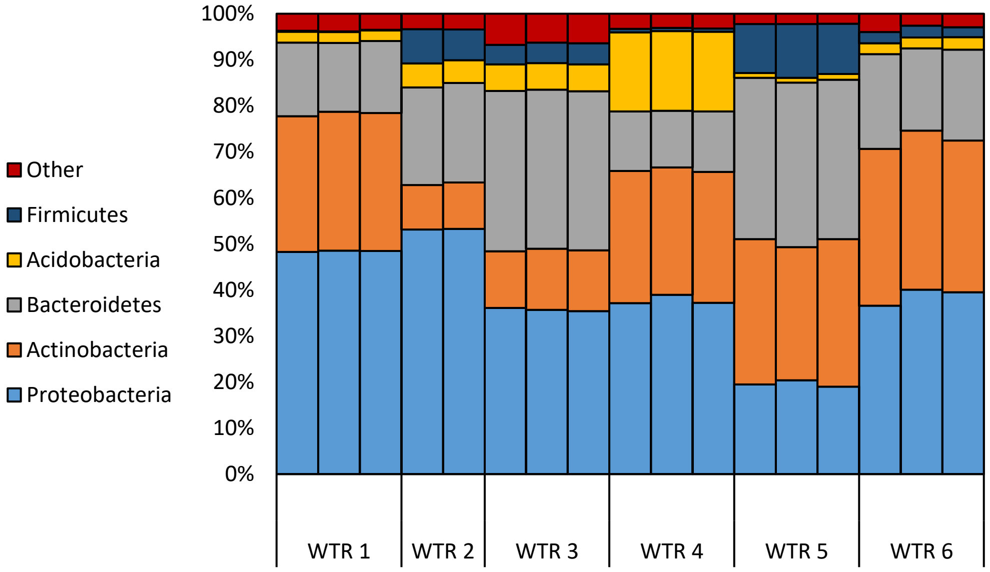

Figure 7-1. The relative abundance of bacterial communities at phyla level in WTR samples.

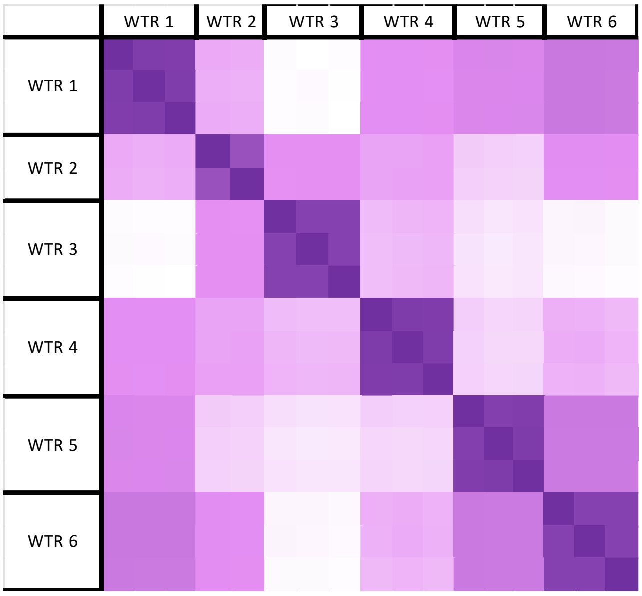

Figure 7-2. The Bray-Curtis dissimilarity coefficient matrix of WTR samples, darker shades indicate more similarity between samples microbial structure.

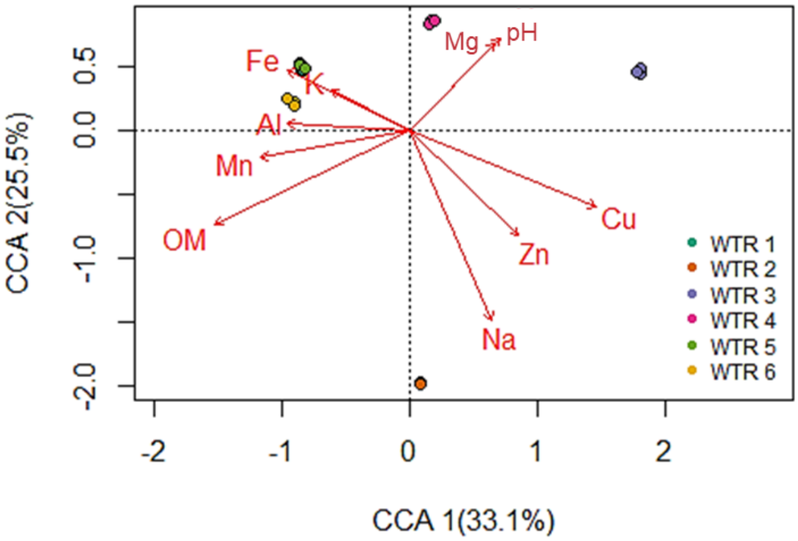

Figure 7-3. A canonical correspondence analysis of the square root transformed microbial community data within WTRs with respect to the measured environmental factors. Arrows indicate the direction and magnitude of a variable's correspondence. Note that WTR 1 and WTR 5 samples overlap, obscuring WTR 1 symbols in the plot.

Relative abundance of specific microbial groups

The most abundant genera in reservoir sourced WTRs were *HgcI* clade (also known as acI) and *CL500-29* marine group (11.09 % ± 3.78 % and 6.77% ± 3.92 % relative abundance respectively in all reservoir samples), both of which are members of the Actinobacteria phyla. The reservoir sourced WTRs were thus rather consistent aside from sample WTR 4, which was an Fe based WTR sourced from a reservoir in Wales, and which aside from HgcI clade was also dominated by *Geothrix* and a not yet cultured genus of the *Nitrosomonadaceae* family (8.78 % ± 0.20 % and 5.03 ± 0.11 % respectively).

While members of the *HgcI* clade were abundant in the reservoir WTRs they were in significantly lower abundance in the river water sourced WTRs (only 0.94 % ± 0.35 %), and members of the *CL500-29* marine group were not present in river sourced WTRs. River sourced samples (one from England and one from Wales, and also differing in principle coagulant type) were found to be widely different from one another in terms of microbial composition, with the only common genera of note having been *Geothrix* and *Geobacter* both of which represented low proportions (each typically representing 1.53 % ± 0.59 % and 1.26 % ± 0.015 % respectively). *Geobacter* was also the only genus that was present in river samples but not in reservoir samples.

