Assessing the fate of Metaldehyde Applied to Arable Soils

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Abstract

Pesticide pollution is a major challenge currently facing many water companies in the UK. Effective management of pesticide transfers from agricultural land to surface waters requires an understanding of the environmental fate of the pesticide active ingredients applied and of their transport pathways. One of the most challenging pesticides for the UK water industry is metaldehyde which seasonally exceeds drinking water standards in many supplies. It is especially problematic because there is currently no economical way of removing it using conventional water treatment processes. In this thesis, aspects of the physical disintegration of slug pellets and the fate of metaldehyde in soils were investigated. Metaldehyde is a molluscicide used in 80% of slug pellets. Three separate studies were performed. The first focused on determining metaldehyde leaching from intact soil cores, assessing differences between loam and clay soil, and between wet-processed and dry-processed slug pellets. The second study compared the half-lives of pelletised and non-pelletised metaldehyde in a laboratory incubation experiment. The final study was split into three sub-experiments focussing on the physical disintegration of slug pellets. The impact of soil moisture content and combined environmental processes was assessed through visible surface area and colour changes over time. The impact of kinetic rainfall energy on pellet visible surface area and weight changes was assessed using a rainfall simulator. The main findings were:

1. Soil moisture is the primary driver of changes in pellet integrity.
2. Clay soils leached more metaldehyde than loam soils after being subject to relatively dry environmental conditions.
3. High soil moisture content led to an increase in the rate of visible surface area reduction and colour change over time.
4. When subject the same environmental conditions, no statistical differences were found between wet-processed and dry-processed pellets in any of the experiments.

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Definitions, Key words and Abbreviations

Definitions and key words

Disintegration – weakening, losing strength and cohesions, coming to pieces

Degradation – deterioration, weakening, becoming more damaged or poorer in quality

In this report, disintegration and degradation are used interchangeably to mean the complete weakening, loss of potency and integrity of metaldehyde and/or the slug pellet casing. The words are used for both chemical and physical affects to metaldehyde forms, except where explicitly stated.

Abbreviations

AW = Anglian Water

B value = Blue value

CV = Coefficient of variance

DCM = Dichloromethane

DWD = Drinking Water Directive

G value = Green value

LoD = Limit of detection

LoQ = Limit of quantification

RGB = Red-Green-Blue

R value = Red value

SEM = Standard error of the mean

SOM = Soil organic matter

SOC = Soil organic carbon

Chapter One

Introduction

1.1 Pesticides and Water

Pesticides are widely used in modern conventional agriculture. They make a valuable contribution to maintaining crop yields and quality by treating a wide range of pests, weeds, fungal infections and other diseases. However, they also have some negative impacts including potential effects on human health, toxicity to non-target organisms in field, such as bees (Cressey, 2017, Desneux et al., 2007), and losses off site, such as to surface waters where they can have negative impacts on the ecosystems of the receiving environment (Tilman et al., 2001, Schäfer et al., 2012, Beketov and Liess, 2008). If rivers are used for drinking water supply the pesticides can also create compliance issues for water companies (Dolan et al., 2014).

In Europe, any pesticides which are licenced for use must undergo a range of laboratory and field testing for their physio-chemical properties, degradation rates and eco-toxicity (European Commission, 2009). They are also tested for their propensity to be transported, for example, via overland flow or leaching, to water environments, including surface and groundwater bodies. These testing protocols are principally designed for chemicals which are applied in liquid spray form because this is the most common mode of application. However, some chemicals are applied in alternative formulations such as pellets or seed treatments. These alternative modes of application are often not well represented in testing and sometimes require different considerations. This could include the mobility of the chemical when in the product rather than in its pure form. For example, pelletised compounds may be tested for sorption and degradation in standard lab tests, such as OECD 307 (OECD, 2002), in which the chemical is introduced into the test system in dissolved form, often within a solvent matrix. Clearly such tests do not realistically investigate the actual behaviour of chemicals considered and there is a need for better characterisation.

1.2 An overview of the metaldehyde problem

As an active ingredient in 80% of slug pellets (Kay and Grayson, 2014), metaldehyde, $(CH_3CHO)_4$, is a contact and systemic molluscicide bait (Lewis et al., 2016) used primarily for controlling slugs and snails. In Europe and the USA molluscs can be responsible for considerable losses in crop productivity, especially during wet and mild seasons when slug activity is highest. Crops targeted include oilseed rape, wheat, corn and soybean, with most damage incurring just after the crop has been drilled, through germination and the initial stages of growth (Simms et al., 2006).

Metaldehyde was discovered by Von Liebig in 1835 (Bieri, 2003). However the first reported use for slug control was not until 1937 (Gimingham and Newton, 1937), after which it attracted the attention of agricultural researchers and the first commercial formulations as a molluscicide appeared. Because it is difficult to penetrate the coating of the slime covering slugs and snails, most molluscicides are used as poisons in palatable baits (Edwards et al., 2009), in which there is a low dose of the active substance. Death occurs once a lethal dose of metaldehyde has been ingested. However, if too much active ingredient is present in each pellet, the pests are able to detect it and stop feeding (Port et al., 2012).

Pesticide pollution primarily occurs through diffuse pollution where entry to water bodies is difficult to regulate and combined management is required from land owners and water companies (Dolan et al., 2012). The European Union Drinking Water Directive (EU DWD) approach to pesticides is based on the principle that no pesticide active substance should be present in drinking water (Dolan, 2013). The maximum allowable concentration for an individual pesticide active substance is 0.1 ug/l (Council Directive, 1998). Metaldehyde is frequently found in raw surface waters exceeding this limit. Metaldehyde is difficult to remove from water using conventional treatment processes. For example, it is not effectively removed by sorption to activated carbon sites, and it cannot be broken down into component parts using ozone or chlorine (Kay and Grayson, 2014, Marshall, 2013).

Diffuse source pollution is now regarded as a larger threat to river water quality than point source pollution, with arable agriculture believed to be the largest pollution

input source (Environment Agency, 2007). In addition to this, catchment management is subject to uncertainty caused by environmental conditions and incomplete knowledge of pollution sources, transformations and transport pathways (Dolan, 2013). Given the inability to effectively remove metaldehyde in water treatment, one of the largest challenges for management is to reduce pesticide transfers to water. Key to this is understanding how pesticides with different characteristics behave within the environment under different conditions.

In the case of metaldehyde, the fact that it is typically applied in pellet form is potentially an important regulator of its behaviour. If pellets remain intact, for example, metaldehyde will not be in close contact with the soil microbial community and may, therefore, breakdown more slowly than the rates suggested by standard laboratory tests (e.g. the OECD (2002) 307 test). This could extend its environmental longevity and increase the potential for leaching loss.

This study investigated the physical disintegration of metaldehyde-based slug pellets in soils, the breakdown of metaldehyde itself and the leading loss of metaldehyde from soils receiving pellets. Two different soil types and two different pellet types were investigated. An environmentally realistic approach was adopted to allow greater understanding of pellet behaviour within the environment and, therefore, inform catchment management.

1.3 Metaldehyde in soils

Metaldehyde is a synthetic organic compound formed from four acetaldehyde monomers (figure 1). Metaldehyde is degraded by microorganisms into acetaldehyde, acetic acid, and finally, water and carbon dioxide (Bieri, 2003). Thomas et al. (2017) identified two bacteria, *Acinetobacter* E1 and *Variovorax* E3, which metabolise metaldehyde. This form of degradation is a strongly exothermic process, which could enhance microbial growth as well as being the result of microbial activity (Thomas et al., 2017).

Metaldehyde is moderately water soluble and has an organic carbon to water partition coefficient (K_{OC}) ranging from 34 to 240 l/kg (table 1)

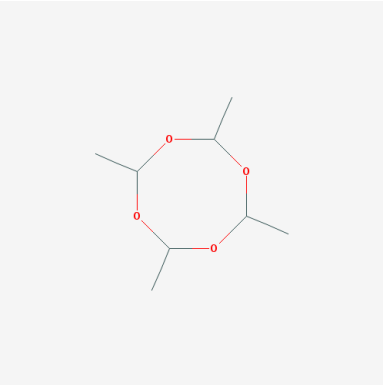


Figure 1: The structure of metaldehyde

Table 1: The physio-chemical properties of metaldehyde (Lewis et al., 2016, Kay and Grayson, 2014)

Property	**Property value**
Molecular mass (g/mol)	176.212
Boiling point (°C)	191
Melting point (°C)	191
Aqueous Solubility at 20°C (mg/l)	188
Solubility in methanol at 20°C (mg/l)	1730
Vapour pressure at 25°C (mPa)	6600
Organic carbon to water partition coefficient, K_{OC} (kg/l)	34 - 240
Henry's law constant at 25°C (Pa m^3 mol^{-1})	3.50

Although sorption and degradation are the two most important processes influencing the fate of pesticides in soils (Boesten and Van der Linden, 1991), there are many related factors involved which stem from the chemical and physical properties of the environment, biological activity, pesticide structure and application method (Edwards, 1975, Kah et al., 2007, Thompson and Goyne, 2012, Kalbe et al., 2008).

1.3.1 Organic carbon content, biological degradation and bioavailability

The rate of degradation of pesticides in soils is often described using first-order kinetics where the rate is proportional to the concentration remaining. This is manifested as an exponential decay over time which can be characterised by a half-life, or DT_{50}.

Metaldehyde is known to depolymerise into acetaldehyde through microbial activity where it's chemical structure is an exploitable carbon source (Simms et al., 2006). Although the calculated K_{OC} of metaldehyde varies (table 1, table 2), it is generally accepted that metaldehyde is 'moderately mobile', allowing it to sorp to the soil organic carbon (SOC) but not so strongly that it is not still mobile and accessible within the soil matrix to microbes. As metaldehyde has been shown to degrade at a reduced rate in sterilised soils (Simms et al., 2006), microbial activity is suggested to be essential to degradation.

*Table 2: Laboratory derived metaldehyde K_{OC} and half-life values, given to 3 significant figures. Calculation procedures for database half-life and K_{OC} values have not been specifically given, although it is highly likely the majority were laboratory-based experiments using laboratory-grade metaldehyde. *value from an in-field study using granules.*

Source	Adsorption Coefficient, K_{OC} (l/kg)	Soil Half-life, D_{50} (days)
PAN Pesticides Database (Kegley et al., 2016)	35.0	67.0 (aerobic soils) to 223 (anaerobic soils)
Pesticide Properties Database (Lewis et al., 2016)	240	5.10
Safety data sheet (Bayer Garden, 2014)	60.4	-
Safety data sheet (Chiltern Farm Chemicals Ltd, 2014)	117	11.9
Assessment of industry data for metaldehyde (Kay and Grayson, 2014)	34.0 to 240	3.17* to 223
OSU extension pesticide properties database (Vogue et al., 1994)	240	10.0

Gevao et al. (2000) state that after a chemical enters the soil and sorbs to the solid phase, it may have reduced bioavailability, and therefore be less likely to degrade. Although sorbed chemicals should not be considered to be permanently bound (Northcott and Jones, 2000), the fact that they are bound may reduce their bioavailability, and therefore make them less degradable. However, Kah et al. (2007) reported that stronger sorption to soils was actually associated with faster degradation probably due to enhanced bioactivity in high organic carbon soil (although for most pesticides tested this relationship was not significant). Soil organic matter (SOM) and microbial population density generally decrease with soil profile depth. Biological activity is therefore less, and there are fewer SOM sorption sites, and less degradation

in the lower horizons (Kookana et al., 2005, Andreu and Picó, 2004). Consequently, if a pesticide is quickly transported to the lower soil horizons, where there is less SOM for sorption and less biological activity, it is more likely to be leached out of the soil profile.

Although it did not specifically focus on metaldehyde, a study of biobeds at Manor Farm, Norfolk, found that the pesticide concentration at 90 cm depth was nearly double that at 45 cm (Cooper et al., 2016). This was reasoned to be due to changes in biobed properties with depth. A clay layer nearer the surface was expected to have higher pesticide retention due to sorption than a lower, sandier layer. Because of this, pore water in the sandier layer would contain higher concentrations. Also, the biobed clay layer could form preferential flow paths, due to drying fissures, allowing leachate to bypass aerobic surface layers where the majority of the biological degradation was expected to occur. The same study concluded that pesticides with low solubility, higher sorption and longer half-lives had the greatest removal rates from the biobed soil matrix (Cooper et al., 2016).

1.3.2 Soil moisture and temperature

Pesticide fate and transport can both be affected by soil temperature and moisture content. It is estimated that 90% of metaldehyde enters water through artificial field drainage (Hewson-Fisher, 2015). Drains are often installed in fields with heavy soils that can become easily saturated (with an associated anaerobic environment with less biological activity). High soil moisture, therefore, has the potential to reduce degradation rate.

Temperature also impacts soil moisture, as evapotranspiration rates tend to be lower in cooler temperatures. This results in an increase in soil moisture contents which increases drainage rates and leaching from fields to surface waters. Temperature has a direct effect on microbial growth and activity where higher temperatures tend to promote higher growth rates and activity if other factors are not limiting (Whelan et al., 2015). Microbial activity, associated with metaldehyde degradation in previous

studies, should be highest in warm, moist environments, typical of optimum mollusc conditions when metaldehyde is most likely to be applied to soils. Moisture content can also affect microbial activity. If the soil is too dry, many soil pores are empty and do not provide a good habitat for many microbes so overall activity decreases. If the soil is too wet oxygen diffusion rates decrease, also reducing microbial activity.

A study on biobeds that highest dissipation occurred when biobed temperature was 20°C in comparison to 10°C and 2°C (Castillo and Torstensson, 2007). Dissipation was also higher when soil moisture content was 60% of water holding capacity in comparison to 30% and 90% (Castillo and Torstensson, 2007). This suggested that maximum rates of pesticide removal would occur in summer as long as moisture content remained relatively high. However, another study on biobeds at Manor farm, Norfolk, showed that there was no seasonal difference in pesticide degradation rates (Cooper et al., 2016).

While field drains can reduce soil water contents and the frequency of saturation (by increasing soil oxygen contents and thereby increasing biological activity and degradation), they also reduce hydrograph lag times and, therefore, have the potential to transport pesticides out of the soil at a faster rate. Although metaldehyde was not included in the study of ionisable pesticides by Kah et al. (2007), the wide variety of laboratory D_{50} and K_{OC} values (table 2) mirrors the correlation indicated by that study, suggesting a relationship between stronger sorption and a lower half-life for metaldehyde.

1.4 Laboratory factors influencing metaldehyde degradation

The potential environmental factors which control degradation and sorption can be monitored and controlled in the laboratory. Laboratory degradation tests are typically conducted at 20 °C in the dark and although this can be used to standardise conditions, thus allowing scientific repetition, it limits environmental realism. Powdered laboratory-grade metaldehyde in solution is characteristically used in such tests instead of pellets or granular metaldehyde forms, which is also not realistic for typical

metaldehyde use in the UK. Inter-laboratory results can also vary widely as a consequence of different soil types, soil pH, and organic matter content. As a result, metaldehyde degradation half-lives calculated under 'controlled conditions' can vary.

In water, metaldehyde degradation from laboratory studies varies significantly. The Pesticide Properties Database records a hydrolysis D_{50} of 11.50 days (Lewis et al., 2016), whereas the PAN Pesticides Database states a D_{50} of 6,150 days (Kegley et al., 2016).

The wide range of different laboratory-quantified K_{OC} and D_{50} values (table 2) may be the result of any of the above environmental or laboratory factors and strongly suggests that metaldehyde can be both persistent and mobile (Kay and Grayson, 2014). As such, it is important to study metaldehyde sorption and degradation in realistic environmental context with an understanding of the numerous possible outcomes.

1.5 Metaldehyde in surface waters

With improvement in analytical methods, metaldehyde has become more readily detectable in surface waters (Gillman et al., 2012). The presence of metaldehyde in raw and treated surface waters was first recognised by Bristol Water in 2007 (Bristol Water, 2009, Pendergrast, 2012) and by 2012, research into general pesticide concentrations revealed consistently high concentrations of metaldehyde. Although relatively few academic studies have been conducted on metaldehyde, it is already a major concern for water companies because of the concentrations measured in routine monitoring challenge DWD compliance. (Kay and Grayson, 2014). In 2009, the Drinking Water Inspectorate (DWI) Annual Report for drinking water quality in England and Wales observed that one third of all water quality failings in drinking water supplies were due to metaldehyde pollution (Colbourne, 2010). The Drinking Water Inspectorate has subsequently given water companies until 2018 to produce tangible reductions in metaldehyde concentration exceedance (Pendergrast, 2014, Purcell). To do this, companies must understand the sources, transportation and transformation of

metaldehyde in the environment before committing to effective management methods.

In a study in Northern France investigating pesticide pressure to barrage ponds which received agricultural runoff (Lazartigues et al., 2012), metaldehyde concentrations were found to be frequently above the EU standard, and sometimes over 1 ug/l. Another study in the River Ugie, Scotland, revealed concentrations up to 0.359 ug/l metaldehyde in raw water (Gillman et al., 2012). In subsequent monitoring on the River Ugie over a further two years, elevated concentrations in pesticides were shown to coincide with increased rainfall and river discharge, indicating that pesticide transport is strongly hydrologically driven (Bloodworth et al., 2015).

Kay and Grayson (2014) used water industry monitoring data from the Ouse catchment, Yorkshire, UK, (April 2008 - August 2011) to compare metaldehyde concentrations in raw waters to catchment characteristics (e.g. percentage cover of crop, crop type, percentage permanent grassland, soils likely to produce quick flow, and mean catchment slope). Metaldehyde concentrations were frequently measured between 0.2 to 0.4 ug/l and peaked at 2.7 ug/l. A seasonal pattern was also shown, with EU regulation exceedance most frequently between October and December, when slug pellet application is common. However, no significant relationship was found between catchment attributes and peak metaldehyde concentrations. Kay and Grayson (2014) hypothesised that although catchment attributes may not be the cause of pesticide loss, individual farm practices such as product used, application rate, technique and timing are likely to influence metaldehyde concentrations in surface waters.

Recently, metaldehyde has been modelled in the Thames catchment, where between 2011 and 2015, metaldehyde average concentration was above the drinking water quality standard at 31 of 140 catchment sites (Lu et al., 2017, Council Directive, 1998). Metaldehyde concentrations in surface waters were strongly linked to application rates, particularly during years where a warm winter was followed by a wet summer and autumn.

1.6 Metaldehyde in arable soils and crops

Metaldehyde residues have also been reported in crops and fauna (Iwata et al., 1982, Moreau et al., 2015), although there is little mention of soil metaldehyde in the academic literature. Only one paper, by Calumpang et al. (1995), specifies metaldehyde in pellet form as opposed to powdered or granular (table 3).

In a study by Zhang and Dai (2006) which sought to determine the behaviour of metaldehyde in granular form applied to tobacco crop and soils in China. Sorption to soils was observed to be highest four days after treatment, declining to near the limit of quantification after twenty-one days. The overall half-life was four days.

Zhang et al. (2011) reported that metaldehyde half-lives in cabbages and soils ranged between 0.75 – 1.02 days at three different locations. They concluded that metaldehyde degradation in soils was not affected by weather, soil type, pH or moisture content. The difference between half-life in this study compared to Zhang and Dai (2006) was suggested to be due to metaldehyde form, where Zhang and Dai (2006) used granules and (Zhang et al., 2011) used wettable powder in solution. However, in research of pellet degradation in rice paddy soils, Calumpang et al. (1995) cited a half-life of 0.27 days, much less than the studies by Zhang and Dai (2006) and (Zhang et al., 2011) and against their theory of longer half-life with larger metaldehyde forms.