Scottish reservoir samples had an abundant presence of *Clostridium sensu stricto 9* and *Flavobacterium* (5.06 % ± 3.17 % and 4.97 % ± 1.87 % respectively). Neither of these were found in other samples aside from Flavobacterium in WTR 2 (1.64 % ± 0.047 %) which, possibly coincidently, was also an Al WTR (as were those from Scotland).

Taxonomic richness and diversity

River sourced WTR samples had significantly higher average bacterial community diversity than reservoir sourced samples (Shannon diversity index of 5.06 versus 3.93; ANOVA $p<0.01$) and total average genus richness (1269 and 735, respectively; Mann–Whitney U test $p<0.01$) was also significantly higher (Figure 7-4). However, coagulant type did not significantly impact either of these parameters (Mann–Whitney U test $p>0.05$) (Figure 7-4).

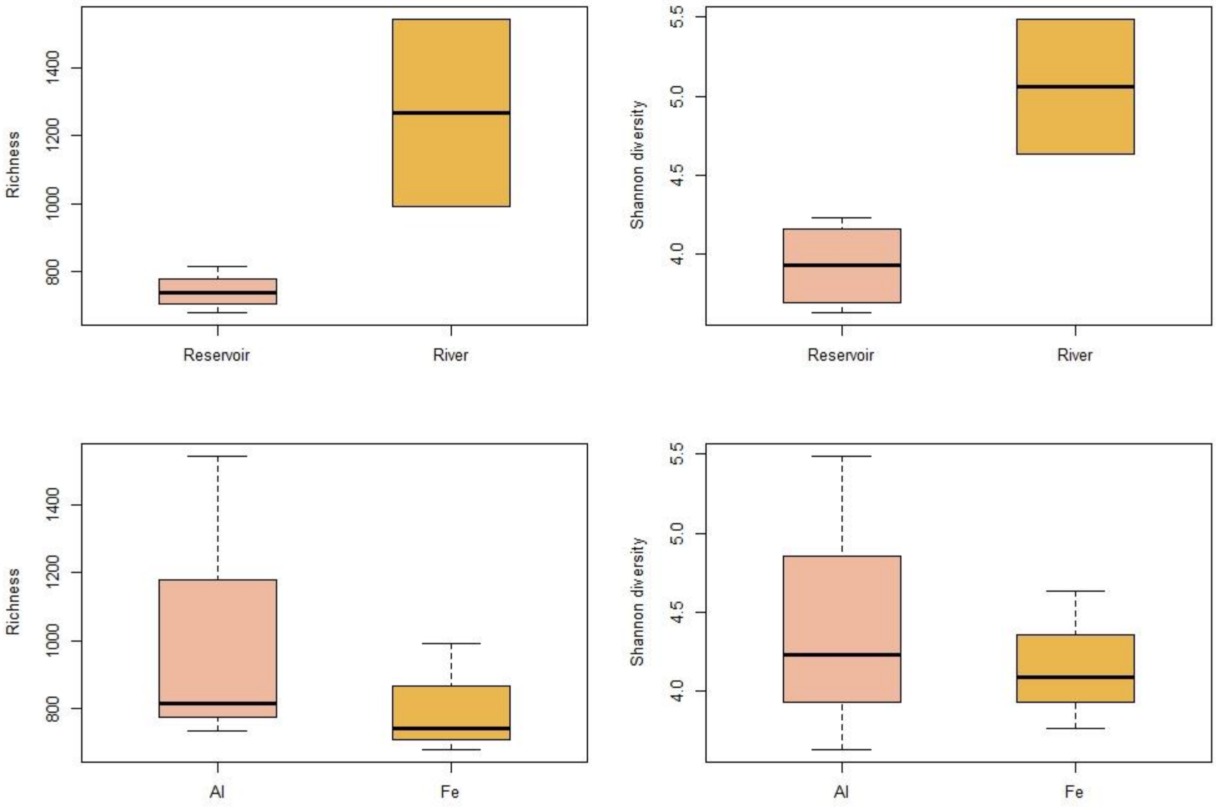

Figure 7-4. The richness and shannon diversity of genera in samples based on: Differing water sources of WTR samples (n=2 river, 4 reservoir) (upper panel), and different coagulants used in the production of WTR samples (n=3) (lower panel). Boxes indicate median (bold, central line) and inter-quartile range, while whiskers indicate 1.5 times the interquartile range.

7.3.2. Comparison of WTR treated soil to untreated control

Variations in community composition

In the soils, the most common phyla were Proteobacteria, Actinobacteria, and Firmicutes, in both treated and non-treated samples (Figure 7-5). Correspondence analysis of soil samples showed that, despite similarities in phyla level composition, at genus level they could be clearly separated by treatment groups along the first dimension (component 1; Figure 7-6), while there was overlap between treatment groups in the second component. However, notably, the first component accounted for 55.1% of the variance, while subsequent components accounted for much less (≤ 13.7%). Due to the low contribution of later components 31.2% of the variance is unaccounted for in this visualisation. Generally, in such plots samples which plot closer together show the most similarity.

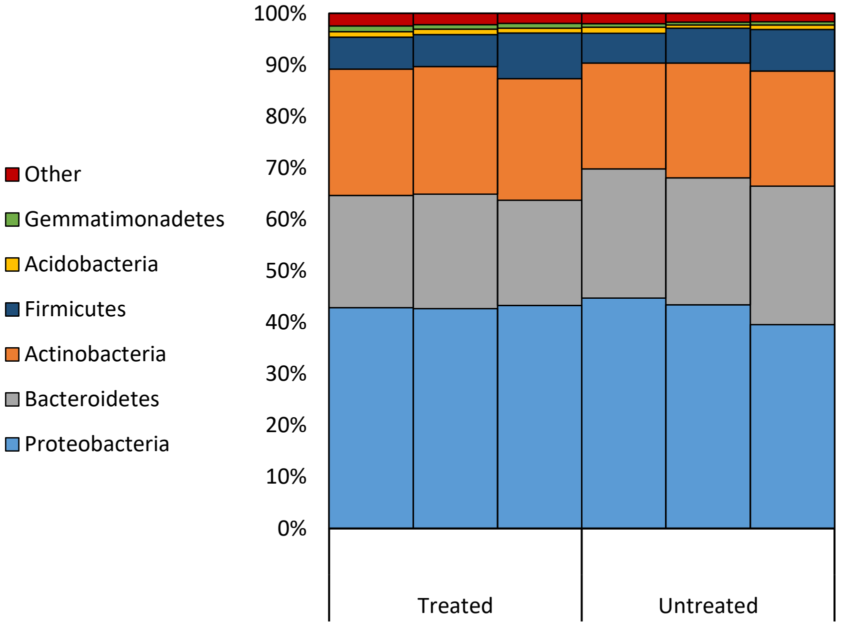

Figure 7-5. The relative abundance of bacterial communities at phyla level in soil samples.

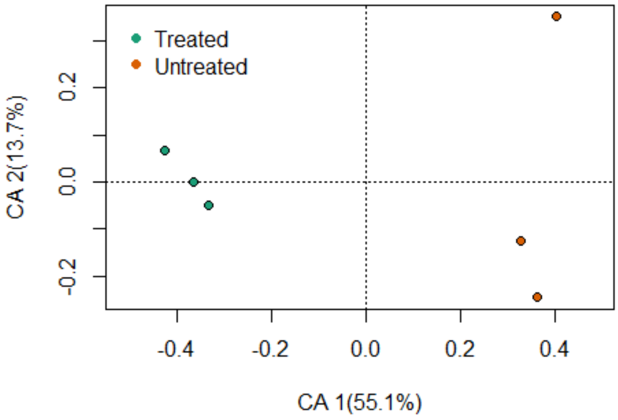

Figure 7-6. Correspondence analysis of square root transformed microbial community data of treated and untreated soil samples (n=3 for each) at the genus level.

Relative abundance of specific microbial groups

There were three noteworthy (greater than 1% relative abundance) significant ($p < 0.05$) increases in the relative abundances of certain bacteria at the family and genus level in treated soils (Figure 7-7). Namely, these were, *Massilia*, *Micromonospora* and *Devosia*, whose relative abundances increased to 7.47 ± 0.35 % from 1.59 ± 0.08 %, 7.41 ± 1.15% from 1.40 ± 0.15 %, and 1.08 ± 0.09 % from 0.539 ± 0.04 % respectively in treated soils. *Massilia*, *Micromonospora* and *Devosia* were present in WTR samples at up to 0.456, 0.115 and 0.340 % of the total abundance respectively, indicating that WTRs were unlikely to be the direct source of the increase observed in treated soils. Whereas there were no noteworthy decreases in in relative abundance in treated soils. The pH of the treated soils was significantly higher than that of the untreated soil (t-test, $p<0.05$).

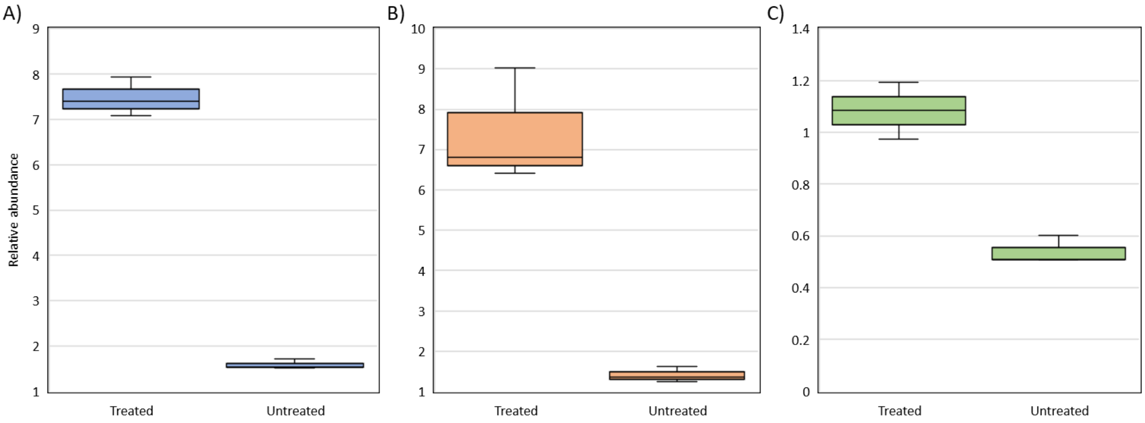

Figure 7-7. The relative abundance of A) Massilia, B) Micromonospora, and C) Devosia in treated and untreated soil samples (n=3). Note change of y axis scale across panels.

Taxonomic richness and diversity

In soil samples the treated soils had a significantly lower ($p < 0.01$) genus richness, i.e., 482 compared to 530 in untreated soils (median value), than the untreated soil but were not significantly different in terms of Shannon diversity index (Figure 7-8).

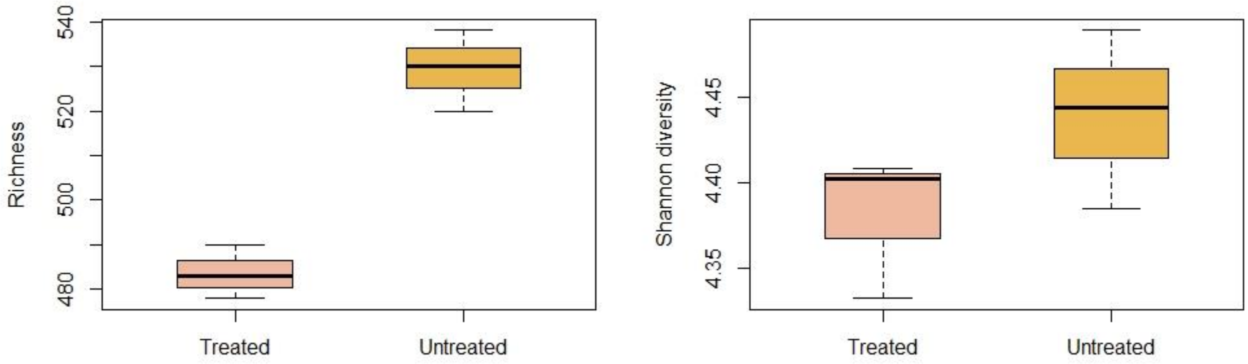

Figure 7-8. The richness and shannon diversity of genera in treated and untreated soil samples (n=3).