Ma et al. (2012) studied the dissipation of metaldehyde granules in cabbages and soils, and reported an average soil half-life of 3.17 days. In this study, metaldehyde was extracted from soils using a centrifuge and analysed using LC-MS-MS. Residues in the soils varied widely across the three study locations, ranging between 0.02 and 7.32 mg/kg at 5 days, 0.01 and 1.00 mg/kg at 7 days and 0.001 and 0.98 mg/kg at 10 days (Ma et al., 2012). After 7 days all of the soil residues were below the maximum residue limit for cabbage crops in China, 1 mg/kg (Institute for the Control of Agrochemicals: Ministry of Agriculture: China, 2017), which is the same as in Europe (European Commission, 2011).

Table 3: Laboratory derived metaldehyde K_{OC} and half-life values

Source	Metaldehyde form	Soil half-life, DT_{50} (days)	Metaldehyde residues in soil (mg/kg) or soil residue information
Residues of metaldehyde in tobacco and soil (Zhang and Dai, 2006)	Granules	4.00	0.04 after 21 days, near LoQ
Dissipation of metaldehyde residues in cabbages and soil (Ma et al., 2012)	Granules	3.17	Detectable up to 7 days after application
Metaldehyde in a rice paddy ecosystem (Calumpang et al., 1995)	Pellets	0.27	1.58 – 1.47 between 1 to 3 days. 0.053 – 0.127 at 3 to 14 days after application. Peak was at day 14.
Residues of metaldehyde in cabbage and soil (Zhang et al., 2011)	Powder solution	0.75 – 1.02	Below 1 mg/kg after 5 days, below LoD after 10 days.
Residues and dissipation of metaldehyde in pakchoi and soils (Dong et al., 2017)	Powder solution	2.30 – 2.40	Below LoQ after 14 days.

1.7 Metaldehyde Pellets

Metaldehyde is most commonly used in a blue-dyed wheat-based pellet form, although it can be found in granular or powder (applied in solution) forms (Cardoso et al., 2015). Slug pellets are typically applied using quad bikes fitted with a hopper which distributes he pellets from the rear of the machine. Current UK commercial pellets use metaldehyde concentrations of either 1.5% or 3% and are processed using a 'wet' or 'dry' method. According to Hewson-Fisher (2015) dry-processed pellets are cheaper but breakdown more quickly in the field, lasting 3-7 days under rainfall in comparison to 21 days recorded during a 'wet pellet' trial. The manufacturing process of the wet pellets gives them some elasticity, enabling them to expand in humid conditions, rather than disintegrating.

The extent to which the physical integrity of the pellet affects the fate and transport of metaldehyde itself is currently unknown. At the time of writing, to the best of the author's knowledge, there is no published information on the effect of physical degradation at the point of application for metaldehyde pollution. Whilst it is possible for metaldehyde to degrade within the pellet itself and to leach out of the pellet matrix, it is possible that these processes may be slower than equivalent processes operating in soils.

Pellets can often be broken during the distribution process. Partially disintegrated pellets will have a greater surface area to volume ratio than intact pellets and may, therefore, be more prone to leaching losses. Physical degradation, therefore, has the potential to affect environmental contamination with metaldehyde. As crops are most at risk during the days following germination, a more persistent pellet would have a larger time frame in which to influence the slug population, thereby extending the crop protection period. However, if a pellet is more stable in the environment, (i.e if it breaks down more slowly) there is a greater chance of metaldehyde leaching to water (if metaldehyde degradation is reduced within the pellet compared to in the soil). Calumpang et al. (1995) suggest that the pellets may "*constantly release*" metaldehyde, insinuating that the pellets do not have to completely degrade before metaldehyde leaching, allowing metaldehyde to enter the soil phase potentially from day zero. Because of metaldehyde' s stability, moderate soil mobility and aqueous

solubility, run-off from treated land is implicated to provide an uncontrolled input of contamination to surface waters which is exacerbated by re-application of pellets after rainfall (Busquets et al., 2014).

1.8 Metaldehyde Management

Particularly high metaldehyde concentrations in rivers were reported in 2012 (Food and Environment Research Agency, 2017). This was a particularly wet, mild summer and autumn which resulted in high mollusc populations in combination with rapid water run-off from treated land (Choi et al., 2004, Marshall, 2013). Although there is no set rule to determine slug abundance, more slugs typically appear at night in wet, mild weather where the temperature is above 5°C and soil is moist (Port et al., 2012). Factors such as air temperature, wind speed, soil moisture, humidity and soil temperature have all been shown to influence population size (Choi et al., 2004). As a result, molluscicide can be applied all year round, although is most commonly associated with drilling of spring and autumn crops when combined conditions support slug presence and crop growth.

Between 2010 and 2015 796 tonnes of metaldehyde was applied to Great British soils (Food and Environment Research Agency, 2017), rising sharply from 1993 when stubble burning, a traditional mollusc-prevention method, was banned (Ministry of Agriculture Fisheries and Food, 1993). Crop coverage also increased during this time from 53,527 ha of treated land in 1990 to 944,378 ha treated land in 2015 (Food and Environment Research Agency, 2017). However it is estimated that in the UK, up to £100 million in product losses could occur annually as a result of ineffective slug control (Castle et al., 2017).

The two main management measures available to reduce metaldehyde contamination of surface waters are improved water treatment and catchment management. Although treatment is often ineffective, it is possible to remove metaldehyde from water sources. The simple chemical structure of metaldehyde in combination with its high polarity makes it difficult to remove using conventional treatment processes such as sorption to activated carbon sites or ozonation (Castle et al., 2017, Busquets et al.,

2014, Autin, 2012). Alternative proposed treatment methods include advanced oxidation using UV/TiO_2 and UV/H_2O_2 (Autin et al., 2013, James et al., 2014); photocatalysis using nano-sized zinc oxide composites (Doria et al., 2013); adsorption using phenolic carbon (Busquets et al., 2014) and coupled adsorption with electrochemical destruction (Nabeerasool et al., 2015). However, significant cost and practical or time limitations have so far prevented large scale, efficient treatment of raw waters.

An alternative to removing metaldehyde in water treatment is to attempt to control the problem at the source (Marshall, 2013). A range of so-called catchment management options exist which involve changing pesticide use or land management practice with the aim of reducing land to water transfers. Current advice for managing metaldehyde includes thinking in terms of 'slope, soil and stream' (Environment Agency, 2016) including monitoring soil drainage, artificial field drain flow, proximity to water courses, meteorology, practices in filling and washing areas for the pellet applicator and taking into account the slope of the land. However, with so many environmental factors that can effect pesticide transport finding the most practical methods of reducing pesticide leaching is vital to lessen pollution.

Pellets are generally applied to soils on an 'as-needed' basis, therefore there is not a strict number of pellet applications a farmer can make per year, although restrictions do apply based on the absolute concentration of metaldehyde applied per hectare per year. Although pellets are used all year round, autumn has a higher metaldehyde application due to winter crop drilling and good mollusc conditions in this period. The decision to apply is generally made based on weather conditions and visible pellet presence, if an application had already been made. The dye in the pellets is therefore an important factor in showing their presence at the soil surface as an indicator of whether re-application is required.

One way of tackling pesticides at source is to increase metaldehyde awareness by producing guidelines for farmers and other pesticide users at the national level about best practice. The Metaldehyde Stewardship Group is an industry-led group which promotes good slug pellet practice for water protection. It has produced guidelines for prevention of water pollution as part of its '*Get pelletwise*' campaign launched in 2009

(Metaldehyde Stewardship group, 2009). These include using the minimum active ingredient application per hectare in order to reduce drainage and runoff losses; not applying pellets within 6 metres of a watercourse; not applying pellets if drains are flowing or if heavy rain is forecast; using a maximum total dose rate of 700g metaldehyde per hectare per calendar year; and using a maximum of 210g metaldehyde per hectare with a recommendation of 160g metaldehyde per hectare (Environment Agency, 2016). Metaldehyde usage statistics suggest that metaldehyde application rates, which peaked in 2009 has since decreased annually (Food and Environment Research Agency, 2017), possibly in direct response to improved management advice.

Another example of a voluntary initiative involving farmers, land owners, agronomists and water companies is the '*Slug it out!*' campaign started by Anglian Water in June 2015. This campaign aims to reduce metaldehyde in regional surface waters before abstraction at treatment works (Anglian Water, 2015). To do this, farmers were offered financial incentives to use alternative pellets containing ferric phosphate rather than metaldehyde. Information and discussion sessions were also given to farmers about sustainable practice. In the first year of the campaign a 60% decrease in reservoir tributary metaldehyde concentrations was observed (Anglian Water, 2016). Although scaling-up voluntary initiatives to large river catchments or a nationwide scheme may prove impractical (Marshall, 2016), '*Slug it out!*' will double in area for its second year. In many studies, catchment management is expected to be the most effective way to reduce excessive metaldehyde concentrations in surface waters given the inability of current treatment techniques (Kay and Grayson, 2014). However, without detailed understanding of metaldehyde application, transportation and transformation by all parties involved, management of the metaldehyde problem will remain an issue.

The success of the '*Slug it out!*' campaign could be attributed to cooperation between all stakeholders, but also to the use of alternative molluscicide pellets which reduced overall metaldehyde input in that region. Water UK advises that although many management techniques have been considered, reducing overall pellet application may be most beneficial way to significantly reduce metaldehyde concentrations in the

environment (Marshall, 2016). They also stated that targeted regulatory mechanisms would be most appropriate to reduce metaldehyde prevalence, and that a nationwide ban of metaldehyde could not currently be justified (Marshall, 2016).

Modelling of metaldehyde inputs and loss rates across catchments with different soil types and varied topography (Lu et al., 2017) is being used by water companies (Nineham et al., Retrieved December 2017) to understand catchment dynamics (e.g. the timing of metaldehyde transfers) and to assess catchment management options (e.g. targeting interventions in the parts of the catchment where loss to water is most likely). This allows many different variables to be considered within a catchment and the impact of simulated management options can provide better advice for land users. However, this process can be time-consuming, costly and inhibited by a lack of data. As it has only recently been implemented publically, the effectiveness of modelling for metaldehyde management is yet unknown, although its potential is large given effective modelling uses elsewhere in the water industry.

1.9 Human Health and Ecology

Metaldehyde has a low toxicity to humans, only having a serious impact at concentrations higher than 100 mg/kg (Ellenhorn, 1997). However, it has been regulated since 1980 (Bullock, 2014) because of harm caused to domestic animals. Cases have also been recorded relating to wild animals (Barnett et al., 2002, Barnett et al., 2003). It is considered moderately toxic in some animals and is the second most common cause of poisoning in dogs (Castle et al., 2017). However, it is also highly specific is believed to have low toxicity to organisms such as earthworms, spiders and ants, who share mollusc habitat, when applied at recommended dose (Cardoso et al., 2015).

1.10 Ferric Phosphate alternative to Metaldehyde

In comparison to metaldehyde, ferric phosphate has its own ecological impacts. Ferric phosphate itself is a relatively benign substance, however the activators within ferric

phosphate which release the toxic iron component are not benign. Chelating agents such as Edetic Acid (EDTA) are known to be toxic to soil biota, including earthworms (National Center of Biotechnology Information, Edwards et al., 2009). It is important, therefore, to note that ferric phosphate is not an alternative without consequence.

In addition to the difference in ecological toxicity, metaldehyde, as an organic pesticide, is subject to specific regulation targets, whereas ferric phosphate, as an inorganic substance comprising phosphorous, iron and chelating agents such as EDTA (all 'non-pesticides'), is not subject to WFD compliance. As inorganic additions to the watercourse, measuring the contribution of ferric phosphate to water quality is almost impossible given other common diffuse phosphate and iron sources, such as fertilisers. As a result, ferric phosphate may be more difficult to regulate than metaldehyde which can be measured, if not currently removed from the watercourse.

1.11 Literature Review Summary

Because of the presence of metaldehyde in raw surface waters and the difficulty in removing it during water treatment, better understanding of how slug pellets function in the terrestrial environment is required in order to generate specific management solutions and advice. Laboratory experiments show metaldehyde to have varying half-life values in soil, between 3.17 and 223 days (Kay and Grayson, 2014) and up to 6150 days in water (Kegley et al., 2016). Some in-field experiments have produced lower soil half-life values ranging between 0.27 and 4.00 days (Calumpang et al., 1995, Zhang and Dai, 2006) which were consistent between studies. Metaldehyde form has also reportedly influenced half-life, although there is not currently a clearly defined relationship between product format and half-life value.

Soil sorption coefficients also widely vary (table 2), with metaldehyde being described as both 'persistent and mobile' (Kay and Grayson, 2014). However, its presence in UK waterbodies suggests that properties gleaned from laboratory practice do not always represent its actual environmental behaviour. As a result, there is a need to further study the fate of metaldehyde in soils, especially when applied in various pelletised

forms which may influence metaldehyde longevity and propensity for mobilisation from soil to waterbodies.

As a relatively 'new' environmental issue, there are many unanswered questions surrounding metaldehyde. It is not known how much metaldehyde leaches from the pellet and whether this can occur immediately after application. It is likely that this will be dependent on environmental conditions, such as soil moisture content, but may require the pellet or breakdown before leaching can occur. Calumpang et al. (1995) suggested that metaldehyde would be consistently released from applied pellets. However, there is currently little further evidence to confirm this. The impact of the pellet itself on metaldehyde half-life is also unknown. Laboratory experiments have tended to use powdered metaldehyde in solution only and solely focused on degradation of the chemical itself. Similarly, the majority of in-field experiments have used powdered or granular forms. Factors influencing pellet application, such as pellet colour and fragility may also influence environmental burdens and fate.

Without understanding how slug pellets are physically broken down, how metaldehyde is degraded and how it is leached in soils, effective management strategies cannot be developed. This research consists of three experiments to determine different factors surrounding metaldehyde breakdown in an arable setting, therefore aims to better-understand the behaviour of pellets containing metaldehyde and of metaldehyde itself in the soil environment.

1.12 Aims and Hypotheses

Experiment One

Experiment one was an integrated fate assessment study to evaluate the combined effect of physical disintegration, sorption to soil solids and leaching under realistic rainfall conditions. Experiments were conducted using intact soil columns. Slug pellets were placed on top of the columns and leachate was collected from beneath them. The experiment compared two different soil types and two different pellet types.

Research questions:

1. To what extent does soil type and pellet type influence metaldehyde leaching under realistic environmental conditions?
2. To what extent does metaldehyde leach from intact soil columns under realistic environmental conditions?
3. Does metaldehyde leach from the pellet before total physical pellet degradation?

Research hypotheses:

1. Higher concentrations of metaldehyde will be leached from clay soil cores in comparison to loam soil cores due to preferential pathway formation and lower organic content
2. Metaldehyde will leach from the soil cores following storm events
3. Initially, higher concentrations of metaldehyde will be leached from dry-processed pellets because they are likely to physically breakdown faster
4. Wet-processed pellets will last for physically longer on the soil surface than dry-processed pellets
5. Metaldehyde will leach from both pellet types before total physical breakdown

Experiment Two

In vitro incubations were conducted to quantify the half-life of metaldehyde in the laboratory. The specific aim here was to compare metaldehyde longevity when applied in solution, as is typically done in standard degradation tests, with metaldehyde fate when applied as pellets. Two different soil types were spiked using powdered metaldehyde in solution and two different types of slug pellets (wet-processed and dry-processed). Although this experiment is not environmentally realistic, it was intended to set a benchmark for slug pellet degradation in comparison with the same substance in solution.

Research question:

1. Does the physical casing of the two pellet types influence metaldehyde half-life, particularly in comparison to powdered metaldehyde?

Research hypothesis:

1. Metaldehyde will have the highest longevity in the wet-processed pellets, followed by dry-processed pellets and then powdered metaldehyde, which will breakdown fastest
2. Metaldehyde longevity will be highest in the clay soil, which has lower organic matter content (therefore an expected lower biological activity to degrade metaldehyde)

Experiment Three

The physical disintegration of pellets under realistic environmental conditions was investigated using a number of sub-experiments. Two pellet types were placed on soils and irrigated to maintain environmentally realistic soil moisture contents. Physical degradation was recorded based on visible pellet surface area and colour. In another sub-experiment, two pellet types were subjected to simulated high-intensity rainfall and physical changes were recorded based on pellet weight and visible surface area. In a third experiment, pellet colour and visible surface area changes were observed when pellets were exposed to integrated outdoor conditions.

Research questions:

1. How quickly do the different pellet casings physically breakdown under realistic environmental conditions, under artificially-irrigated conditions and under simulated rainfall conditions?
2. Does the colour of the different pellet types change over time under irrigated and environmentally realistic conditions, and to what extent?

Research hypothesis:

1. Soil moisture will be a driver for physical degradation where increased soil moisture will result in faster degradation
2. Kinetic energy from rainfall impact will be a driver for physical degradation where prolonged exposure to high intensity rainfall will cause faster pellet degradation

3. Pellets will expand in visible surface area immediately following storm events, before decreasing in visible surface area. Wet pellets will expand more than dry pellets.
4. Dry-processed pellets will reduce in visible surface area faster under all experiment conditions in comparison to wet-processed pellets
5. Colour for both pellet types will change over time

Each experiment is described as a discrete chapter of this thesis. Each chapter contains the methodology, results and discussion specific to that research, including answering the research questions and hypotheses specific to that chapter. A final chapter, chapter five, brings the results of all the experiments together and attempts to integrate them in a general discussion as well as drawing conclusions on the overall investigation.

Chapter five will discuss the following overarching research questions:

1. What is the overall influence of soil type on metaldehyde pellet degradation (physical casing and chemical metaldehyde degradation)
2. What is the overall influence of metaldehyde pellet type (wet-processed verses dry-processed pellets) on degradation?
3. Is metaldehyde effected by residuality?

Recommendations will also be made for metaldehyde management based on the conclusions drawn from this study.

1.13 Sampling Location, Soil Type and Pellets

Soil was excavated from Lyndon Farms, Rutland (LE15 8TW), shown in figure 2. The land is in the Upper Welland catchment, and is part of the Anglian Water region, situated within 2km of Rutland Water. The River Chater, a tributary of the River Welland, flows through Lyndon Farms.

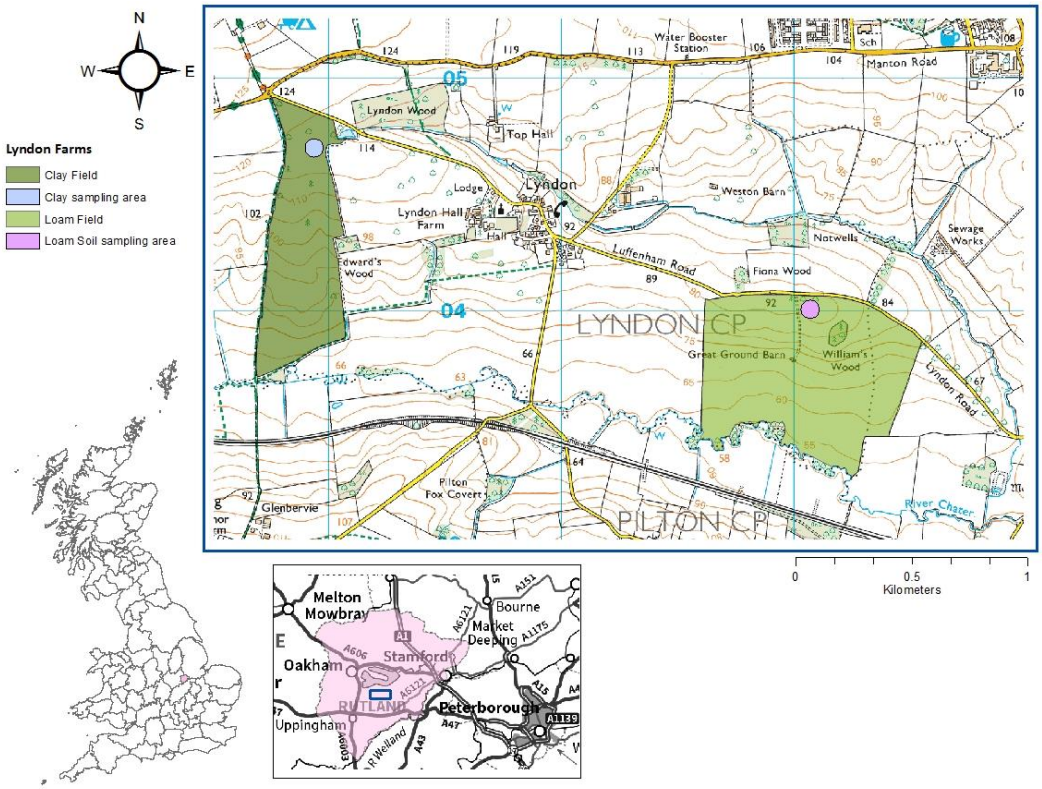


Figure 2: Map of Lyndon Farms soil sampling sites. Clay soil was excavated from 0°40'16.022"W 52°37'56.271"N and Loam soil was excavated from 0°38'23.248"W 52°37'32.668"N.

Two sampling sites were chosen to represent different arable soil types; a Loam and a clay. The loam field, to the east of Lyndon village, last received metaldehyde in November 2014. Whereas the clay field, to the west of Lyndon, last received metaldehyde in November 2015.

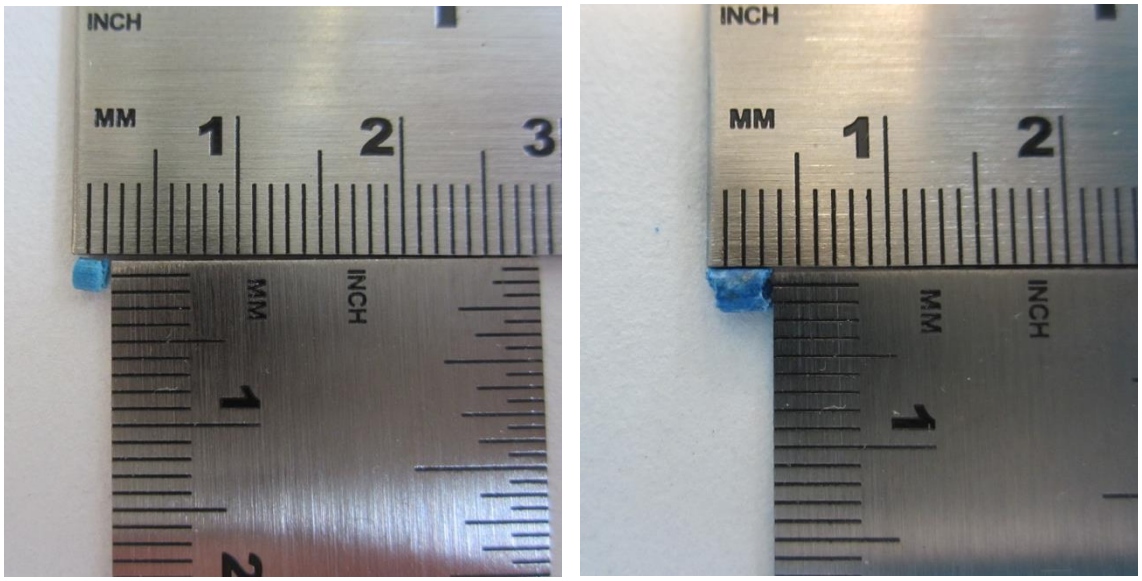
A loss on ignition test showed that the dried loam soil was, on average, 11.20 ± 0.5 % organic matter and the dried clay soil was 09.80 ± 0.1 % organic matter.

To represent commonly used agricultural slug pellets, two pellet types were chosen:

- A dry-processed pellet, brand-named '*Trigger 3*'
- A wet-processed pellets, brand-named '*Carakol 3*'.

Throughout this thesis wet-processed pellets are referred to as 'wet pellets' and dry-processed pellets are referred to as 'dry pellets'.

Dry pellets are physically larger than wet pellets (figure 3). On average, wet pellets weighed 7.5 ± 0.1 mg (CV = 12.74%) and dry pellets weighed 22.2 ± 7.0 mg each (CV = 31.45%). Dry pellets are more variable in size than wet pellets.



Carakol 3: Wet-processed slug pellet	Trigger 3: Dry-processed slug pellet

Figure 3: Wet-processed and dry-processed slug pellet size difference

Soil sampling methods have been included in the methodology specific to each experiment, but soil types are from the same sampling location throughout.

Chapter Two

Experiment 1: Integrated fate assessment

2.1 Introduction

An integrated fate assessment was conducted with the aim of quantifying the combined effect of physical disintegration, sorption to soil solids, biodegradation and leaching under realistic environmental conditions. It sought to compare the rate at which metaldehyde leached through two different soil types as it would in an arable setting. Two pellet types were used and control treatments were also employed.

2.2 Reagents

Laboratory grade, 99% metaldehyde, purchased from Acros Organics.

Wet-processed pellets, brand name *Trigger 3* were purchased from Certis as 3% metaldehyde pellets. Pellets were stored in sealed opaque bags at room temperature, away from sunlight.

Dry-processed pellets, brand name *Carakol 3* were purchased from Adama as 3% metaldehyde pellets. Pellets were stored in sealed opaque bags at room temperature, away from sunlight.

HPLC grade methanol and Laboratory grade dichloromethane were purchased from Fischer Scientific.

A 1000 mg/l metaldehyde in methanol stock calibration standard was made by weighing 50 mg ± 0.5 mg laboratory grade metaldehyde in an amber-glass 100ml Pyrex bottle. To this, 50 ml methanol was added to the metaldehyde using a 50ml glass pipette. The stock calibration standard was capped and mixed to dissolve, then stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

2000 ug/l, 1500 ug/l, 1000 ug/l and 500 ug/l working calibration standards were made by pipetting 100 ul, 75 ul, 50 ul, and 25 ul of the stock calibration standard into

individual 50 ml volumetric flasks and topping up to 50 ml with dichloromethane. Fully-dissolved solution was transferred to a screwcap glass bottle and stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

Low-concentration working calibration standards were made by first producing a 1 mg/l solution from the 1000 mg/l stock calibration standard. The 1 mg/l standard was made by adding 50 ul 1000 mg/l stock calibration standard to a 50 ml volumetric flask and topping up to 50 ml.

200 ug/l, 150 ug/l, 100 ug/l, 75 ug/l, 50 ug/l, 25 ug/l and 20 ug/l working calibration standards were made by pipetting 10 ml, 7.5 ml, 5 ml, 3.75 ml, 2.5 ml, 1.25 ml and 1ml of the 1 mg/l standard into individual 50 ml volumetric flasks. Each flask topped up to 50 ml with dichloromethane, transferred to a screwcap glass bottle and stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

Working internal standard was acquired from the Anglian Water central laboratory, Huntingdon. The 50 mg/l internal standard was made by measuring 40 ml methanol into a 100 ml pyrex bottle using a glass pipette. Using a 5ml calibrated syringe, 4ml methanol was withdrawn and discarded from the pyrex bottle. Using another 5ml calibrated syringe, 4ml stock internal standard was added to the pyrex bottle. The working internal standard was capped and mixed to dissolve, then stored in a refrigerator at 1 - 10°C.

2.3 Apparatus

2.3.1 Making the soil cores:

- 500 ml amber-glass bottles
- uPVC underground pipe cut into 160mm x 280mm pieces and finished with a bevelled edge at the bottom the pipe and an 11 mm diameter hole drilled 15 mm from the top of the pipe
- Flexible plastic tubing, 15 mm external diameter and 11 mm external diameter, and solid PEX pipe cut to 15 mm x 45 mm
- 160mm diameter end caps to fit to uPVC pipe cuttings. A 6 mm diameter hole was drilled into the centre of each cap.
- Evo-stick rapid epoxy resin glue - and pipe insulation fixing tape

2.3.2 Leachate storage and extraction:

- Cold storage room or fridge for sample storage
- Solid phase extraction cartridges. Bakerbond SDB1, 200 mg, 3 ml.
- Water purifier – Elga DV25, producing deionised water
- Vacuum manifold with attachments that will fit the solid phase extraction cartridges
- Blow-down apparatus capable of directing a gentle stream of air into a GCMS vial – Techne sample concentrator with compressed air

2.3.3 Analysis:

Gas chromatograph and mass spectrometer – Perkin Elmer Clarus 500:

Column: 30m x 250 µm diameter

Carrier gas: Helium, 1 ml per minute

Injection temperature: 280 °C

Injection volume: 1 µl (pulsed split-less injection)

Temperature programme: Oven

Initial temperature 40.0 °C for 2 minutes, then 20.0 °C per minute to 250 °C and hold for 0.00 minutes.

Equilibration time: 1 minute

Total run time: 12.50 minutes

SIM: Solvent delay 0.00 to 4.90 minutes

SIM of 4 masses, monitored 6.35 minutes to 6.58 minutes in EI+ ionisation mode

Using these conditions, the following applies:

Table 4: Metaldehyde retention times for GCMS

Compound	Approximate retention time (minutes)	Ions monitored	
D16 metaldehyde	6.41	50.0	98.0
Metaldehyde	6.46	45.0	89.0

TurboMass software was used to tune and calibrate the GCMS, and calculate area responses from chromatogram sample peaks.

Method

2.4 Soil column creation and leachate collection

Eighteen soil columns were created from one length of uPVC underground pipe. Each column measured 160mm in diameter and 280 mm in length with one edge bevelled to aid soil extraction. Eighteen soil cores were extracted from two different fields at Lyndon Farms (figure 2), with nine cores for each soil type; loam soil and clay soil. Cores were extracted by carefully pushing the columns into the ground bevelled edge first and digging around the columns, maintaining the soil structure and repeating as necessary until the soil was 40 mm from the column top. Any excess soil at the bottom of the core was cut off with a palette knife. The complete cores were then extracted from the ground and fitted with a 160 mm end cap which acted as a base for the core (figure 4). The end caps were pre-drilled with a 6 mm hole in the centre to allow the core to drain whilst fitting snugly to the column pipe. Pipe insulation fixing tape was used to seal the end cap to the column, further preventing water entry or leaking at that join (figure 5). A 10 mm layer of sand was positioned between the end cap and the soil horizons to promote free draining.

Figure 4: Soil column extraction process. From bottom left, clockwise: pushing the columns carefully into the ground using a mallet and wood; digging around the columns before pushing them deeper into the ground or extraction (loam soil, left; clay soil, right); cutting excess soil from the column base; adding a 10 mm layer of sand to the base of the soil core.

To collect leachate, a 15 x 46 mm length of PEX piping was resin-glued to the base of the end cap, completely surrounding, without covering, the 6 mm drainage hole. Pipe insulation fixing tape was also used to strengthen the bond between the PEX piping and the end cap. Once secured, flexible 15 mm external diameter tubing (12 mm internal diameter) was stretched over the PEX piping to create a watertight seal. The other end of the flexible tubing was inserted into a 500 ml amber-glass bottle with a 15 mm hole drilled into the screwcap top, also making a watertight seal to ensure only leachate entered the bottle.

An overflow system was added to the soil cores to prevent water or pellet loss from over the column top. An 11 mm hole was drilled into the soil core, with the centre 15 mm from the lip of the column into which an 11 mm external diameter (8 mm internal) tube was inserted, connecting the top of the column to an overflow collection bottle. The overflow flow bottles were the same as the leachate collection bottles, except with an 11 mm hole drilled into the screwcap top. Cores were spaced evenly apart.

Figure 5: Soil core set-up at Brookfield. From bottom left, clockwise: Gluing PEX piping over the drainage hole; Bottle attached to PEX piping using flexible tubing; Completed set-up for the clay soil cores; overflow bottles attached to cores using flexible tubing. The Loam soil set-up up was identical to the clay soil set-up.

Completed soil cores were labelled and placed in an open, outdoors location at the University of Leicester Brookfield Campus (LE2 1RQ) and left subject to weather conditions. For each soil type, three cores received dry-processed pellets, three received wet-processed pellets and three were 'control' cores with no pellets. Cores were labelled 'C' for clay soil or 'L' for loam soil and numbers were used to indicate pellet application. Cores 1, 2 and 3 received dry-processed pellets, cores 4, 5 and 6 received wet-processed pellets and cores 7, 8 and 9 received no pellets.

Each pellet-receiving soil core received double maximum dose per hectare per year. Maximum total dose for both pellet types is 700g metaldehyde/ha/year, equivalent to 23.33 kg pellets/ha/year for the 3% metaldehyde pellets used, assuming each pellet contained exactly 3% metaldehyde. Each soil core was equivalent to 0.00000201 ha, therefore 0.093829 g pellets/soil core was equivalent to double the maximum total dose per annum. Pellets were weighed out as close as possible to 0.093829 g (table 5).

Soil cores were erected on 16/03/2017 and pellets were applied on 24/03/2017. Bottles were unattached on 28/07/2017 before being re-attached on 06/09/2017 and re-applying pellets on 26/09/2017. Sampling phase two finished on 26/10/2017.

Table 5: Pellets weights per soil core for the integrated fate assessment,, equivalent to double the maximum total dose per hectare per year. Pellets weighed 0.09383 ± 0.0033 g. Mean pellet weight applied to cores = 0.09312 g, standard deviation = 0.001544 g. Mean metaldehyde dose = 0.002793 g, standard deviation = 0.00004633 g. Applications refer to the two sampling phases

First Application	Loam Core number	Pellet weight applied (g)	Number of pellets	Metaldehyde dose to core; 3% active substance (g)	Clay core number	Pellet weight applied (g)	Number of pellets	Metaldehyde dose to core; 3% active substance (g)
Dry pellet cores	L1	0.0948	4	0.002844	C1	0.0962	5	0.002886
	L2	0.0937	4	0.002811	C2	0.0914	4	0.002742
	L3	0.0929	5	0.002787	C3	0.0916	3	0.002748
Wet pellet cores	L4	0.0920	13	0.002760	C4	0.0953	13	0.002859
	L5	0.0916	13	0.002748	C5	0.0946	13	0.002838
	L6	0.0924	14	0.002772	C6	0.0919	12	0.002757

Second application	Loam Core number	Pellet weight applied (g)	Number of pellets	Metaldehyde dose to core; 3% active substance (g)	Clay core number	Pellet weight applied (g)	Number of pellets	Metaldehyde dose to core; 3% active substance (g)
Dry pellet cores	L1	0.0916	4	0.002748	C1	0.0934	4	0.002802
	L2	0.0923	4	0.002769	C2	0.0913	5	0.002739
	L3	0.0937	5	0.002811	C3	0.0932	5	0.002796
Wet pellet cores	L4	0.0906	13	0.002718	C4	0.0957	12	0.002871
	L5	0.0938	14	0.002814	C5	0.0919	12	0.002757
	L6	0.0937	13	0.002811	C6	0.0953	13	0.002859

Leachate was collected depending on rainfall events. A rain gauge at the University of Leicester Main Campus, 996 metres as-the-crow-flies from Brookfield, was used to record rainfall. Overflow bottles were collected only when necessary. Bottles were removed and replaced with equivalent bottles, ensuring the same two bottles were always used on the same core and washed with deionised water and dried between uses. Once collected, leachate and overflow bottles were stored in the cold store and prepared for GC-MS as soon as possible, then stored in GC-MS vials in the fridge for a maximum of three weeks until analysis. Metaldehyde is thought to have a hydrolysis half-life of 6,150 days (Kegley et al., 2016), therefore variation in sample storage time before elution, both at Brookfield and in the cold store, was assumed to have negligible effect.

2.5 GC-MS preparation and analysis

Using solid phase extraction (SPE), leachate was prepared for GC-MS analysis. Baker SDB1 200mg 3ml cartridges were eluted with 10 ml methanol, followed by 2 ml deionised water, a variable but known volume of sample and 2 ml deionised water respectively, using a vacuum manifold within a fume hood. 5 ul of working internal standard was added to the cartridge before sample elution. During elution the meniscus of the eluent was prevented from falling below the cartridge packing material and the volume of eluted sample was noted for each individual cartridge. Cartridges were dried by passing air through them using the vacuum manifold within the fume hood and all elution waste was discarded via chemical waste disposal.

Once dried, cartridges were stored in a fridge until elution into GCMS vials. To elute into GCMS vials, cartridges were fixed above the vials using a clamp and stand, and 2 ml of dichloromethane (DCM) was pipetted into the top of the cartridge, draining under gravity. Using a stopper attached to some tubing and a syringe, any remaining DCM in the cartridge could be gently pushed out under pressure. The total DCM fraction in the GCMS vial was capped and stored until GCMS analysis. Just before analysis, DCM was evaporated in room conditions, or very gently by passing air over the vials, to 0 ml. 1 ml DCM was subsequently added to each vial to ensure the same volume solution in each for analysis. 1 ml of each individual working calibration standards was pipetted into 1 ml GCMS vials with 5ul of working internal standard in each and analysed alongside the sample vials.

Samples and calibration standards were analysed on the GCMS using selective ion monitoring (SIM). This isolates peak responses for metaldehyde and deuterated metaldehyde (the internal standard) by only targeting ions of interest and therefore maximising sensitivity. A DCM wash was used between each sample to prevent cross-contamination.

2.5.1 LoD and LoQ

Limit of detection (LoD) and limit of quantification (LoQ) were calculated using the following equations, described in ICH Validation of Analytical Procedures (European Medicines Agency, 2005) using the standard deviation of response (σ_R) and the slope of the calibration curve (m):

$$LoD = 3.3\frac{\sigma_R}{m}$$

$$LoQ = 10\frac{\sigma_R}{m}$$

The standard deviation of the response was approximated by the standard error of the intercept of the calibration curve, which was derived using the LINEST function in MS Excel. A statistical explanation of LoD and LoQ can be found in Appendix A.

2.5.2 Metaldehyde concentration calculation

Following GCMS analysis, each sample and working calibration standard had two response peaks if metaldehyde was present. The first peak, at 6 minutes 40 seconds was the deuterated metaldehyde peak and the second peak at 6 minutes 41 seconds was the metaldehyde peak. Integrating these peaks, the response area was noted and used to calculate metaldehyde concentration.

To create the known concentration calibration curve the ratio between the metaldehyde response area and the deuterated metaldehyde response area for each of the working calibration standards was calculated and plotted against its nominal concentration (figure 6). Each calibration curve was also used to calculate LoD and LoQ for its associated sample lot.

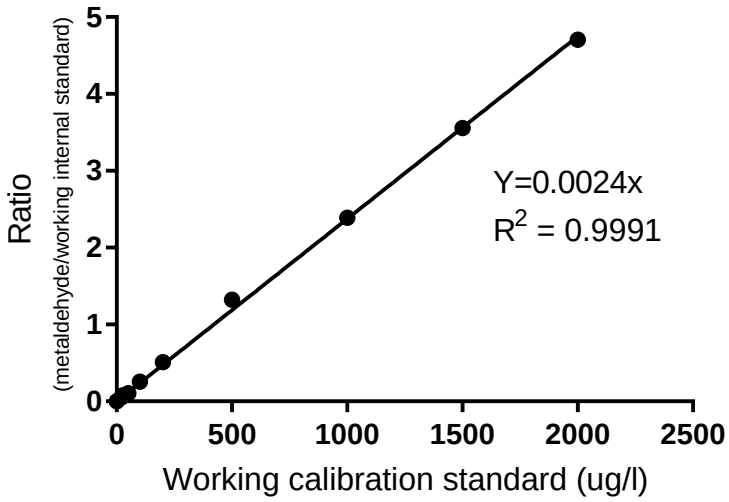


Figure 6: Example of a calibration curve taken from the integrated fate assessment, sampling period 8. Nominal concentrations were plotted against the area response ratio of metaldehyde to working internal standard. The equation of the linear line of best fit is Y=0.0024x when the intercept is 0. This equation is used to calculate sample concentration from sample response area. Without a forced intercept the linear line of best fit is Y=0.0024 + 0.0269 and R^2 = 0.9993. This equation is used to calculate LoD and LoQ

For each sample, the ratio between the two response peaks was calculated. From this the concentration of metaldehyde in the GCMS vial was calculated using the equation of the calibration curve, assuming the equation intercept was 0. By assuming an intercept of 0, the concentration in DCM reflected by the calibration curve would be 0 ug/l if there was no response peak for metaldehyde; thus 'ghost concentrations' could be eliminated. The calculated concentration in DCM (ug/l) was multiplied by the volume of sample in the GCMS vial (0.001 L) to find the mass of metaldehyde in DCM. From this the concentration of metaldehyde in the original sample could be found by dividing the mass in DCM (ug) by the water sample volume extracted (L). Applying the LoD and LoQ values for that sample lot's calibration curve, sample metaldehyde concentrations could be stated to a 95% statistical significance.