7.3.3. Functional profiling

The functional profile of WTRs and soils were determined using PICRUSt, which predicts the KEGG Orthology (KO) functional groups based upon the QIIME predicted taxonomic data. While the bacterial genera present in samples varied (Figure 7-1 and Figure 7-5), when considering the KO groups samples had similar functional profiles throughout (Figure 7-9).

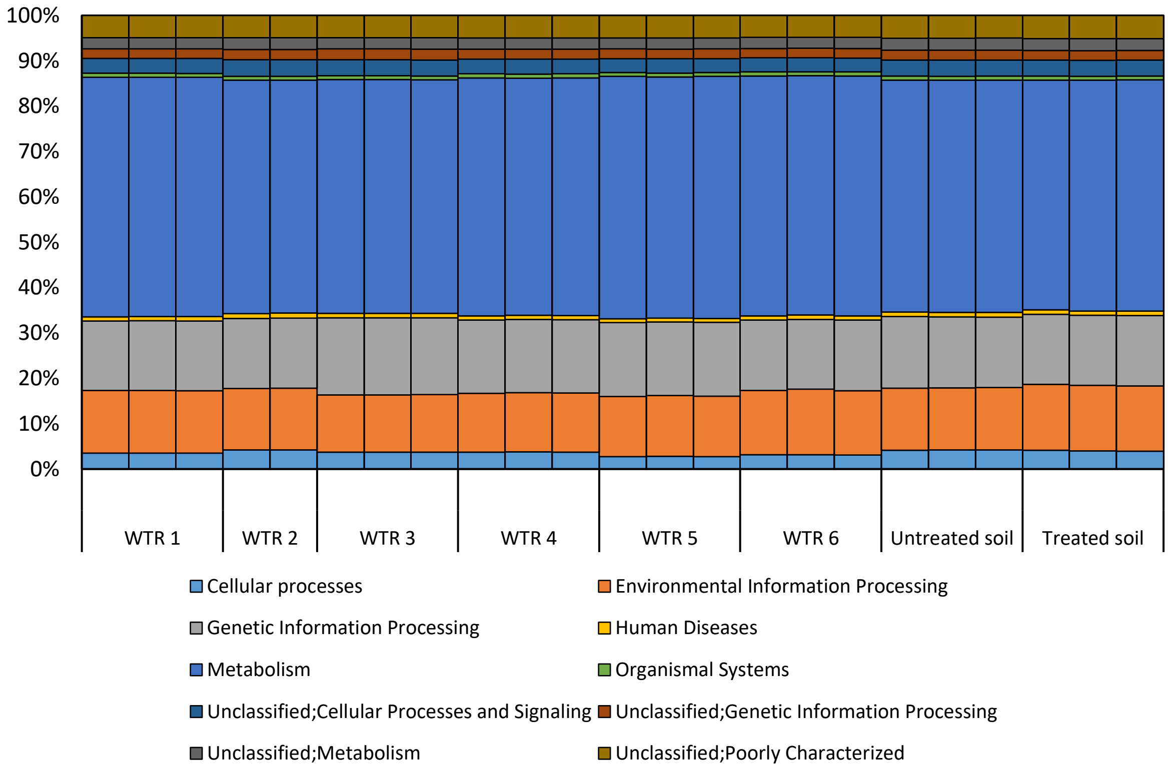

Figure 7-9. Level 2 predicted functional profiling results from PICRUSt analysis of samples. Percentage indicates relative abundance of level 2 functional groups grouped by KEGG Orthology groups.

7.4. Discussion

7.4.1. Water treatment residuals

Community composition

WTRs collected in this experiment had been stored on site at the various water treatment plants in differing conditions (i.e. outdoor lagoons or indoor tanks) and for different lengths of time (<1 day - months), the variability of which is a typical feature of the practise of spreading these materials to land. However, due to the variable and often unspecified length of storage and the variation in lagoon/tank design (or a combination of both), these were not included as independent variables in this study (i.e. effects of storage time or manner were not investigated). Storage or stockpiling of the material may enable establishment and stabilisation of the bacterial communities that will eventually be introduced into the soil environment after land application as discussed in section 2.20 of this thesis. The relative homogeneity of community composition observed amongst subsamples (replicates) of each separate WTR indicates that a microbial community had effectively become established and that the sample processing steps (which included thorough mixing by hand before subsampling) were sufficient to ensure subsamples were representative. Homogeneity within WTR samples has also been reported previously (Xu *et al.*, 2018), while Wang *et al.* (2021b) found that WTRs that originally differed in microbial community structure became more similar after dewatering or drying (the WTRs in the present study had all been partially dewatered or dried before storage and sampling). To our knowledge, the present study is the first to examine bacterial communities in such previously stored WTRs that have likely developed stable communities, with the handful of previous studies that have been reported on the subject having focussed on freshly produced WTRs (Xu *et al.*, 2018; Ai *et al.*, 2019).

Nevertheless, the predominant phyla in WTR samples analysed in this study were similar to those found by Xu *et al.* (2018) in WTRs from China, however that study found a higher relative abundance of Planctomycetes and Cyanobacteria in some samples (e.g. up to 18 % versus 0.039 % and up to 49.7 % versus 0.37 %, respectively) and a lower relative abundance of Actinobacteria and Acidobacteria. The lower abundance of Cyanobacteria in WTRs from the UK found in the present study compared with the Chinese studies may simply reflect cooler temperatures and lower solar irradiance levels in the UK (Solargis, 2020). This is important from a management perspective as many species of Cyanobacteria have been found to produce toxins that can pose a risk to human health and are often encountered in freshwater environments (Falconer, 1996; Briand *et al.*, 2003). Of the Cyanobacteria found in samples of the present study, none were associated with common potentially toxic bacteria forms. In a similar fashion, the potential pathogens found by Xu *et al.* (2018) (*Bacteroides ovatus, Prevotella copri, Escherichia coli*, and *Rickettsia*) were not found in any of the samples from the current study. It is possible that this may be attributed to the effectiveness in water treatment plants in the UK. However, this may also be attributed to the season in which samples were collected (February in this study vs October by Xu *et al.* (2018)), and the length of storage time, therefore further seasonal/temporal sampling could be used to explore this hypothesis.