An overview of the integrated fate assessment method development can be found in Appendix B.

2.6 Results

In the following results section, treatment types are referred to using acronyms.

Loam soil = L; Clay soil = C; Wet pellets = W; Dry pellets = D; Laboratory grade metaldehyde = M.

Therefore a loam soil with laboratory grade metaldehyde treatment type would be referred to as 'LM'.

Samples obtained during the integrated fate assessment were split into two sampling phases. The first phase was between 16/03/2017 and 28/07/2017 (total duration 135 days, 127 days with pellets applied) and the second phase was between 06/09/2017 and 26/10/2017 (total duration 50 days, 30 days with pellets applied). Within each sampling phase, sampling periods differentiated between collected sample lots over time.

2.7 Sampling Phase One: All data

Metaldehyde samples obtained in the first sampling phase were treated as discrete sample lots. Cores were established on 16/03/2017 and pellets were applied to the cores on 24/03/2017 (taken as day 0), therefore sample one is pre-pellet application. There were eight sample collections during sample phase one, referred to as sampling periods 1 to 8.

Only samples collected on the 18/05/2017 (day 55) and 22/05/2017 (day 59) contained any statistically measurable metaldehyde and these samples also had the lowest LoD and LoQ values (table 6). As such, these two sample sets can be viewed with respect to time also.

On the 18/05/2017 (day 55), all clay leachate bottles had metaldehyde present except for core C7. Only leachate bottles from L4, L8 and L9 loam soil cores contained metaldehyde.

On the 22/05/2017 (day 59), all pellet-receiving clay cores and all pellet-receiving loam cores, except L6, contained metaldehyde.

Control cores L8, L9, C8 and C9 all had measurable metaldehyde responses on the 18/05/2017 (day 55), although only core C9 had a recordable response on 22/05/2017 (day 59).

Table 6: Sampling Phase One collection information, including collection dates, total rainfall, field duration, average temperature, leachate volume, LoD/LoQ and metaldehyde concentrations

Sample period			1	2	3	4	5	6	7	8
Collection date (dd/mm/2017)			24/03	03/04	18/05	22/05	06/06	09/06	07/07	28/07
Total rainfall in sampling period (mm)			0	5.4	58.2	23	42.4	13.4	19.2	54
Sampling period field duration (days)			3	10	45	4	15	3	28	21
Cumulative days since pellets applied (days)			0	10	55	59	74	77	105	126
Average Temperature (°C)			8.24	9.53	9.96	11.4	16.8	13.3	17.8	17.1
Leachate volume range (ml)			12.20 – 20.00	3.900 – 30.80	15.20 – 195.1	41.90 – 258.7	24.10 – 214.0	9.300 – 283.6	38.00 – 86.00	70.00 – 280.0
LoD in DCM (ug/l)			235.5	168.8	12.10	12.10	62.30	62.30	62.30	62.30
LoD range in water (ug/l)			11.775 – 19.30	5.481 – 43.28	0.0620 – 0.7961	0.0468 – 0.2888	0.2911 – 2.585	0.2197 – 6.699	0.7244 – 1.639	0.2225 – 0.8900
LoQ in DCM (ug/l)			713.5	511.6	36.50	36.50	188.8	188.8	188.8	188.8
LoQ range in water (ug/l)			35.68 – 58.48	16.61 – 131.2	0.1871 – 2.401	0.1411 – 0.8711	0.8822 – 7.834	0.6657 – 20.30	2.195 – 4.968	0.6742 – 2.697
Treatment type										
Concentration in sample (ug/l)	L1	Dry	<LoD	<LoD	<LoQ	45.95		<LoD	<LoD	<LoD
	L2	Dry	<LoD	<LoD	<LoD	33.45			<LoD	<LoD
	L3	Dry	<LoD	<LoD	<LoD	16.62			<LoD	<LoD
	L4	Wet	<LoD	<LoQ	3.028	51.07			<LoD	
	L5	Wet	<LoD	<LoD	<LoD	21.06		<LoD	<LoD	
	L6	Wet	<LoD		<LoD	<LoD		<LoD	<LoD	<LoD
	L7	Control	<LoD	<LoD	<LoD	<LoD			<LoD	<LoD
	L8	Control	<LoD		3.310	<LoD			<LoD	<LoD
	L9	Control	<LoD		1.641	<LoD			<LoD	<LoD
	C1	Dry	<LoD		134.7	153.5			<LoD	<LoD
	C2	Dry	<LoD	<LoD	27.65	153.2	<LoD		<LoD	<LoD
	C3	Dry	<LoD	<LoD	403.9	237.8			<LoD	<LoD
	C4	Wet	<LoD		7.716	1.111	<LoD		<LoD	
	C5	Wet	<LoD		83.81	311.6	<LoD			<LoD
	C6	Wet	<LoD		0.6079	6.205				<LoD
	C7	Control			<LoD	<LoD	<LoD	<LoD	<LoD	
	C8	Control	<LoD		0.5920	<LoD	<LoD			<LoD
	C9	Control	<LoD		3.835	<LoQ	<LoD	<LoD	<LoD	<LoD

2.7.1 Rainfall and Temperature

Across 135 days in sampling phase one only 50 days had rainfall (figure 7). The highest monthly rainfall was during May, totalling 83.8 mm in comparison to 63 mm and 65.6 mm in June and July respectively, and 7.6 mm and 11 mm rainfall in March and April. The largest rainfall event occurred on 17/05/2017 (day 54), with 21.8 mm rain occurring in 9 hours at an intensity of 2.4 mm/hour.

Across all months the average rainfall was 1.6 mm per day, although in March and April the average was <0.48 mm/day and in May, June and July the average was >2.1 mm/day. A Kruskal-Wallis test found no significant difference between mean rainfall for each month ($p>0.05$). A Kruskal-Wallis test was used because a Bartlett's test was found to be significant ($p<0.0001$) and, therefore, the data did not fit a Gaussian distribution (ANOVA requirements). A one-way ANOVA also found no significant difference between mean rainfall for each sampling period ($p>0.05$).

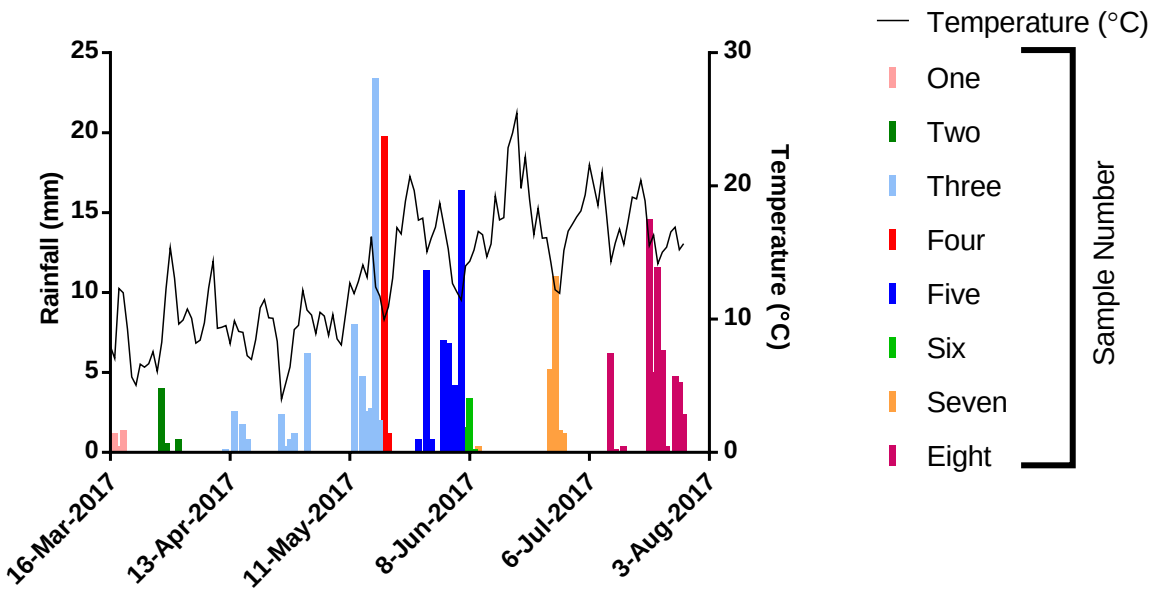


Figure 7: Rainfall and temperature for sampling phase one

Temperature also varied across sampling phase one, ranging from an average 8.9 °C in March to 17.4 °C in July (figure 7). Differences between months and sampling periods were shown to be significant. A one-way ANOVA found a significant difference to the 99.99% confidence interval ($p<0.0001$) between mean temperature for each sampling period. Using a Tukey's multiple comparisons test with single pooled variance, the following sampling periods had significantly different mean temperatures (table 7):

*Table 7: Tukey's comparison showing significant differences in temperature between sampling periods within sampling phase one. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Sample Period Number (one)	Average temperature for that sample period, °C (one)	Sample Period Number (two)	Average temperature for that sample period, °C (two)	Significance level
1	8.24	5	16.8	****
1	8.24	7	17.8	****
1	8.24	8	17.1	****
2	9.53	5	16.8	****
2	9.53	7	17.8	****
2	9.53	8	17.1	****
3	9.96	5	16.8	****
3	9.96	7	17.8	****
3	9.96	8	18.1	****
4	11.4	5	16.8	**
4	11.4	7	17.8	***
4	11.4	8	17.1	**

A Kruskal-Wallis test also found a significant difference to the 99.99% confidence interval ($p<0.0001$) between mean daily temperature for each month. A Bartlett's test was significant ($p<0.001$) and therefore a one-way ANOVA was inappropriate for the distribution. A Dunn's multiple comparisons test found significant differences in temperature between months during sampling phase one (Table 8):

*Table 8: Dunn's comparison showing significant differences in temperature between months within sampling phase one. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Month one	Average temperature one	Month two	Average temperature two	Significance level
March	8.85	May	13.3	**
March	8.85	June	16.7	****
March	8.85	July	17.4	****
April	9.26	May	13.3	**
April	9.26	June	16.7	****
April	9.26	July	17.4	****
May	13.3	July	17.4	**

2.7.2 Rainfall and Drainage Rate

Drainage rate was highest during sampling period four for the loam soil, with an average of 2.47 mm/day in the LD treatment, and highest during sampling period six for clay soil, with an average of 3.04 mm/day in the control treatment (figure 8). Although pellet type would have obvious no effect on drainage rate, the drainage rate within treatments will affect calculated mass flux of metaldehyde. Drainage was therefore analysed by treatment type.

A two-way ANOVA showed that 60.99 % of variation occurred due to sampling period, significant to the 99.99% significance level ($p<0.0001$), and only 1.22 % of variation occurred due to treatment type ($p>0.05$).

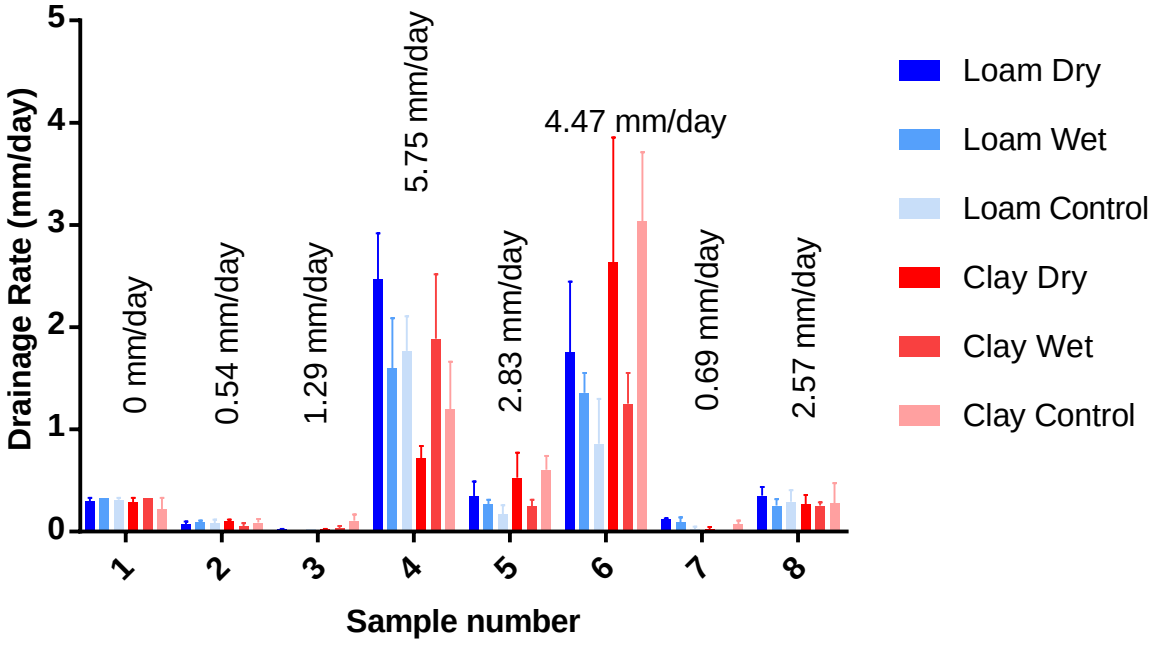


Figure 8: Sampling Phase One drainage rate with standard error of the mean. The mean average rainfall rate per day for the sampling period is written above each sampling period

A Tukey's comparison test between treatment types within the same sampling period showed that there were some significant differences in drainage rates (table 9).

*Table 9: Tukey's comparison showing significant differences in drainage rates between treatment types for sampling periods 4 and 6 within sampling phase one. Drainage rates shown are average drainage rates for that treatment type. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Sampling Period	Treatment type (one)	Drainage rate, mm/day (one)	Treatment type (two)	Drainage rate, mm/day (two)	Significance
4	LD	2.47	CD	0.717	**
	LD	2.47	CC	1.198	*
6	LD	1.75	CC	3.04	*
	LW	1.36	CD	3.64	*
	LW	1.36	CC	3.04	**
	LC	0.861	CD	2.64	***
	LC	0.861	CC	3.04	****
	CD	2.64	CW	1.25	*
	CW	1.25	CC	3.04	***

Another Tukey's comparisons test within treatment type showed that there were some significant differences between sampling periods (Table 10, clay; Table 11, loam).

*Table 10: Tukey's comparison showing significant differences in drainage rate for clay core treatment types between sampling periods of sampling phase one. Drainage rates shown are average rates for that treatment type. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Sampling period (one)	Drainage rate, mm/day (one)	Sampling period (two)	Drainage rate, mm/day (one)	Significance level
CD	1	0.288	6	2.64	****
	2	0.104	6	2.64	****
	3	0.023	6	2.64	****
	4	0.717	6	2.64	***
	5	0.527	6	2.64	****
	6	2.64	7	0.022	****
	6	2.64	8	0.268	****
CW	1	0.332	4	1.89	**
	2	0.055	4	1.89	***
	3	0.037	4	1.89	***
	3	0.037	6	1.25	*
	4	1.89	5	0.25	**
	4	1.89	7	0	***
	4	1.89	8	0.255	**
	6	1.25	7	0	*
CC	1	0.221	6	3.04	****
	2	0.089	6	3.04	****
	3	0.109	6	3.04	****
	4	1.20	6	3.04	***
	5	0.607	6	3.04	****
	6	3.04	7	0.073	****
	6	3.04	8	0.276	****

*Table 11: A Tukey's comparison showing significant differences in drainage rate for loam core treatment types between sampling periods of sampling phase one. Drainage rates shown are average rates for that treatment type. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Sampling period (one)	Drainage rate, mm/day (one)	Sampling period (two)	Drainage rate, mm/day (one)	Significance level
LD	1	0.298	4	2.47	****
	1	0.298	6	1.75	**
	2	0.072	4	2.47	****
	2	0.072	6	1.75	**
	3	0.024	4	2.47	****
	3	0.024	6	1.75	***
	4	2.47	5	0.350	****
	4	2.47	7	0.124	****
	4	2.47	8	0.353	****
	5	0.350	6	1.75	*
	6	1.75	7	0.124	**
	6	1.75	8	0.353	*
LW	1	0.332	4	1.60	*
	2	0.091	4	1.60	**
	2	0.091	6	1.36	*
	3	0.021	4	1.60	**
	3	0.021	6	1.36	*
	4	1.60	5	0.272	*
	4	1.60	7	0.092	**
	4	1.60	8	0.255	*
	6	1.36	7	0.092	*
LC	1	0.312	4	1.77	**
	2	0.079	4	1.77	**
	3	0.024	4	1.77	***
	4	1.77	5	0.172	**
	4	1.77	7	0.025	***
	4	1.77	8	0.292	**

Drainage rate for both the loam and clay soils increased with rainfall volume (figures 7 and 8). A two-way ANOVA showed 90.6% of variation occurred between sampling periods, statistically significant to the 99% confidence interval ($p<0.01$). Only 0.2 % of total variation was due to soil type which was not statistically significant. A matched pairs t-test showed that there was no significant difference between loam and clay drainage rate for the first sampling phase ($p>0.05$).

Regression analysis within each soil type showed that neither loam nor clay soils produced a statistically significant relationship between rainfall and drainage rate, however both relationships had a significantly non-zero slope ($p<0.05$, figure 9).

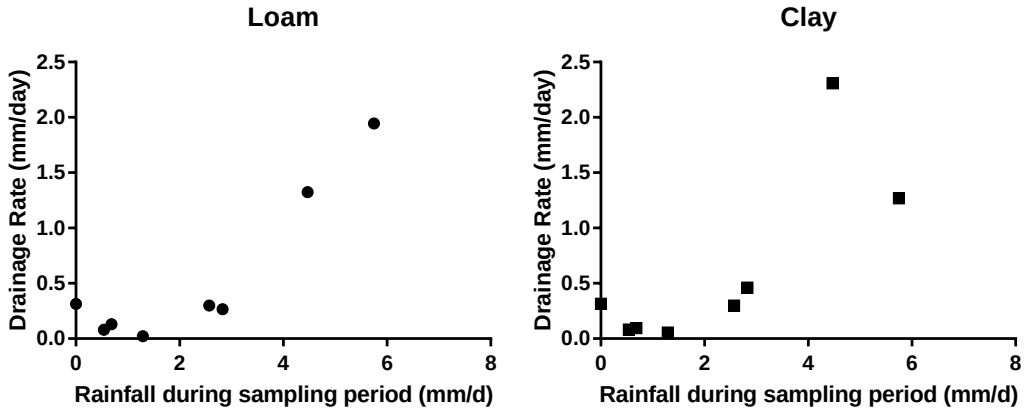


Figure 9: Drainage rate (mm/day) against rainfall (mm/d) for each sampling period in sampling phase one. Each data point represents one of the eight sample lots collected in sampling period one, 16/03/2017 to 28/07/2017.

2.7.3 Leachate Volume

Leachate volume varied by both soil type and treatment type. Although pellet application would not impact leachate volume, it will impact mass flux and LoD calculations. A Pearson's correlation coefficient showed a statistically significant relationship between leachate volume and rainfall for all treatment types (LD, LW, CW $p<0.01$; LC, CC $p<0.05$) except CD ($p>0.05$). When comparing leachate volume to rainfall using just soil type, rather than treatment type, a Pearson's correlation coefficient shows a statistically significant relationship for both soils ($p<0.05$, figure 10).

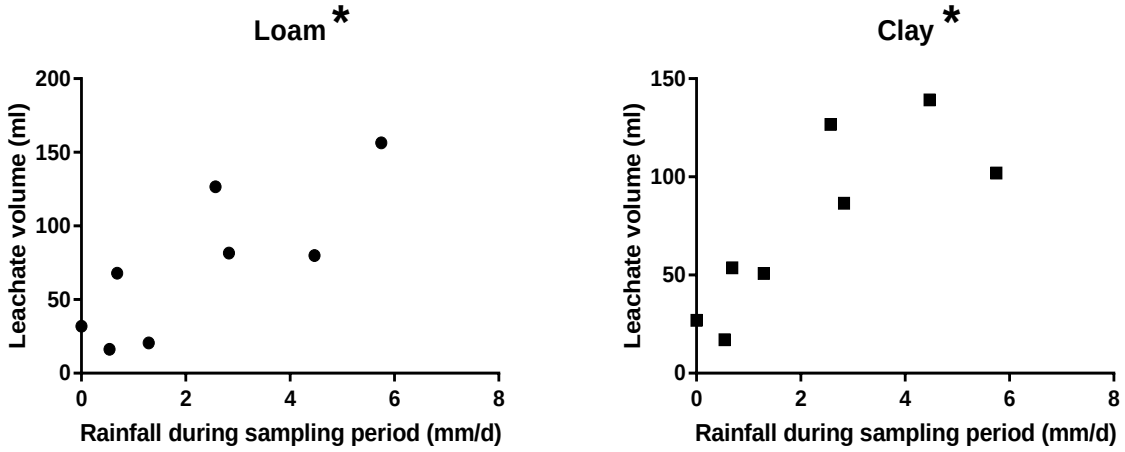


*Figure 10: Average leachate volume, ml, by average daily rainfall, mm/day, for each sampling period in sampling phase one. * = p<0.05*

The highest leachate volume recorded was 283.6 ml (CD, sampling period 6, figure 11). Visually, leachate volume was much higher in sampling periods 4, 5, 6 and 8. This corresponds with increased rainfall, which is statistically correlated to leachate volume (figure 10).

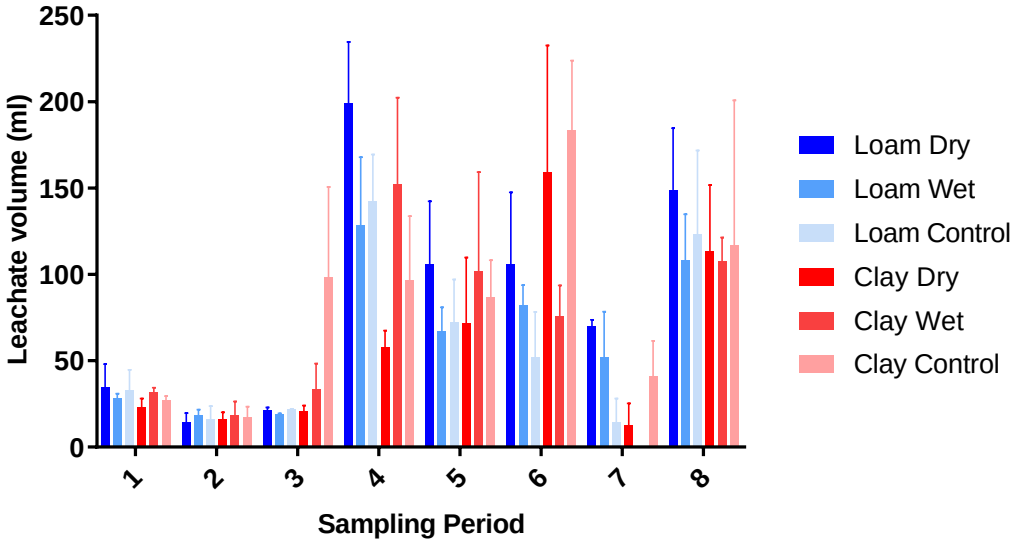


Figure 11: Leachate volume per treatment type for each sampling period in sampling phase one. Standard error is shown in error bars.

A two-way ANOVA showed that 42.19% of the variation in leachate volume occurred due to sampling period ($p<0.0001$). However there was no statistical significant variation due to treatment type, and therefore also between soil type. A Tukey's multiple comparison test showed that there was some statistically differences between sampling periods within treatment types (table 12).

*Table 12: A Tukey's comparison showing significant differences in leachate volume for treatment types between sampling periods of sampling phase one. leachate volumes shown are average volumes per treatment type to 3 s.f. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment type	Sampling period (one)	Leachate volume, ml (one)	Sampling period (two)	Leachate volume, ml (one)	Significance level
LD	1	34.6	4	199	**
	2	14.5	4	199	***
	2	14.5	8	149	*
	3	21.3	4	199	***
	3	21.3	8	149	*
	4	199	7	69.8	*
LC	2	15.9	4	142	*
	3	21.8	4	142	*
	4	142	7	14.1	*
CD	1	22.8	6	159	*
	2	15.7	6	159	**
	3	20.8	6	159	*
	6	159	7	12.7	**
CW	1	31.4	4	152	*
	2	18.6	4	152	*
CC	1	26.8	6	183	**
	2	17.0	6	183	**
	6	183	7	41.0	**

Between treatment types within the same sampling period, another Tukey's comparison test showed a significant different in leachate volume obtained in sampling period four between treatments LD and CD ($p<0.05$) and in sampling period six between LC and CC ($p<0.05$). All other leachate volumes for all other sampling periods were not significantly different.

2.7.4 Qualitative analysis

Photos were taken of the soil core surface on each visit to the site, regardless of whether leachate was collected. Although the change in soil surface and pellet size and visibility cannot be quantifiably measured, the observed difference over time can be linked to metaldehyde leachate concentration.

For the clay cores, cracking was clearly visible on the soil surface, particularly in the earlier stages (figure 12). The loam cores had much less cracking across the surface, although the soil did pull away from the plastic core casing (figure 13).

Pellets were clearly visible on the soil surface on the 03/04/2017, 10 days after pellet application, for both soil and pellet types. Although the blue colour had faded to black, dry pellet shapes could be seen on the loam soil surface on the 22/05/2017 (day 59). Dry pellet colour also seemed to last longer, where the blue colour was still visible on the 16/05/2017, 53 days after pellet application, and the wet pellets were not clearly detectible. This could also have been from wet pellets falling between cracks in the soil due to their smaller size.

Colour change and pellet disappearance – possibly due to degradation, camouflage to the soil or transport beneath the soil surface - appeared most rapid between 16/05/2017 (day 53) and 22/05/2017 (day 59), when the cores were subject to rainfall.

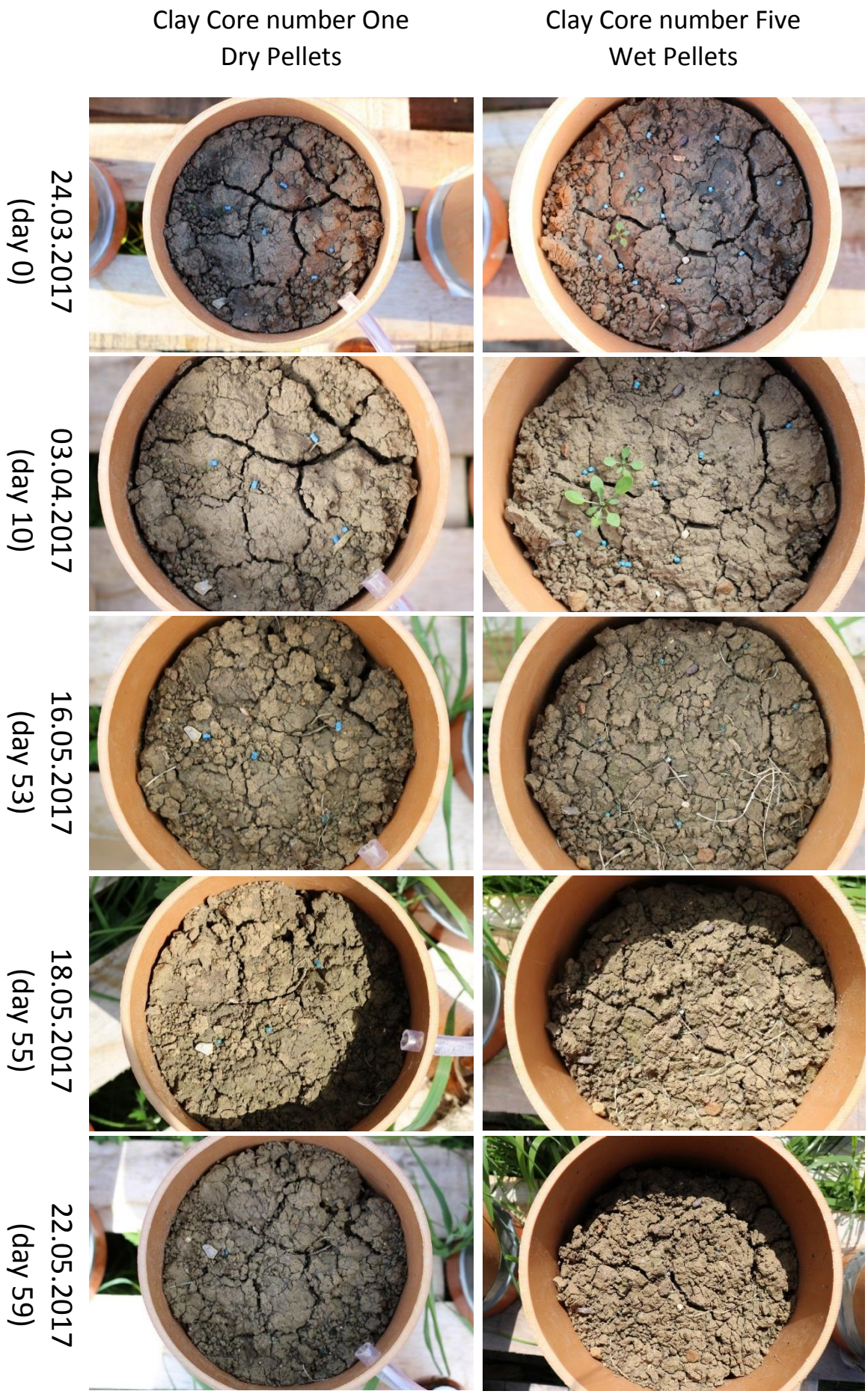


Figure 12: Photographs of Clay soil cores from sampling phase one

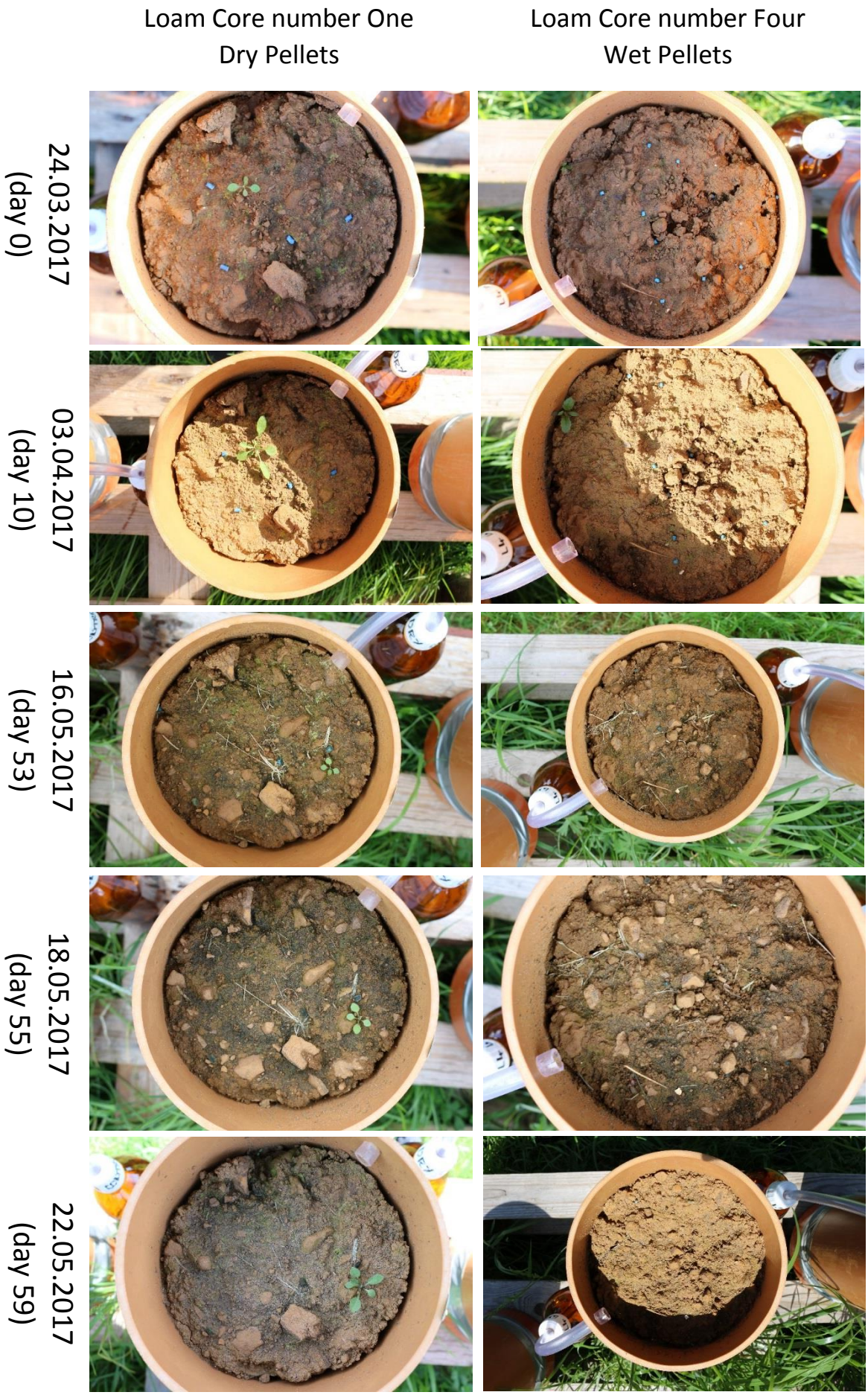


Figure 13: Photographs of Loam soil cores from sampling phase one

2.8 Sampling Phase One: Sample Periods Three and Four

2.8.1 Mean concentration

Combining data from sample periods three and four (03/04/2017, day 10, to 22/05/2017, day 59), clay soil cores visually had more metaldehyde present in the leachate than loam soil cores (figure 14). Statistically measurable metaldehyde concentration ranged between 0.5920 ug/l and 403 ug/l for the clay cores, and between 1.641 ug/l to 3.310 ug/l for the loam cores (table 6) over 18/05/2017 (day 55) and 22/05/2017 (day 59). A Welch's t-test, which assumed the standard deviation was unequal between two data sets, showed that clay soil leached statistically significantly higher concentrations of metaldehyde than the loam soil (figure 14; One-tailed, Welch-corrected t = 2.435; $p<0.05$).

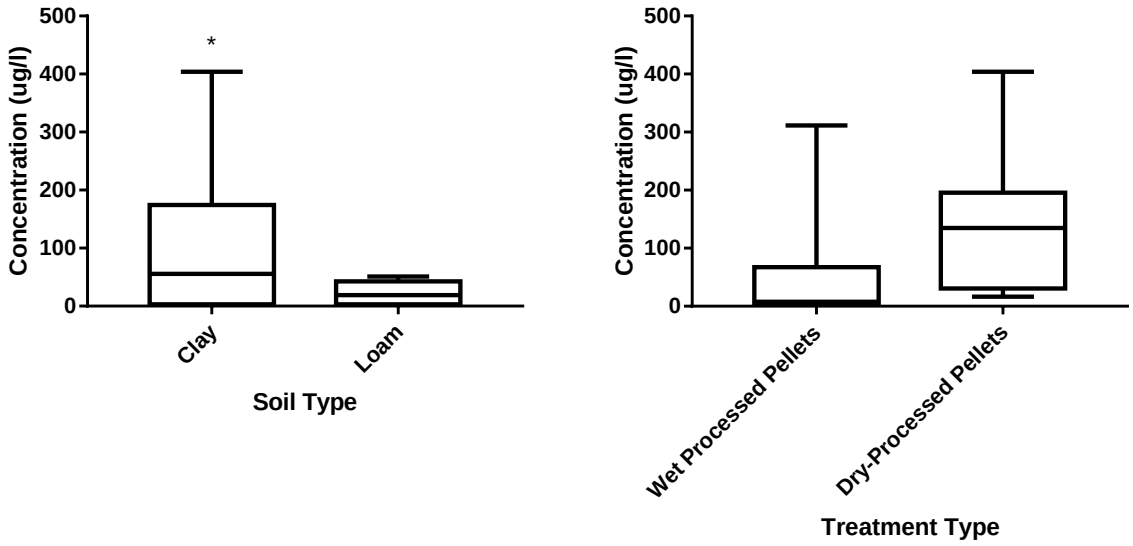


*Figure 14: Left; Combined Metaldehyde concentration per soil type for sampling periods three and four within sampling phase one. Clay soil leached significantly more leachate tham the loam soil ($p<0.05$). Right; Combined metaldehyde concentration leached for wet-processed and dry-processed pellet types for sampling periods three and four within sampling phase one. * = $p<0.05$.*

Dry-processed pellet cores contained more metaldehyde in their leachate than wet-processed pellet cores. Statistically measurable metaldehyde ranged between 0.6079 ug/l and 311.6 ug/l for wet-processed pellets and between 16.62 ug/l and 403 ug/l for dry-processed pellets. However, a Welch's t-test at the 95% significance level showed that metaldehyde leached was not statistically significantly different between the two pellet types (Figure 14; Welch-corrected t = 1.488; $p>0.05$).

2.8.2 Rainfall, Drainage Rate and Leachate

During the 45 day field duration for sample period three, 58.2 mm rainfall fell with 72.6% of that rainfall in the six days leading up to sample collection on the 18/05/2017 (day 55). On the 17/05/2017 (day 54) there was a total of 23.4 mm rainfall, 39.0% of the field duration rainfall (figure 15).

During the 4 day period following the sample collection on the 18/05/0217 (day 55), 23.0 mm rainfall fell.

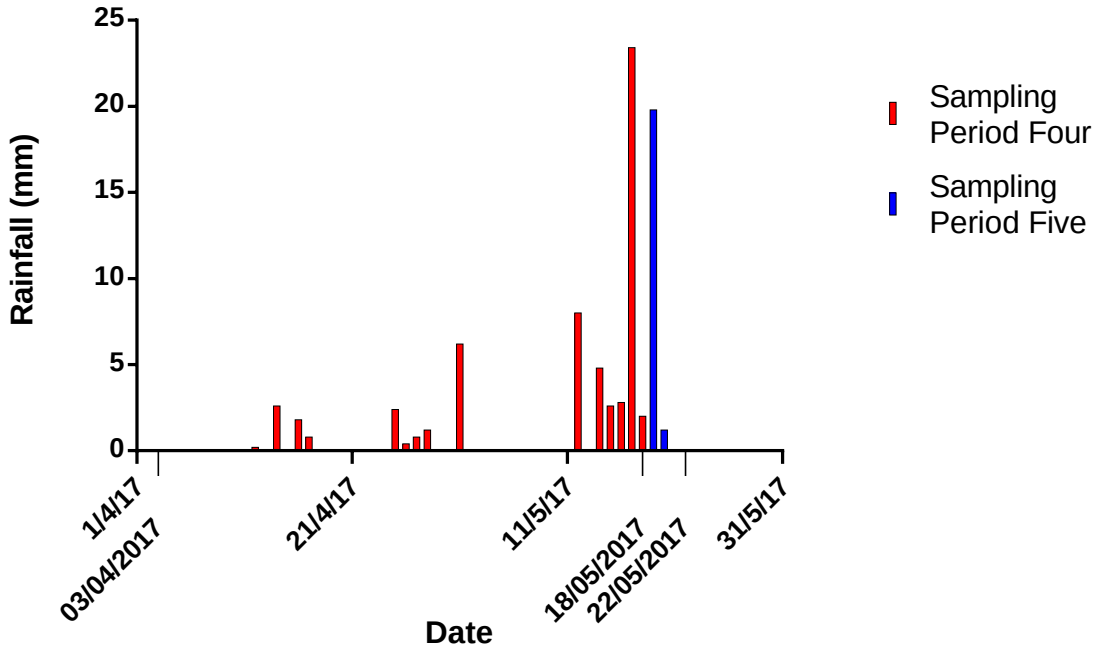


Figure 15: Total Rainfall per day (mm) for sampling periods three and four within sampling phase one

No significant relationship was found between rainfall and drainage rate for sampling phase one (figure 8, figure 9), however drainage rate (mm/day) was clearly higher during the field duration leading up to 22/05/2017 (day 59) than 18/05/2017 (day 55) (figure 9). This corresponds with rainfall and drought during sampling period one (figure 7) and with significant differences in drainage rate (Tables 10 and 11).