The two genera found to be dominant in WTRs generated from raw water abstracted from reservoirs, namely the HgcI clade and CL500-29 marine group, match with what is known about their distributions in that they are common and abundant in a wide range of freshwater habitats and particularly in reservoirs and lakes (Warnecke *et al.*, 2004; Ram *et al.*, 2019). HgcI genera are associated with nutrient cycling (Ghylin *et al.*, 2014), whereas members of the CL500-29 clade are known to be generalist that can utilize different carbon sources (Lindh *et al.*, 2015). Their greater relative abundance in reservoir samples may be due to longer water residence times and subsequent increased nutrient cycling levels in reservoirs relative to rivers.

The two river water sourced WTR samples (WTR2 and WTR3) differed from one another in microbial composition, which could be due to differences in the original raw water linked to their different geographical locations and/or to some effect linked to the differing coagulant used. The two genera present that were common between river samples, *Geobacter* and *Geothrix* (the only identified species of which is *G. fermentans*), are both strictly anaerobic and found in Fe reducing environments (Coates *et al.*, 1999; Lovley *et al.*, 2011) suggesting that anaerobic microsites existed at some stage during WTR formation. Geobacter species reportedly may also play a role in the reduction of As and increase its mobility from sediments (Wang *et al.*, 2019).

The *Flavobacterium* genus which was present in Al based WTRs is commonly found in most aquatic ecosystems varying from freshwater to saline, and plays a role in denitrification (Boone *et al.*, 2001; Schaechter, 2009). The lack of other highly abundant genera being significantly affected by WTR coagulant type is likely due to coagulant type having little influence on important parameters such as available element concentrations and organic matter content. For example, WTR 1, which is Fe based, had similar concentrations of available Al as WTR 2 which is Al based. Similarly, WTR 3 had no detectable Fe in its leachate although it is Fe salt based.

Clostridium sensu stricto 9 which was abundant in only the WTRs samples from Scotland (both of which were reservoir based and of the Al-WTR type) may be important as the *Clostridium* genus, while also containing many commonly occurring and harmless freeliving bacteria, contains species that are harmful to human and animal health including *C. botulinum* and *C. tetan* which are causative of botulism and tetanus respectively (Schaechter, 2009). Therefore, further examination of the Clostridium to a species level for any potential negative associations is warranted.

7.4.2. Farm soils

Massilia and *Micromonospora,* both of which were enriched in WTR treated soils relative to controls, have both been previously related to plants and the rhizosphere (Hirsch and Valdés, 2010; Ofek *et al.*, 2012). *Massilia* is known to occur alongside grass (Chaudhary and Kim, 2017), whilst *Micromonospora* are major components of nitrogen fixation in root nodules (Trujillo *et al.*, 2015). Therefore, these enrichments may relate to increased biomass generated in treated soil mesocosms, or to the differing previous land usage of soils. Members of the *Devosia* genus are often found in contaminated soils or waters due to their ability to degrade several toxic organic compounds, including aromatic and xenobiotic compounds, but they also commonly thrive in healthy soil environments rich in nutrients (Talwar *et al.*, 2020). It is difficult to comment on the nutrient content of soils as available N and P were not measured, however WTRs are known to potentially immobilise both (Turner *et al.*, 2019). However, the treated soils had higher concentrations of available K than the untreated equivalent (Table 1). Species of *Massilia*, *Micronmonospora* and *Devosia* have been shown to prefer neutral pHs, with a pH of 7 being optimal for growth (Weon *et al.*, 2010; Zhang *et al.*, 2012; Carro *et al.*, 2016; Lu *et al.*, 2020). Therefore, the pH of the treated soil is more favourable to these species than the untreated soil (pH 7.05 ± 0.00 vs 5.79 ± 0.01, Table 1). The influence of land use prior to collection (i.e. arable vs pasture) and land use intensity could possibly play a role in microbial community development in the soils (Estendorfer *et al.*, 2017). For example Haynes *et al.* (2003) found that microbial biomass (determined by fumigation–extraction) of soils under natural grasslands were greater than those under arable crops and annual ryegrass. However, Méndez *et al.* (2009) found that α-diversity (the variation of microbes in a single sample) and beta diversity (the variation of microbial communities between samples) of pasture and agricultural land were similar. Furthermore, soil treatment after initial collection (prior to usage in the mesocosm experiment), particularly the drying at 105 °C, may have also influenced microbial populations determined, as such pre-treatments have been shown to influence microbiomes in some soils (Schroeder *et al.*, 2021). That is, although treatment at 105 °C would not remove microbial DNA from the soil or prevent it from being identified by the methods used, it may alter the abundance of some microbial groups in the period between heating and sample collection for analysis.

7.4.3. Functional profiling

The similar functional profiles between WTR samples may be an example of functional redundancy within a microbial community. That is, the same functional roles can be fulfilled by multiple species. Functional redundancy is present in soils, and therefore may also be present in WTRs due to their physical and chemical similarity (Grządziel, 2017). The similarity between WTR and soil bacteria functional profiles would support this idea. Any influence on soil functions and processes primarily driven by fungal groups, as opposed to bacterial and related groups examined here, warrant investigation for the same reasons that motivated this study.

7.5. Conclusions

Analysis of sequenced DNA of WTRs from the UK revealed that the predominant phyla of bacteria and associated microbes were similar to those found in studies of WTRs from China, showing some level of universality. However, important differences in cyanobacteria and pathogenic microbe abundance were identified that can be linked to water quality, treatment processes and climatic parameters. Based on these results, it is unlikely that the WTRs from the UK investigated in this study would negatively impact soil microbial functions when utilised for agricultural land application. While the specific microbial composition of WTRs samples varied, the functional profiles were similar to each other and to soils, likely due to the process of functional redundancy.

Two factors that control the chemical composition of WTRs, river source and coagulant used, were explored as controlling variables on microbial composition. River sourced WTR samples had significantly higher bacterial community diversity and genus richness than reservoir samples, however coagulant type did not play a significant role in species diversity and richness. Furthermore, canonical correspondence analysis of microbial composition in WTRs clearly separated samples based on their water source type (river, reservoir), but not principal treatment salt used in their production (Al or Fe). Additionally, the environmental variables explored in this study produced a model that could predict the microbial structure of WTRs. The presence of *Geobacter* and *Geothrix* within river samples is indicative of Fe reducing anaerobic environments. However, the possibility of these genera increasing As mobility is an avenue for further research.

Comparison between treated and untreated soils showed that their microbial composition was comparable but that untreated soils had a significantly higher genus richness and they could also be separated into clear clusters by correspondence analysis (CA). Increases in relative abundance of genera in treated soils, notably *Massilia* and *Micromonospora* and *Devosia* were also noted.

Future avenues of research identified from the present study deserve further exploration:

- Samples collected over a range of seasons, as seasonal effects on water sources and storage lagoons could play a key role in microbial community development.
- Further experiments with WTRs applied to soils and on treated and untreated soils plus soils treated with other organic matter supplements (e.g. manure), to confirm whether the elevated presence of *Massilia*, *Micromonospora* and *Devosia* observed in WTR treated soils in this study is due to WTR additions of microbial populations or whether the enrichment of these families and genera is attributable to general microbial

responses to additional available organic matter contributed by amendments such as WTRs or manure and linked increased plant growth. This exploratory experimentation and analysis could involve a variety of soils and WTRs (and alternative organic amendment) combinations in the presence and absence of plants.

- Tests on microbial community effects when WTRs are applied to sandy and/or acidic soils with typically limited (in terms of abundance, diversity and complexity) indigenous microbial populations.
- Field trials on treated and untreated agricultural land that have a common land use history.
- Exploration of the effect of river sourced WTRs, which contained the genera *Geobacter* and *Geothrix*, on As mobility within soils after land application.
- Experiments to assess any influence of WTR application on other microbial organisms in soil beyond those examined in this study (e.g. fungi).

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Chapter 8 – General discussion and conclusions

8.1. Summary of aims

This thesis aimed to answer the overarching research question below, which was highlighted in section 2.20 as the result of a comprehensive review of the literature pertaining to the physical, chemical and biological characteristics of WTRs from varying sources and the impacts of their application on related facets in agricultural soils:

> *What impact do water treatment residual amendments have on soil health and ecology and is this a feasible future disposal route?*

Thus, the research reported in this thesis set out to explore gaps in the literature through the receptors highlighted in the conceptual framework of chapter 2.20.: soils, porewaters, earthworms and microbes. Each of this thesis' chapters aimed to explore one or more of the chosen quantifiable outputs outlined in figure 2-16 through field- or lab-based experiments, or a combination of both. These experiments involved multiple WTRs with differing chemical and biological characteristics sourced from the UK with the aim of providing a broad understanding of how these characteristics may influence any observed impacts.

8.2. Chapter summary

Chapter 2- WTR Literature review

In chapter 2, a thorough review was conducted into the literature based on WTRs and their uses, particularly land spreading. Findings suggest that WTRs have multiple feasible end of life uses, however some uses, particularly land spreading, are better developed than others. Additional advancements in research into the reuse and recycling of WTRs as coagulants are constantly being reported. Based on the physical and chemical variability of WTRs their potential uses will vary from case to case. Land application of WTRs has already proven to be a logistically and commercially successful end of life use for WTRs. Nevertheless, it is fair to say that, to date, biological and ecological effects of land applications of WTRs are under-researched and so the later chapters of this thesis aimed at addressing that gap. The most commonly cited concern with land spreading WTRs, namely the leaching of pollutants (particularly Al), has been proven repeatedly to be an unlikely problem in most natural environmental settings when WTRs are applied at rates compatible with typical agricultural practise. However, there remains a disconnect between the scientific consensus, the business practices and the governing environmental policy regarding WTRs. Additionally, there is also a lack of publicly available data regarding disposal figures for WTRs.

Chapter 3- Paper and pulp mill sludge literature review

A review of another recovered waste product with potential for environmental applications was conducted as part of the industry focused aspect of this CASE study PhD project. Pulp and paper mill sludge (PPMS) use faces some similar issues to those of WTRs and the conclusion is that benefits of land application can be achieved but that PPMS use also warrants case-by-case analysis prior to choosing end of life use. Of the two major types of PPMS (primary and secondary), secondary PPMS has more appeal to agricultural land owners due to its more desirable C:N ratio and greater concentration of P and thus capacity as a fertiliser alternative. The coapplication of a N source alongside PPMS, or pre-treatment of PPMS via composting can help alleviate the N immobilisation which can occur particularly after primary PPMS land application.

Chapter 4- The effects of land application of WTRs on soil ecology: Fieldwork

In chapter 4 the effect of land spreading WTRs at three active farm sites on ecological receptors were explored. It was hypothesised, based on this thesis' conceptual model, that earthworm and microbial populations size and activity could be stimulated by the organic matter additions from WTRs. Treated areas of a field were compared to those of an untreated portion of the field, or treated fields were compared to adjacent untreated fields. Two chosen ecological receptors were assessed, firstly earthworm population size, diversity and species richness were explored. And secondly microbial activity was measured by proxy through soil respiration.

There were relatively low numbers of earthworms found at site 2 and 3 and during the first sampling period at site 1. However, this was in both the treated and untreated soils, and was attributed to adverse (hot and dry) weather conditions. Soils from the untreated and treated potion of site 3 were under different land management which may have limited the robustness of comparisons. Continued measurements at site 1 found that earthworm populations were in the typical range, and had the usual dominant species for earthworm populations in the UK in both the untreated and treated portions of the site, disproving any hypothesised potential benefits to earthworm species. Similarly, CO_2 efflux remained similar between sampling sites and seasons. Earthworm populations and soil respiration measurements did not differ between treated and untreated areas.

A comparison of two earthworm collection methods, hand sorting and electrical octet collection, found that the two methods produced similar earthworm samples and thus results obtained by each method are comparable.

During these studies the control and impacted soils used in chapters 6 and 7 were collected at site 3. These soils differed in that the control soil was collected from arable land and the impacted soil was collected from pasture land. This adds an extra variable to consider when interpreting the results from the section of this chapter pertaining to site 3 and the subsequent sections of chapter 6 and 7 that rely on these soils for their control impact soils, and therefore may limit the robustness of conclusions drawn experiments using these soils.

Chapter 5- Batch extraction and column leaching

In chapter 5 the results from a series of leaching studies based on WTRs were discussed. As highlighted in section 2.20 there have been varying results in WTR sorption and leaching experiments. Many of these previous experiments have been smaller in scale and therefore the results of this chapter were relatively unprecendented. This was one of the most comprehensive batch extraction studies of WTRs. Batch extraction results from a large variety of WTR samples from around the UK showed that they could benefit soils through additions of K, Mg and Mn. Furthermore, notable concentrations of Al leaching from WTRs were not found, although some WTRs leached higher amount of Fe relative to other samples in this study.