Leachate volume, which was statistically significantly correlated to rainfall in sampling phase one (figure 10), was much higher in sampling period three and four than it had been in previous sampling periods (figure 11). Sampling period three also had the highest leachate volumes for loam soils.

2.8.3 Mass Flux

Flux was calculated using the complete raw data, including values for metaldehyde concentrations <LoD and <LoQ, without modification. This was to prevent skewing the data in favour of higher average mass flux for any one treatment type. Fraction of the mass applied was also calculated without modifying or excluding raw data.

Clay cores had higher mass fluxes; up to 7.16 ug/m^2/day for the CD treatment on 18/05/2017 (day 55) and up to 164.1 ug/m^2/day for the CW treatment on 22/05/2017 (day 59). The two largest recorded mass fluxes also had the largest associated SEM, 5.06 ug/m^2/day for the 18/05/2017 (day 55) and 25.44 ug/m^2/day for 22/05/2017 (day 59) (figure 16D).

In comparison to this, loam cores had much smaller mass fluxes, all below 0.07 ug/m^2/day for the 18/05/2017 (day 55) and up to 54.60 ug/m^2/day (LD treatment) on 22/05/2017 (day 59). SEM was also lower for the loam cores, with SEM lower than 0.032 ug/m^2/day for all treatments on 18/05/2017 (day 55) and up to 16.56 ug/m^2/day (LW treatment) on 22/05/2017 (day 59) (figure 16C).

As fractions of the mass applied, very little metaldehyde leached from the soil cores in a measurable quantity. Based all soil cores, the highest fraction of the mass leached was 0.36% for core C3, a clay, dry pellet treatment on 18/05/2017 (day 55) and 1.6% for core C5, a clay, wet pellet treatment on 22/05/2017 (day 59) (figure 16C and 16D).

The cumulative total mass of metaldehyde leached over the whole experiment is difficult to estimate due to the relatively high LoD and LoQ values obtained and the associated difficulty in determining low cocnetrations during some periods.

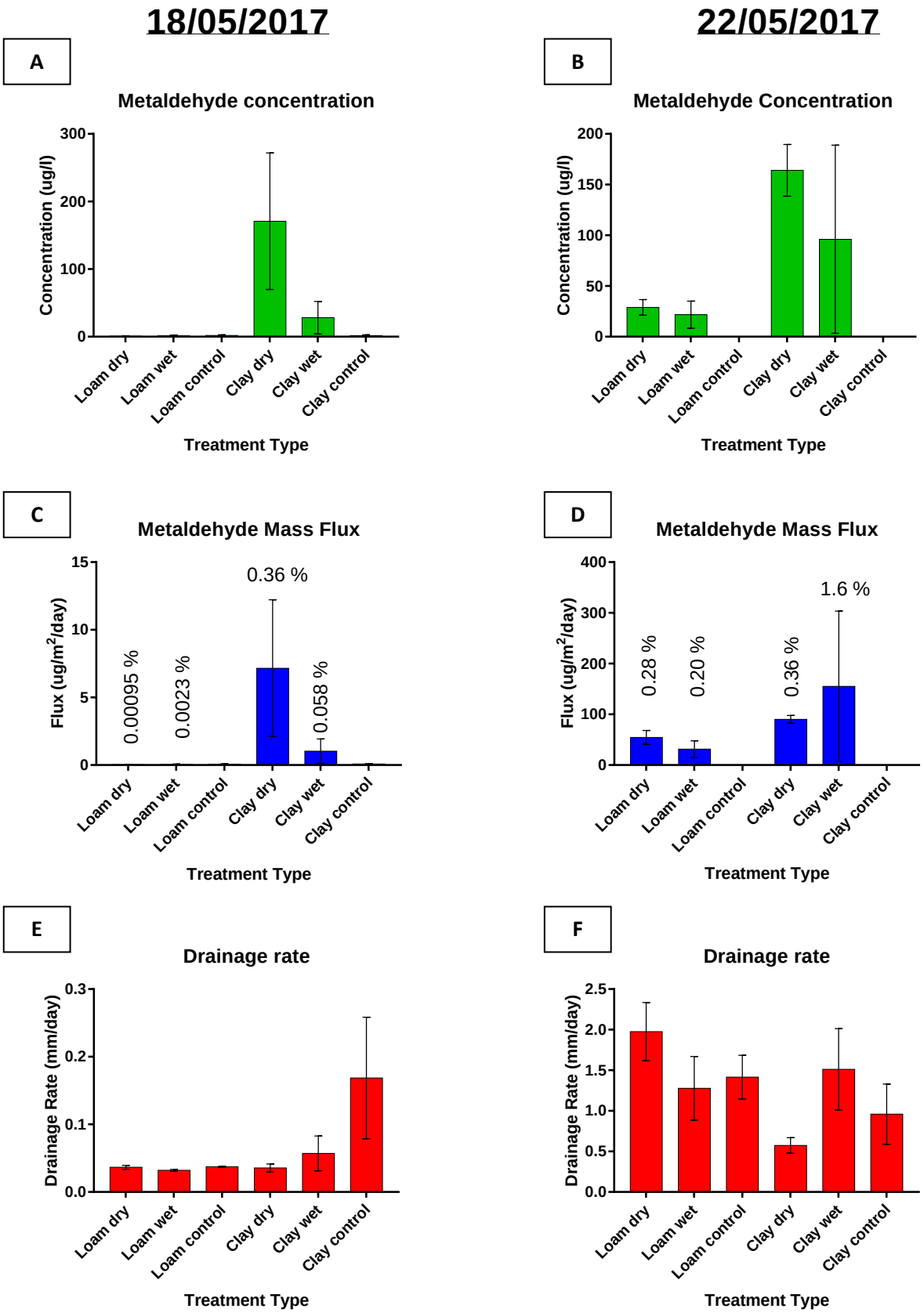


Figure 16: Comparison between sampling period 3 (collected 18/05/2017, day 55) and sampling period 4 (collected 22/05/2017, day 59). A and B: Metaldehyde concentration, ug/l, per treatment type. C and D, Metaldehyde mass flux, ug/m²/day, per treatment type with the percentage of the mass applied written above each treatment type. E and F: Drainage rate, mm/day, per treatment type. For all graphs, error bars represent the standard error of the mean.

2.9 Sampling Phase Two: All Data

Metaldehyde samples obtained during sampling phase two (06/09/2017 – 26/10/2017) could all be viewed with respect to time as LoD and LoQ were consistently lower during this sampling phase and fewer samples were 'lost' due to systematic error. Leachate collection bottles were attached to the soil cores on 06/09/2017 and pellets were applied on 26/09/2017 (taken as day 0). Therefore all of the first sample lot collected were control samples (although there was a possibility of residual metaldehyde leaching from pellets applied in phase one). Total sampling duration was 50 days, in which pellets were applied for 30 days. There were four sample collections during sample phase two, referred to as sampling periods A to D (table 13).

Table 13: Sampling Phase Two collection information, including collection dates, total rainfall, field duration, average temperature leachate volume, LoD/LoQ and metaldehyde concentrations

Sample Period			A – no pellets	B	C	D
Collection date (dd/mm/2017)			26/09	29/09	09/10	26/10
Total rainfall in sampling period (mm)			55.2	9.2	9.4	11
Sampling period field duration (days)			20	3	10	17
Cumulative days since pellets applied (days)			0	3	13	30
Average temperature (°C)			16.0	12.9	12.7	13.4
Leachate volume range (ml)			15.20 – 195.1	23.20 – 213.7	22.60 – 133.75	6.300 – 107.85
LoD (ug/l)			37.07	13.31	38.06	38.06
LoD range in water (ug/l)			0.1900 – 2.439	0.06229 – 0.5737	0.2845 – 1.684	0.3529 – 6.041
LoQ (ug/l)			112.3	40.33	115.3	115.3
LoQ range in water (ug/l)			0.5757 – 7.390	0.1887 – 1.739	0.8622 – 5.103	1.069 – 18.31
	Treatment type					
Concentration in sample, 4 sf (ug/l)	L1	Dry	<LoD		<LoD	<LoD
	L2	Dry		<LoD		<LoD
	L3	Dry		<LoD	<LoD	<LoD
	L4	Wet		<LoD	<LoD	
	L5	Wet	<LoD	<LoD	<LoD	<LoD
	L6	Wet		<LoD	<LoD	
	L7	Control	<LoD	<LoD	<LoD	<LoD
	L8	Control	<LoD	<LoD	<LoD	<LoD
	L9	Control	<LoD	<LoD	<LoD	<LoD
	C1	Dry		0.4150	<LoD	<LoD
	C2	Dry		<LoD	<LoD	<LoD
	C3	Dry	<LoD	<LoD	<LoD	<LoD
	C4	Wet	<LoD	1.210	1.442	<LoQ
	C5	Wet	<LoD			<LoD
	C6	Wet	<LoD	0.7551	<LoQ	<LoD
	C7	Control		<LoD	<LoD	<LoD
	C8	Control		<LoD	<LoD	<LoD
	C9	Control	<LoD	<LoD	<LoQ	<LoD

Few samples had any measurable metaldehyde, with all measurable responses being from clay soils (table 13). Core C4 had a consistent metaldehyde response, increasing until 09/10/2017 (day 13)and then decreasing to <LoQ on 26/10/2017 (day 30). Core C6 also had a similar response, showing measurable metaldehyde on 29/09/2017 (day 3) before presenting <LoQ on 09/10/2017 (day 13) and <LoD on 26/10/2017 (day 30).

Of the measurable responses, three were from wet-processed pellet cores and one, the smallest measurable concentration, is from a dry pellet core. Mass flux ranged from 0.846 ug/m^2/day (C4, 09/10/2017, day 13) to 1.54 ug/m^2/day (C6, 29/09/2017, day 3) and all measurable samples were <0.003 % of the mass applied. For the samples on 29/09/2017 (day 3), the dry pellet core had the lowest mass flux, 1.13 ug/m^2/day and lowest fraction of the mass applied, 0.0024 %.

Core C9, a control core, as in sampling phase one, had a metaldehyde response although it was not measurable. None of the loam control cores had a measurable metaldehyde in leachate response.

2.9.1 Rainfall and Temperature

The majority of rainfall events for sampling phase two were below 5 mm/d, averaging 1.67 mm rain per day with a standard deviation 3.58mm and a median of 0.2mm (figure 17). There was one low-intensity storm event on 25/09/2017 (day -1, before pellet application) which produced 24.6 mm rainfall total at 3.446 mm/hour. There were no statistically significant differences in rainfall between sampling periods ($p>0.05$). However a Kruskal-Wallis test showed a statistically significant difference between daily average rainfall between August, September and October ($p<0.05$).

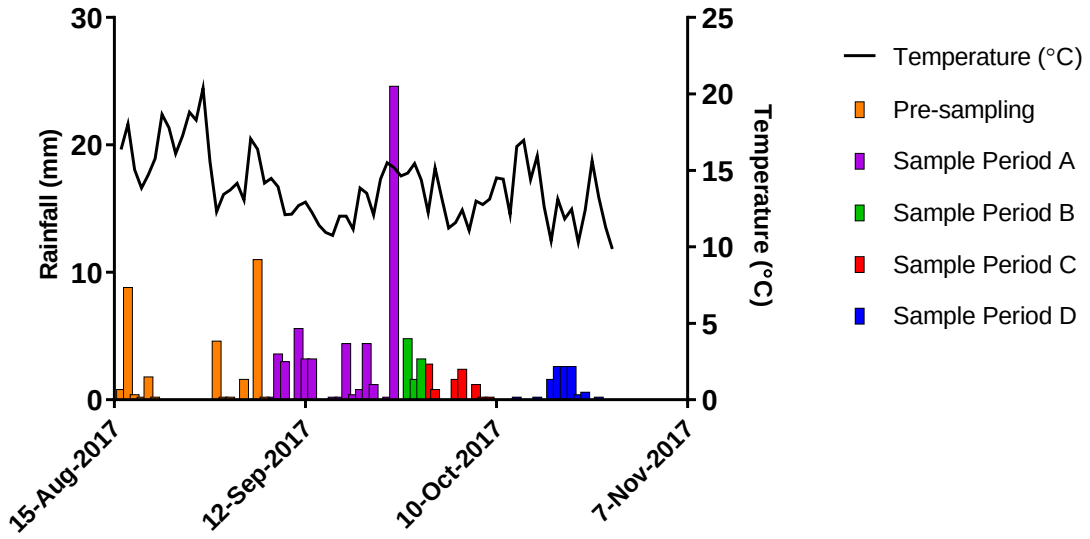


Figure 17: Daily average rainfall and temperature for sampling phase two. No pellets were applied to cores during sampling period A and during pre-sampling.

Average daily temperature was 16.40 °C in August pre-sampling and the mean maximum daily temperature was 21.29 °C. Average daily temperature decreased by 2.95 °C between August and September with mean maximum daily temperature decreasing by 3.32 °C; and between September and October, mean daily temperature decreased by just 0.42 °C, however the average maximum daily temperature decreased by 2.08 °C.

In comparing daily average temperature between sampling periods, a Kruskall-Wallis test showed a statistically significant difference in temperature ($p<0.0001$). A Bartlett's test for unequal variances was statistically significant ($p<0.05$) and therefore a one-way ANOVA was unsuitable. A Dunn's multiple comparison test also showed a statistically significant difference in mean temperature for periods pre-sampling and A ($p<0.001$), pre-sampling and C ($p<0.01$) and pre-sampling and D ($p<0.01$), table 14.

*Table 14: A Dunn's multiple comparisons test showing significant differences in temperature between sampling periods within sampling phase two. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Sample Period (one)	Sampling period average temperature, °C (one)	Sample Period (two)	Sampling period average temperature, °C (two)	Significance level
Pre-sampling	16.0	A	12.9	***
Pre-sampling	16.0	C	12.7	**
Pre-sampling	16.0	D	13.4	**

When comparing daily temperature between August, September and October, a one-way ANOVA could be used because variances across data points were considered statistically homogenous (Bartlett's; $p>0.05$). Daily mean temperatures were found to be statistically significantly different between August, September and October ($p<0.0001$). A Tukey's multiple comparisons test with single pooled variance showed statistically significant differences in mean between August and September ($p<0.0001$) and August and October ($p<0.0001$), table 15.

*Table 15: A Tukey's comparisons test showing significant differences in temperature between months within sampling phase two. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Month one	Average temperature one	Month two	Average temperature two	Significance level
August	16.4	September	13.4	****
August	16.4	October	13.0	****

2.9.2 Rainfall and Drainage Rate

Drainage rate was highest during sampling period B, 26/09/2017 (day 0) to 29/09/2017 (day 3), following the 24.6 mm rainfall event on the 25/09/2017 (day -1) (figures 17 and 18). Total rainfall during sampling period B was 9.2 mm, equivalent to 3.07 mm/day, in comparison to 2.76 mm/day for period A, 0.94 mm/day for period C and 0.65 mm/day for period D. A two-way ANOVA showed that 92.58 % of variation occurred between sampling periods, significant to the 99% confidence interval ($p<0.01$). However, no statistical relationship was found between rainfall and drainage using a Pearson's correlation test ($p>0.05$).

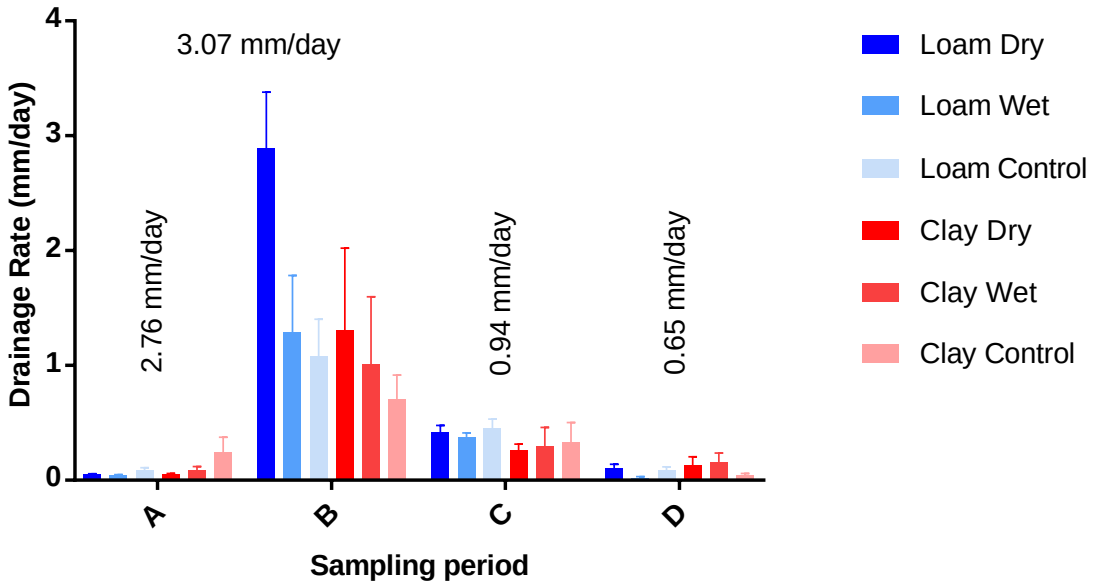


Figure 18: Sampling Phase Two drainage rate with standard error of the mean. The mean average rainfall rate per day for the sampling period is written above each sampling period

Drainage rate varied by soil type and within soil type, split into treatment application categories. Pellet application itself would not obviously affect drainage rate, however drainage rate may affect mass flux of metaldehyde. A matched pairs t test showed that there was no statistically significant difference between clay and loam average drainage rate within each sample period ($p>0.05$).

Sampling period B had the highest drainage rates, coinciding with the highest rainfall rate per day, 3.07 mm. Drainage was highest for the LD treatment which averaged 2.89 mm/day, however all other treatments were within 0.244 mm/day to 1.30 mm/day, less than half the drainage rate of the LD cores. A two-way ANOVA with respect to

time showed that the loam soil, dry pellet cores were statistically significantly higher than all other treatment types for sampling period B (LW, LC, CD; $p<0.001$; CW, CC, $p<0.0001$). All other treatment types had no significant difference within sampling periods.

During sampling period A, clay control cores had the highest drainage rate, up to 0.485 mm/day for core C7. All other cores had a mean drainage rate of <0.09 mm/day. Drainage rate decreased between periods B to C and C to D, reflecting rainfall intensity, which decreased from 3.07 mm/day to 0.94 mm/day to 0.65 mm/day. During period C, drainage rate ranged between 0.263 mm/day (CD) to 0.447 mm/day (LC). For period D, drainage rate was similar to period A, ranging between 0.041 mm/day (CC) to 0.159 mm/day (CW).