Incorporated column leachate (i.e. leachate from columns in which WTRs were incorporated, i.e. mixed through, soil) was reduced in Fe, Co and Mn, and Cr, Co, and Ni compared to control and surface applied columns respectively. Furthermore, column leachate TOC concentrations were reduced by the incorporation of 5% and 10% WTRs (w/w), however they were not by the surface application of 5% and 10% WTRs. Overall, column leaching results highlight the importance of incorporation of WTRs into the soil column for best results in terms of C and nutrient retention and reducing the availability of harmful elements.

Chapter 6- Mesocosms

This chapter focussed on the two mesocosm experiments, the first was outdoors in an open enclosure, the second was conducted indoors in a climate-controlled cabinet. Between these experiments a picture of the effects of WTR application on earthworms, porewater chemistry and soil respiration was obtained. The goal of this experiment was to determine whether any of the potentially negative impacts on the chosen receptors highlighted in section 2.20 such as increased mortality or limited growth of earthworms were observed when WTRs were applied at high application rates. Through the measurement of both chemical and ecological receptors these studies had the potential to determine whether changes in the availability of nutrients or potentially harmful elements could be source of any negative effects on earthworms or microrganisms, if any where observed.

Outdoor experiments produced a trend of increased earthworm mass with increasing WTR application. A similar trend was produced with CO_2 efflux. Soil porewater was not significantly enriched in Al, however As was enriched when applying one Al based WTR at rates higher than regulatory limits. The results from aged farm soils (treated and untreated) differed from those of freshly laboratory amended soils, which could be due to the effects of aging or due to the different land uses prior to collection.

In the indoor study earthworm survival rates and weight change was not significantly different from the control treatments, although CO_2 efflux was higher in week one from the treated soils.

Overall, it can be concluded that the WTRs used in these studies did not negatively impact earthworm populations or soil respiration even when applied at or above the regulatory limits within the UK. Moreover, WTR application could positively affect earthworm weight gain. Leached amounts detected in soil porewaters obtained via rhizon samplers were relatively low compared to total WTR elemental concentrations indicating that most of the elemental contents of the WTRs are not ecologically active in the short to medium term. Longer term experiments with a larger variety of WTRs could be conducted to support and further test this hypothesis.

Chapter 7- DNA extraction

In chapter 7 analysis was conducted on Illumina MiSeq sequenced DNA extracted from WTRs and WTR treated soils (with a control soil for comparison). This chapter aimed to explore whether WTRs had similar microbial population compositions to soils, and the potential that WTR additions to soils could either introduce new microbes to soils, alter their overall microbial community structure, or stimulate the growth certain microbes' populations due to their high organic content. The WTRs in this study had a similar relative abundance of phyla to those in the limited number of previous WTR studies reported in the literature, but with a lower abundance of cyanobacteria. WTR microbial profiles were then analysed through CCA to determine whether and how production and/or environmental variables influenced results. WTR samples could be separated into clear clusters based on their water source type, but not principal treatment salt. River sourced samples contained significantly higher community diversity and genus richness than reservoir samples. A combination of readily available elemental concentrations and pH could predict the microbial profiles, indicating an association between these factors and the microbial suites. Furthermore, treated and untreated soils could be clearly separated by canonical analysis. *Massilia*, *Micromonospora* and *Devosia* had a higher relative abundance in treated soils. Although this could be attributed to differing land usage prior to soils collection. While the specific microbial composition of WTRs samples varied, the functional profiles were similar to each other and to soils, likely due to a degree of functional redundancy in the microbes present across substrates. WTRs from the UK were unlikely to pose a risk to the soil microbiome in terms of introducing or promoting pathogenic bacteria or related groups.

8.3. General discussion and conclusions

This thesis has explored the effects of WTRs on ecological and chemical receptors as summarised set out in the conceptual model of section 2.20 of this thesis and presented in figure 2-16. Based on the results of chapters 4-7 a revised conceptual model is presented in (Figure 8-1) which summarises this thesis' observed results. In summary, while statistically significant changes were observed in ecological and chemical receptors, these were not notable drawbacks to the application of WTRs to soils when considering the variable nature of these receptors in natural soils. For example, the increase of Ni in WTR soil's porewaters found in chapter 6's outdoor mesocosm experiments were relatively meaningless in terms of ecotoxicology. Further, within that same chapter As concentrations above certain toxicity thresholds were observed in porewaters of WTR treated soils, however this was already raised during chemical testing of these WTRs and they are subsequently limited in their application rates in an agricultural setting. Furthermore, WTRs had similar functional profiles and genus relative abundance to soil samples. Due to the previous lack of WTR studies focussing on soil ecology, the lack of negative impacts discovered in this thesis are encouraging. While there were negligible or positive effects on the *Lumbricus terrestris* survival and weight change, and mixed earthworms' population diversity and species richness, the effects of WTR application on other terrestrial invertebrates such as enchytreids (pot worms) and springtails remain unexplored. Overall, this study agrees with what previous studies have found concerning Al leaching not being a concern to soils ecology following WTR land application under typical conditions.