Between sampling periods, drainage rate had some significant differences within treatment type. LD had the highest significant differences, centring on the drainage rate spike for sampling period B. All other significant differences in drainage rate also incorporated the high period B drainage with most differences being between loam soil cores (table 16).

*Table 16: A Tukey's comparison showing significant differences in drainage rate for treatment types between sampling periods of sampling phase two. Drainage rates shown are average rates for that treatment type. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Sampling period (one)	Drainage rate, mm/day (one)	Sampling period (two)	Drainage rate, mm/day (two)	Significance level
LW	A	0.046	B	1.28	**
	B	1.28	D	0.016	**
LD	A	0.053	B	2.89	****
	B	2.89	C	0.421	****
	B	2.89	D	0.101	****
LC	A	0.090	B	1.08	*
	B	1.08	D	0.085	*
CD	A	0.052	B	1.30	**
	B	1.30	C	0.263	*
	B	1.30	D	0.126	*

2.9.3 Leachate Volume

Leachate volume varied by both soil type and treatment type (figure 19), where, similar to drainage rate, pellet application would not impact leachate volume but would affect mass flux and LoD calculations. A Pearson's correlation showed no statistically significant relationship between leachate average volume per treatment and rainfall ($p>0.05$). This was different to sampling phase one which did have a significant relationship (figure 11). The highest leachate volume was recorded was 213.7 ml

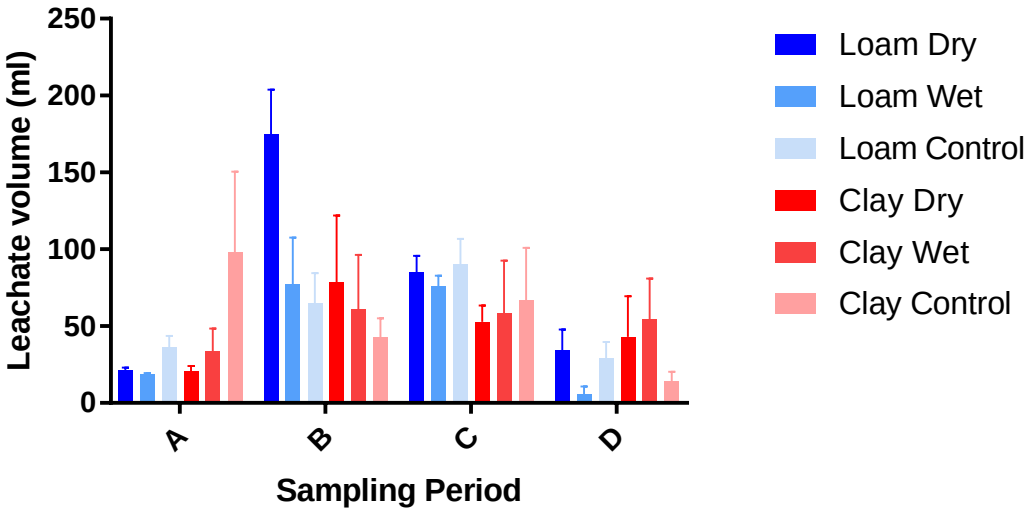


Figure 19: Leachate volume per treatment type and sampling period for sampling phase two. Standard error is shown in error bars.

A two-way ANOVA showed that 20.98% of variation in leachate volume occurred due to sampling period ($p<0.001$). However, there was no statistical significant variation due to treatment type, therefore also between soil type. A Tukey's multiple comparison test showed that there was some statistically differences between sampling periods within treatment types (table 17).

*Table 17: A Tukey's comparison showing significant differences in leachate volume for treatment types between sampling periods of sampling phase two. leachate volumes shown are average volumes per treatment type to 3 s.f. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Sampling period (one)	Leachate volume, ml (one)	Sampling period (one)	Leachate volume, ml (two)	Significance level
LD	A	21.3	B	174	****
	B	174	C	84.6	*
	B	174	D	34.6	***
CC	A	98.3	D	14.0	*

Between treatment types within the same sampling period, a two-way ANOVA showed a significant different in leachate volume obtained in sampling period two between treatments LD and LC ($p<0.05$), LD and CW ($p<0.05$) and between LD and CC ($p<0.01$). All other leachate volumes for all other sampling periods were not significantly different.

2.9.4 Qualitative analysis

Soil cores during the second sampling phase were much less cracked, although there was more debris on the soil surface (figures 20 and 21). Pellets could be seen throughout the 31 day application to the cores in both soil and pellet treatments. Migration below the soil surface was also clearer, particularly for the wet pellet, clay cores in which pellets can be seen in the zoomed-in image. As with the first sampling phase, dry pellets were visible for longer on the soil surface.

Mollusc activity was documented in the second sampling phase more than in the first, evidenced by the clay core, dry pellet photo on 29/09/2017 (day 3) and the loam core, wet pellet photo on 09/10/2017 (day 13).

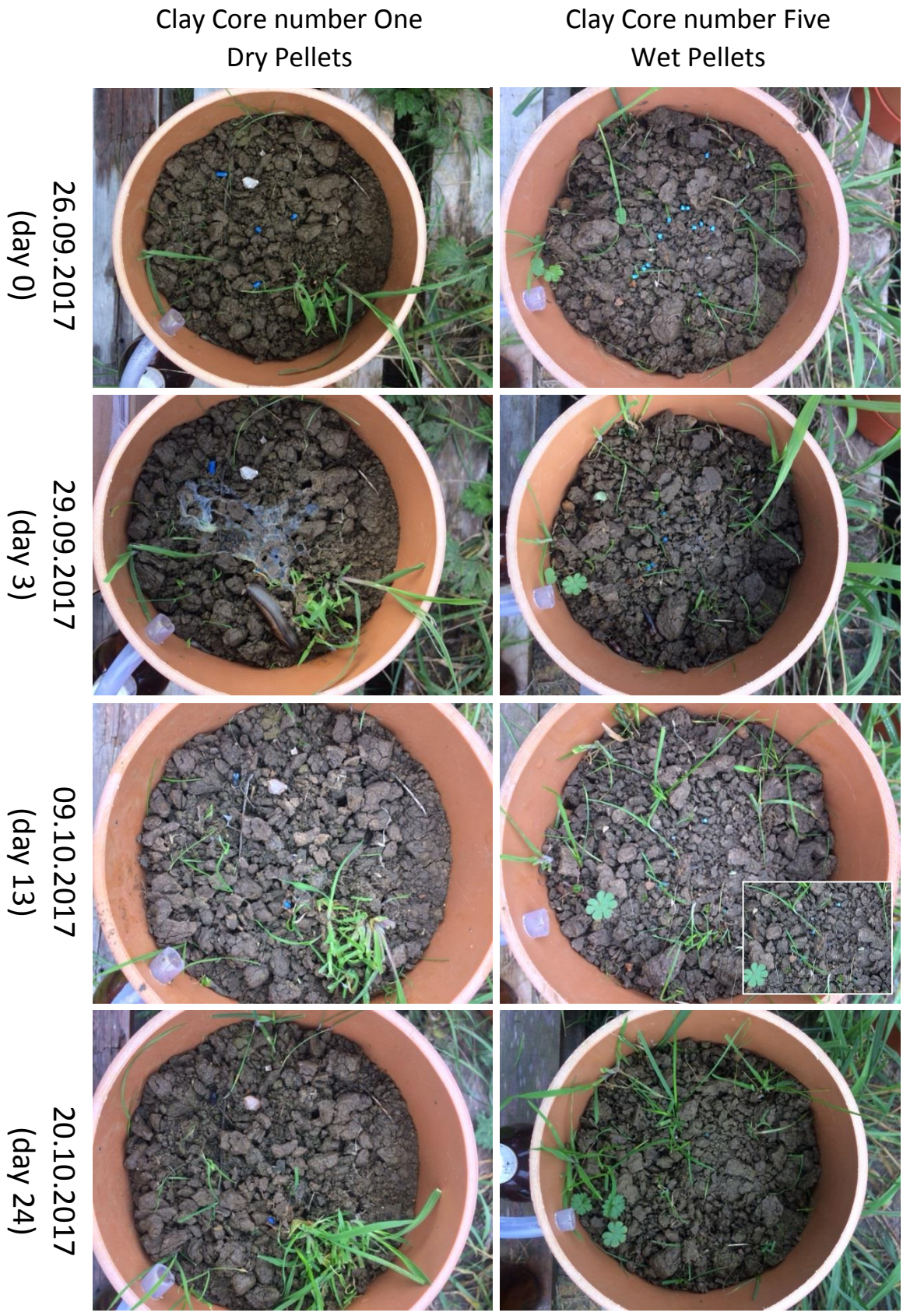


Figure 20: Photographs of Clay soil cores during sampling phase two

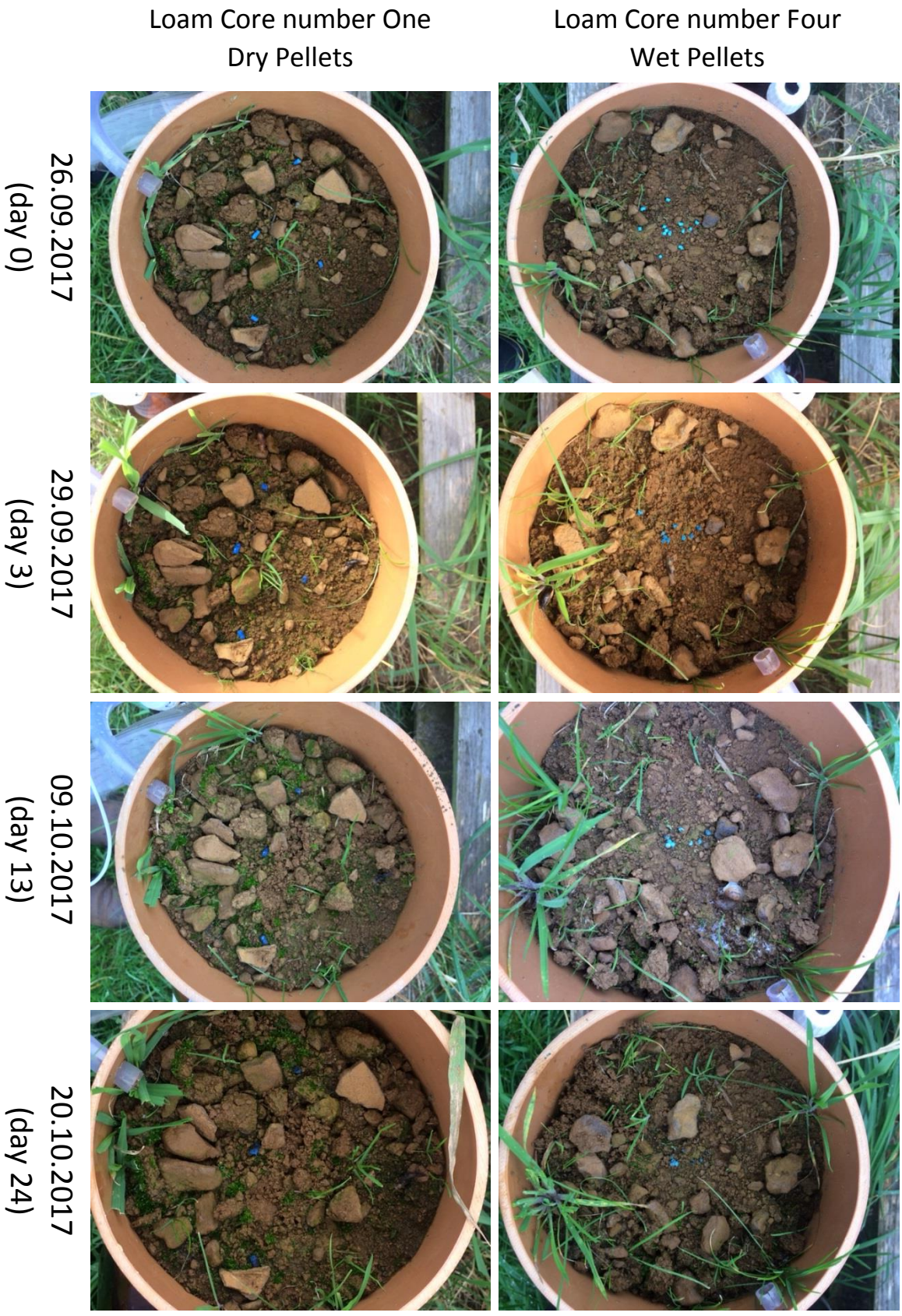


Figure 21: Photographs of Loam soil cores during sampling phase two

2.10 Results Summary

Table 18: Integrated fate assessment results summary

	Sampling Phase One	Sampling Phase Two
Leachate Samples	• Only sample periods 4 and 5 contained measurable metaldehyde responses • Clay cores had visually and statistically higher metaldehyde concentrations than loam cores • Dry pellet cores visually produced more metaldehyde, but this could not be proved statistically	• Only four samples contained any measurable metaldehyde, all within clay cores. • Of the measurable metaldehyde samples, 3 were wet-pellet cores and 1 was a dry pellet core. • Too few sample points to do statistical analysis on pellet or soil differences
Mass flux	• Mass flux was highest in the clay cores, up to 164.1 ug/m2/day and much lower in the clay cores, up to 54.60 ug/m2/day. • As fractions of the mass applied, up to 1.6% of the metaldehyde was leached.	• For the samples on 29/09/2017 (day 3), the dry pellet core had the lowest mass flux and lowest fraction of the mass applied. • The lowest measurable mass flux was on 26/09/2017 (day 3). Drainage rate was also lower here. • As fractions of the mass applied, all samples were <0.003% of the original mass.
Rainfall	• 135 days total sampling, only 50 days rain • Average 1.62 mm/day • No significance between either sampling periods or months •	• 50 days total sampling, 35 days rainfall • Most rainfall events were <5 mm/day, averaging 1.70 mm/day • No statistically significant differences in rainfall between sampling periods • A Kruskal-Wallis test showed statistically significant difference between daily average rainfall between months ($p<0.05$)
Pellets	• Pellets were visibly less clear on the soil surface after the rainfall events, although dry pellets were visible for longer	• Both Pellet types were visible throughout the experiment duration • Mollusc presence was seemingly higher
Temperature	• Significant differences in both sampling periods and months • An ANOVA showed a significant difference in daily average temperature between sampling periods ($p<0.0001$). • A Kruskal-Wallis test showed a significant difference in daily average temperature between months ($p<0.0001$)	• Significant differences in both sampling periods and months • A Kruskal-Wallis test showed a significant difference in daily average temperature between sampling periods ($p<0.0001$) • An ANOVA showed a significant difference in daily average temperature between months ($p<0.0001$)
Drainage	• No significant correlation between rainfall and drainage rate • No significant difference between loam and clay drainage rates • Sampling period 4 had the highest loam soil drainage rate; sampling period 6 had the highest clay soil drainage rate • Significant differences in drainage rates between treatment types of the same sampling period and within treatment types for different sampling periods	• No significant correlation between rainfall and drainage rate • No significant difference between loam and clay drainage rates • Sampling period B had the highest drainage rate for both soils • Significant differences in drainage rates within treatment types for different sampling periods. Sampling period B had significantly higher drainage rates than all other treatment types in sampling period B.

2.11 Discussion

The integrated fate assessment experiment aimed to show the combined effects of disintegration of slug pellets, microbial degradation of metaldehyde and its pellet casing, and sorption effects, showcasing any differences between soil type and pellet type. The following research questions and hypotheses were tested:

Research questions:

4. To what extent does soil type and pellet type influence metaldehyde leaching under realistic environmental conditions?
5. To what extent does metaldehyde leach from intact soil columns under realistic environmental conditions?
6. Does metaldehyde leach from the pellet before total physical pellet degradation?

Research hypotheses:

6. Higher concentrations of metaldehyde will be leached from clay soil cores in comparison to loam soil cores due to preferential pathway formation and lower organic content
7. Metaldehyde will leach from the soil cores following storm events
8. Initially, higher concentrations of metaldehyde will be leached from dry-processed pellets because they are likely to physically breakdown faster
9. Wet-processed pellets will last for physically longer on the soil surface than dry-processed pellets
10. Metaldehyde will leach from both pellet types before total physical breakdown

In response to question 1 and hypothesis 1, clay soils were shown to leach statistically significantly higher concentrations of metaldehyde than the loam soil cores. This may have been due to a variety of environmental factors, given that pellet type was not a measurably significant factor. The clay soil had a lower SOM content and, therefore, would have been less likely to retain metaldehyde by sorption. Lower organic matter levels are often associated with fewer active biodegrading bacteria (Kookana et al., 2005) which are suggested as the primary cause of metaldehyde breakdown (Simms et

al., 2006). Clays are often more varied in soil moisture, draining less freely than loam soil, but also cracking during the long periods without moisture input. This was visible in the soil cores used in the experiment (figures 12 and 13). Pellets on the soil surface were also noted to fall between the clay soil cracks, being transported to lower soil horizons which are often less biologically active (Kookana et al., 2005). With little soil moisture, both pellets and metaldehyde would be expected to degrade at a slower rate. Metaldehyde present lower in the core would also have less distance to leach before reaching the extraction bottle. In arable fields clay soils are frequently under-drained. Thus, if soils cracks are connected to the drainage network, metaldehyde residence time is decreased and leaching is enhanced. In this way, the environmental conditions, and how they affect clay over loam, have a significant impact on pellet leaching irrespective of pellet type. The cracks formed during the experiment duration were not believed to be an artefact of coring, being entirely due to environmental conditions alone, where cracking is known to occur in soils, even in arable fields (Øygarden et al., 1997).

In response to research question 2 and hypotheses 2, few soil cores leached measurable concentrations of metaldehyde during the duration of the integrated fate assessment experiment. Of those that did measurably leach metaldehyde, the flux was a maximum of 1.6% of the nominal metaldehyde applied in each period. During sampling phase two, which was conducted between September and October, when slug pellets are typically applied to arable cropland, metaldehyde flux was consistently <0.003% of the mass applied. This shows that only a small fraction of the applied metaldehyde is leached under environmentally realistic conditions.

The difference in metaldehyde flux between the two sampling phases might be explained by the rainfall input to the soils. Although average rainfall intensity in the two phases was approximately the same, phase one had less frequent, heavier storm events (of 135 days, only 50 had rainfall). Because of this, soils were dry and able to form cracks. In combination with the above theory about leaching in cracked soil, one heavy rainfall event would have been enough to leach metaldehyde through these cores. In comparison to the first sampling period, the second sampling period had 35 days of rain over 50 days. This means that soil moisture content may have been higher

(evapotranspiration rates typically decline in September), with increased biological activity and fewer soil cracks. As a result, a lower metaldehyde flux was recorded

Although the first sampling period appears to show a clear patter between recorded metaldehyde concentration and rainfall events, a correlation cannot be confirmed because leachate was collected on a sample volume basis. This meant that multiple storm events were represented per sample and therefore one sample concentration of metaldehyde cannot be associated with one storm event for this experiment. In addition, other factors discussed below may also have influenced leaching from the soil cores.

Low percentage metaldehyde flux rates suggest that much of the metaldehyde either degrades in the soil or is bound to the soil solids. If the metaldehyde became a bound residue it may be biologically unavailable, and therefore have a much slower in-soil degradation rate (Gevao et al., 2000).

With increased applications and higher soil concentrations, more bound residues are likely to be formed (Gevao et al., 2000). This would imply that the applied rate of 1400g metaldehyde per hectare (double the maximum annual total dose) to the soil cores may have led to only a fraction of metaldehyde being available for degradation or leaching. Freeing up bound residues may be one explanation for the concentrations of metaldehyde measured in the control cores, where metaldehyde had not been applied since November 2014 (loam soil) and November 2015 (clay soil).