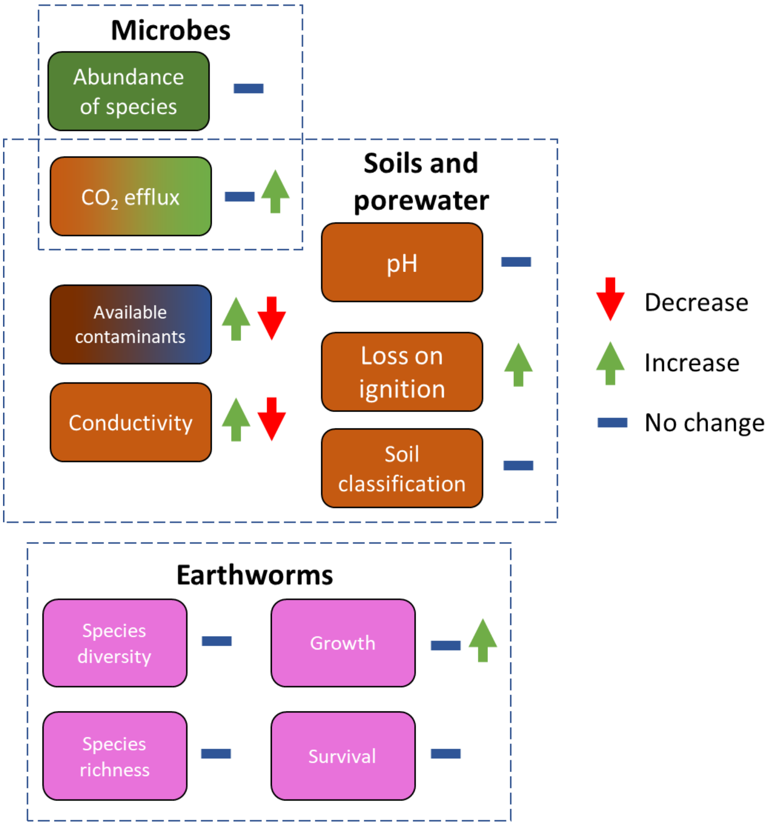

Figure 8-1. A summary of the results of this thesis in relation to the original conceptual framework from chapter 2. Where multiple symbols are present, a mixture of results were observed either in the same or over multiple experiments.

The regulatory limits to WTR application in the UK (up to 250 T ha^{-1} depending on solids content and prior chemical analysis) remain appropriate based upon the results of this thesis where treatments of up to 480 t ha^{-1} produced negligible results on the measured variables (earthworms, soil respiration and porewater chemistry). The benefits of these regulations were further highlighted by the significant As leached from the outdoor mesocosm experiments which was predicted by prior chemical screening in advance of the study, although even the produced porewater sample concentration of mesocosms treated with this WTR remained below the typical porewater As toxicity thresholds. However, the studies within did not explore the effects of pH and the suitability of the limits imposed in the UK (i.e. a soil pH higher than 6.5 for Al WTR application).

The reduced carbon leached from incorporated WTR columns highlights a potential for increasing soil carbon stocks through the addition of WTRs. Carbon storage within soils is important as soil account for a majority of the earth terrestrial carbon stores and are under threat from increasing global temperatures (Crowther *et al.*, 2016). Furthermore, land use as cropland and pasture can reduce soil organic matter (SOM) (Sanderman *et al.*, 2017). Therefore, the potential for WTRs to reduce soil carbon losses, as highlighted in chapter 5, could be seen as an environmentally positive benefit to land application of WTRs. Importantly, this carbon retention was only achieved in incorporated column studies and not surface applied columns. However, further studies would be required to adequateley model these carbon storage dynamics and to confirm the influence of incorporating WTRs into the soil column.

The findings of this research may not apply to WTRs from other countries, as the properties of the water source and treatment process employed are likely to differ. Furthermore, the lab based studies of this thesis were relatively short term and the longer-term field studies were limited in scope by Covid restrictions. Therefore, many of the findings of this research need testing over longer periods of time to confirm that their results remain similar after longer periods.

8.4. Wider Importance of Thesis Findings

Chapter 2 summarised the existing literature over a timeframe that had not been done previously and with a greater focus on landspreading including information that is only available in grey literature and based upon the knowledge of industry experts. Subsequently, this chapter has been published and is now highly cited (47 citations on google scholar as of 20/12/21). Many of these citations are from authors living in developing countries, highlighting the interest in the reuse of WTRs and the methods employed in more developed countries and the importance of disseminating this information.

The reduced leaching of carbon from columns found in chapter 5 is a property of WTRs that has not been reported previously in the literature and may therefore encourage further work in this direction to explore the dynamics of this change and what are the other controlling factors.

In chapter 7, the DNA extraction and analysis via ilumina sequencing of WTRs is an early addition to a new field of study in WTR research. This work complements other recent national and subnational studies of WTR DNA such as Xu *et al.* (2018), Wang *et al.* (2021b), Garau *et al.* (2019) and Wang *et al.* (2021a) and revealed that potentially toxic cyanobacteria found in these past studies are more likely due to the water source or treatment processes employed in the authors countries.

8.5. Future recommendations

This research produced many significant and novel findings, however it also highlighted key areas that require further research. Suggested areas for further research are as follows:

- The analysis of DNA and elemental leachability of WTR samples collected over a range of seasons to explore whether there are seasonal effects that influence WTR properties through their effects on source water and outdoor storage lagoons. There is the potential for the seasonal algal blooms in source waters to contribute potentially toxic cyanobacteria and pathogens the WTRs. Furthermore, seasons with higher rainfall and increased surface runoff can increase the turbidity of water sources and WTR production, but it is unknow what affect this may have on WTR physicochemical properties (Ahmad *et al.*, 2017).

- Additionally, further analysis focussing on WTRs and the impact that storage type (i.e. indoors or outdoors, partially dewatered or fresh) and duration have on their chemical, physical and biological properties.
- Explore the impacts of WTRs on other common terrestrial invertebrates such as enchytreids (pot worms) and springtails and freshwater invertabrates such as daphnia and chironomidae larvae (due to the potential for leaching and runoff). This could lead to the development of toxicity thresholds such as predicted no effect concentrations based on experiments exploring survival, reproduction, and growth.
- Further studies of the land application of WTRs based on soils collected directly from agricultural land where soil types and WTRs vary, specifically examining soil chemistry, and invertebrate and microbial ecology. This would build upon the work in this thesis which was limited by the differing land usage of collected agricultural soils.
- There is a need for more long-term (> 1 year) experiments using a larger range of WTRs than previous long-term studies. Past studies rarely focus on more than two WTRs and/or soil types limiting how representative these can be of the large variability in agricultural soils and WTR’s properties.
- Based on the results of chapter 5 there is the potential for further exploration of the carbon capture potential of WTRs within the soil column. This could initially consist of similar column experiments using WTRs with different origins and based on Al coagulants in a variety of soil types. The fate of this retained carbon is also of interest,

References for general discussion and conclusions

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Supplementary material

Supplementary material 1. The chemical composition of the water treatment residuals (WTRs) used in this study. Values given are in mg/kg.

Element	Fe WTR	Al WTR
K	1030	717
Mg	3360	481
Al	8810	139591
As	5.4	53.5
Hg	0.23	0.1
N	5430	11400
P	1700	717
S	1190	5808
Cd	0.11	0.43
Cr	15.9	11.5
Cu	13.3	27.3
Ni	57.7	22.2
Pb	16.3	8.35
Zn	176	63
Fe	351000	5301