Temperature varied significantly between both sampling periods and months for both sampling phases. Temperature influences evapotranspiration, and therefore, soil moisture, which is a primary control over drainage rate and pesticide leaching (Pullan et al., 2016). Both temperature and soil moisture also influence degradation (and to some extent, sorption). However, due to the long duration of both sampling phases and the significant differences in temperature over time it is impossible to measure the direct influence of temperature on leaching. Temperature did appear to affect slug presence. Molluscs were visibly more active during the second sampling phase, as seen in soil core photographs (figures 20 and 21). Molluscs thrive under mild, moist conditions and may be more prevalent at lower temperatures with more frequent

rainfall. During the second sampling period, more pellets were visibility consumed by molluscs, and therefore the metaldehyde available for leaching may have been reduced at the soil surface. This could have been another reason for lower metaldehyde flux during the second sampling period.

Unlike temperature, monthly rainfall only varied significantly in the second sampling phase which transitioned across summer to autumn. Rainfall did not correlate with drainage rate, which is more related to water storage. However, increased rainfall rate and higher leachate volumes, which were correlated, did increase metaldehyde detectability within the method. The LoD and LoQ calculations involved converting the LoD/LoQ for metaldehyde in DCM to the LoD/LoQ of metaldehyde in water using the sample leachate volume. A higher volume lowered the LoD and LoQ. During GCMS analysis, some chromatographs produced two visible peaks even when the metaldehyde concentration was below LoD. In these cases, increased leachate volume may have resulted in quantifiable detections.

The lowest LoD calculated in this experiment was 12.1 ug/l metaldehyde in DCM, equivalent to 0.0468 ug/l in water with a 258.7 ml leachate sample, below the drinking water directive (DWD) limit of 0.1 ug/l metaldehyde in water. However, the majority of samples had a LoD higher than 0.1 ug/l. It is likely that some metaldehyde leaching occurred during periods when the concentration was <LoQ. However, it is not possible to estimate the flux during these periods. The total cumulative leaching loss is, therefore, highly uncertain.

Drainage rate was not statistically different between soil types. However, statistical differences were found between sampling periods and between treatments within sampling periods. This clearly demonstrates that there was significant environmental variability within the same soil type subject to the same environmental conditions. Small variations in soil horizon structure, including the presence of stones, compaction, cracking and organic layers can alter drainage and therefore impact leachate pathways.

In further response to research question 1 and in response to question 3 and hypotheses 3, 4 and 5, no statistical difference was found between pellet type and leached metaldehyde concentration. However, dry pellet cores appeared to generate higher metaldehyde concentrations which may have been due to the more fragile nature of the dry pellets, which crumbled more easily. This evidence lends weight to hypothesis 3 although does not confirm it as correct.

Photographs of the pellets show that they did not completely physically disintegrate over time, however they did change colour and gradually reduce in size. Pellets also moved beneath the soil surface (perhaps washed down the macro-pores in storm events or during disturbance due to solifluction or bioturbation). This was particularly true for the wet pellets, which were smaller in size than the dry pellets. Therefore, the physical presence of pellets in the photographs was not necessarily an indicator of physical degradation at the surface.

Dry pellets visually lasted for longer on the soil surface than the wet pellets during the first sampling phase and were still visible on the 16/05/2017 after 54 days. This goes against hypotheses 4, in which wet-processed pellets were predicted to physically last longer on the soil surface. In the second sampling phase (31 days), both pellet types were still blue and visible on the soil surface at the end of the phase, although in reduced visible quantity (due to mollusc consumption). In answer to research question 3 and hypothesis 5, given that metaldehyde was observed to leach from the soil cores when pellets were visible on the soil surface and when they were not visible, metaldehyde leaching is not likely to be dependent on physical pellet disintegration.

Chapter Three

Experiment Two: *In vitro* incubations

3.1 Introduction

In vitro incubations compared the degradation half-lives of wet- and dry- processed slug pellets with metaldehyde introduced in dissolved form, in conditions which are comparable to standard soil tests. A principle aim was to show if there was a difference in metaldehyde degradation rate when present in different application forms, rather than to exactly quantify changes in the concentration of metaldehyde over time. Although not environmentally realistic, the experiment was designed to highlight whether there is delayed degradation as a consequence of the pellet casing and whether the pellet type influenced degradation rate.

3.2 Reagents

Laboratory grade, 99% metaldehyde, purchased from Acros Organics.

Wet-processed pellets, brand name *Trigger 3* were purchased from Certis as 3% metaldehyde pellets. Pellets were stored in sealed opaque bags at room temperature, away from sunlight.

Dry-processed pellets, brand name *Carakol 3* were purchased from Adama as 3% metaldehyde pellets. Pellets were stored in sealed opaque bags at room temperature, away from sunlight.

HPLC grade methanol and Laboratory grade dichloromethane were purchased from Fischer Scientific.

A 200 mg/l metaldehyde in methanol solution was made by weighing out 100 mg laboratory grade metaldehyde into a 500 ml volumetric flask and topping up to 500 ml with methanol. The solution was capped and shaken until dissolved. Fully-dissolved solution was transferred to a screwcap glass bottle and stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

A 1000 mg/l metaldehyde in methanol stock calibration standard was made by weighing 50 mg ± 0.5 mg metaldehyde in a 100ml Pyrex bottle. To this, 50 ml methanol was added to the metaldehyde using a 50ml glass pipette. The stock calibration standard was capped and mixed to dissolve, then stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

2000 ug/l, 1500 ug/l, 1000 ug/l and 500 ug/l working calibration standards were made by pipetting 100 ul, 75 ul, 50 ul, and 25 ul of the stock calibration standard into individual 50 ml volumetric flasks and topping up to 50 ml with dichloromethane. Fully-dissolved solution was transferred to a screwcap glass bottle and stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

Low-concentration working calibration standards were made by first producing a 1 mg/l solution from the 1000 mg/l stock calibration standard. The 1 mg/l standard was made by adding 50 ul 1000 mg/l stock calibration standard to a 50 ml volumetric flask and topping up to 50 ml.

200 ug/l, 150 ug/l, 100 ug/l, 75 ug/l, 50 ug/l, 25 ug/l and 20 ug/l working calibration standards were made by pipetting 10 ml, 7.5 ml, 5 ml, 3.75 ml, 2.5 ml, 1.25 ml and 1ml of the 1 mg/l standard into individual 50 ml volumetric flasks. Each flask topped up to 50 ml with dichloromethane, transferred to a screwcap glass bottle and stored in a refrigerator at 1 - 10°C to prevent solution concentration by evaporation.

3.3 Apparatus

3.3.1 Solvent extraction:

100 ml amber-glass vessels with screwcap PTFE lids

Incubator, HeraTherm IGS60

Freezer, set to -15.6 °C

Freeze dryer, LTE Scientific Mini Lyotrap

Orbital shaker, Stuart SSL1, set to 160 rpm

Water purifier, Elga DV25, producing deionised water

3.3.2 Analysis:

Gas chromatograph and mass spectrometer – Perkin Elmer Clarus 500:

Column: 30m x 250 µm diameter

Carrier gas: Helium, 1 ml per minute

Injection temperature: 280 °C

Injection volume: 1 µl (pulsed split-less injection)

Temperature programme: Oven

Initial temperature 40.0 °C for 2 minutes, then 20.0 °C per minute to 250 °C and hold for 0.00 minutes.

Equilibration time: 1 minute

Total run time: 12.50 minutes

SIM: Solvent delay 0.00 to 4.90 minutes

SIM of 4 masses, monitored 6.35 minutes to 6.58 minutes in EI+ ionisation mode

Using these conditions, the following applies:

Table 19: Metaldehyde retention times for GCMS

Compound	Approximate retention time (minutes)	Ions monitored	
D16 metaldehyde	6.41	50.0	98.0
Metaldehyde	6.46	45.0	89.0

TurboMass software was used to tune and calibrate the GCMS, and calculate area responses from chromatogram sample peaks.

3.4 Method

The method was developed based on OECD test 307 (OECD, 2002). 15 (± 0.15) g moist soil was placed in each of one hundred and fifty 100 ml amber glass cylindrical vessels. Half of the vessels contained loam soil and half clay soil, and total vessel weight was noted to monitor soil moisture content. Soil was extracted from Lyndon Farms (figure 2). For each soil type, three control bottles did not receive metaldehyde, twenty-four received 22.2mg of dry-processed pellets, twenty-four received 22.2mg of wet-processed pellets and twenty-four received 3.3 ml of a 200 mg/l laboratory-grade metaldehyde in methanol solution (table 20). The 22.2 mg total pellet weight per vessel was chosen as it was equivalent to approximately one dry pellet per vessel, where dry pellets are larger and heavier than wet pellets. Excluding the control vessels, approximately 0.667 mg metaldehyde was applied per vessel assuming each pellet was 3% metaldehyde. This was approximately equivalent to 51.4 mg/kg of metaldehyde applied to loam soil and 57.3 mg/kg metaldehyde applied to clay soil. Before pellets were added to their respective vessels, 3.3 ml methanol was mixed into the soil to ensure a consistent soil environment between treatment types.

Treatment type	Soil type	Average dry soil weight from 15 g moist soil (g)	Number of vessels
Control	Clay	11.65	3
Wet pellets			24
Dry pellets			24
Laboratory grade metaldehyde			24
Control	Loam	12.98	3
Wet pellets			24
Dry pellets			24
Laboratory grade metaldehyde			24

Table 20: Vessel soil weights for the in vitro incubation experiment

Vessels were placed in a dark incubator at 20 °C without lids. Vessels were weighed weekly to maintain consistent soil moisture content, and deionised water was added if necessary. On days 0, 2, 5, 7, 12, 19, 26, and 33, eighteen vessels were sacrificially

removed from the incubator, three from each treatment type. Control vessels were all removed on day 0.

On removal, vessels were placed in a fume hood for one hour to evaporate any excess methanol. Following this, vessels were put in a freezer for at least 4 hours then freeze-dried for 24 hours to remove all water. After freeze-drying, 25 ml dichloromethane (DCM) was added to each vessel, then vessels were capped and put on an orbital shaker for 24 hours at 160 rpm.

Once shaken for 24 hours, vessels were left to settle for one hour keeping the lids on the vessels to maintain metaldehyde equilibrium between the solvent and solid phases. 5 ml of supernatant was extracted and filtered using a 0.45 μm syringe filter into a glass beaker, then 1 ml of the filtered solution was pipetted into a GCMS vial.

From each working calibration standard, 1 ml was pipetted into GCMS vials. A blank standard of 1 ml DCM was also produced. Standards were analysed alongside the samples during GCMS analysis, using selective ion monitoring mode and a DCM wash.

Following GCMS analysis, each sample and standard had one response peak if metaldehyde was present. Integrating these peaks, the response area was noted and used to calculate metaldehyde concentration. The response area from the working calibration standards was plotted against their respective nominal concentrations and regression equation calculated. Inputting the response peak area into this equation, metaldehyde concentration for each sample could be back-calculated considering the volume of filtered sample per GCMS vial. Over time, concentrations were plotted in a DT50 degradation curve and treatment methods could be compared.

An overview of the incubation experiment method development and justification can be found in Appendix C.

3.5 Results

In the following results section, treatment types will be referred to using acronyms.

Loam soil = L; Clay soil = C; Wet pellets = W; Dry pellets = D; Laboratory grade metaldehyde = M.

Therefore a loam soil, laboratory grade treatment type would be referred to as 'LM'.

3.5.1 Recovery

667 ug metaldehyde was applied to each vessel with 15.0 g moist soil in each. This was approximately equivalent to 13.0 g dry loam soil and 11.6 g dry clay soil. Therefore, nominally, 51.3 mg/kg metaldehyde was applied to the loam soil vessels and 57.5 mg/kg was applied to the clay soil vessels.

Overall recovery increased between day 0 and day 7, then decreased until day 30. The maximum recovery was up to 66.9 % of the applied nominal metaldehyde for loam soil and up to 59.7 % of the applied nominal metaldehyde for the clay soil, both from day 7 (figure 22). Recovery was higher in the loam soil, by percentage applied, until day 19 when clay had higher recovery and recovery for both soils was <17%.

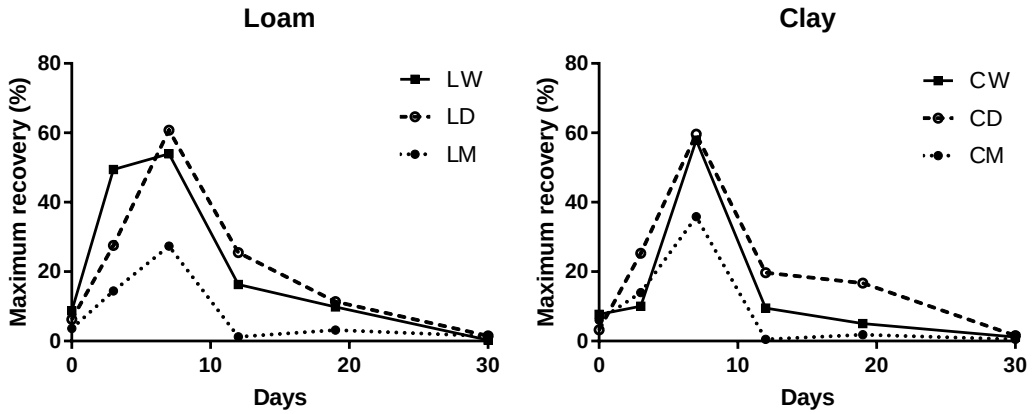


Figure 22: Maximum recovery of metaldehyde applied to loam (left) and clay (right) soils over the 30 day incubation.

For the loam soil, maximum recovery was initially higher for the wet pellet treatments, with dry pellets having a higher recovery from day 7. In the clay soil, maximum recovery was highest for the dry pellets with the exception of day 0.

3.5.2 Average

As with the percentage of maximum recovery, for all treatment types the average concentration increased between day 0 and day 7, then decreased until day 30 (figure 25).

In the loam soil, wet-processed pellets initially had higher concentrations than the dry pellets, although dry pellets had higher concentrations from day 7 until day 30. In the clay soil, dry pellets also had higher concentrations than the wet pellets for the majority of time, although wet pellets had higher concentrations on day 0. The highest recorded concentration was 27.00 mg/kg for dry-processed pellets in clay soil on day 7. The highest in loam soil was 25.56 mg/kg, also for dry pellets on day 7.

Using a two-way ANOVA, treatment type was compared by extraction day. There were no statistically significant differences between treatments, including pellet type and soil type, for day 0, 3, 12, 19 or 30. However, day 7 concentrations were significantly different between 5 treatment types (table 21).

*Table 21: Tukey's comparison showing statistical significance in concentration (mg/kg) between treatment types on extraction day 7. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001*

Treatment one	Concentration (mg/kg)	Treatment two	Concentration (mg/kg)	Significance level
LW	21.52	LD	25.56	*
LW	21.52	CD	27.00	*
LD	25.56	LM	9.41	***
LM	9.41	CW	21.77	*
LM	9.41	CD	27.00	****

Within treatment types, concentration change was compared between extraction days (table 22). With the exception of the LM treatment, all treatment types contained statistically significant changes in concentration over time. All of the treatment types statistically significantly changed in concentration between day 0 and day 7, day 7 and day 19, and day 7 and day 30. LD, CW and CD treatments had statistically significant increases in concentration between day 3 and 7; and LD, CW, CD and CM treatments all had significant decreases in concentration between day 7 and day 12. LW also had statistical significance between days 3 and 19, and 3 and 30.

*Table 22: Tukey's comparison showing statistical difference between extraction days within specific treatment types. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Day number A	Concentration (mg/kg)	Day number B	Concentration (mg/kg)	Significance level
LW	0	2.757	3	13.95	*
	0	2.757	7	21.52	*
	3	13.95	19	21.52	*
	3	13.95	30	0.1064	**
	7	21.52	19	3.059	*
	7	21.52	30	0.1064	**
LD	0	1.413	7	25.56	****
	3	7.715	7	25.56	****
	7	25.56	12	10.37	***
	7	25.56	19	3.653	****
	7	25.56	30	0.3774	****
CW	0	3.096	7	21.77	****
	3	5.482	7	21.77	***
	7	21.77	12	2.985	****
	7	21.77	19	1.438	****
	7	21.77	30	0.4238	****
CD	0	1.536	7	27.00	****
	3	10.19	7	27.00	***
	7	27.00	12	6.439	****
	7	27.00	19	5.283	****
	7	27.00	30	0.4335	****
CM	0	2.510	7	16.65	**
	7	16.65	12	0.1641	***
	7	16.65	19	0.8939	***
	7	16.65	30	0.2040	***

3.5.3 LoD effect on averages

To calculate the average concentration of metaldehyde for each treatment type per extraction day, the mean average was taken using the complete raw data, without excluding or modifying concentrations based on LoD or LoQ If LoD and LoQ values had been entirely excluded, average concentration would change by -100% to +126% mg/kg, particularly impacting day 30 samples where all treatment types had some samples less than LoQ

If concentrations had be modified so that values which were less than LoD equalled zero and values which were less than LoQ equalled the LoD, all impacted averages were reduced, up to 100% less than originally calculated. By modifying results so that concentrations less than LoD equalled 0.5 LoD and concentrations less than LoD equalled LoD, the impacted averages were also reduced, up to 41% less than the original concentration.

By maintaining the complete raw data to calculate averages error could be reduced which would have skewed the data to higher or lower concentrations based on fabricated values. By reporting SEM alongside the averages, the uncertainty in the estimate of the mean was included and therefore error acknowledged.

3.5.4 Standard error of the mean

In this report, standard error of the mean (SEM) was used to quantify the uncertainty of the estimate of the mean, as opposed to the dispersion of the data from the mean, indicted by standard deviation (Barde and Barde, 2012). As with concentration, the SEM was highest on day 7 with the exception of the LW treatment for which SEM was highest on day 3. For all treatments, the lowest SEM was for day 30.

For clay soil, the highest SEM was for the dry pellets with SEM consistently above 1.9 mg/kg. The other clay treatments had an SEM of <1 mg/kg for all days other than day 7.

In the loam soil, SEM was highest in the wet pellets until day 7, after which the dry pellets had the highest SEM. Wet pellets had the highest recorded SEMs, up to 5.252 mg/kg on day 3, over double all other treatment types for that extraction.

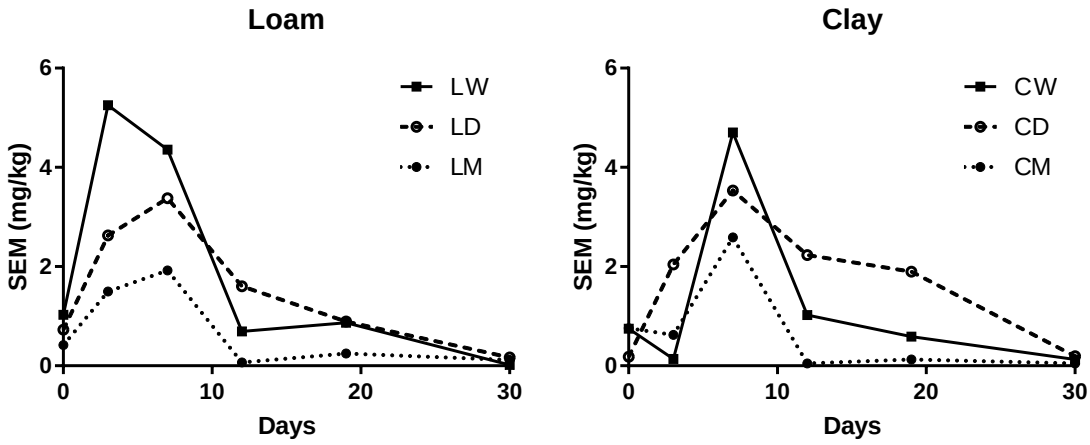


Figure 23: Standard error of the mean for metaldehyde recovered for loam (left) and clay (right) soils from incubation day 0 to day 30.

Conducting a two-way ANOVA with a no-matching experiment design, treatment type SEM was compared by extraction day. 60.61 % of total variation in SEM was the result of variation in extraction day, which was highly statistically significant ($p<0.0001$), where only 12.27 % of variation occurred due to treatment type. No statistically significant differences in SEM between treatment types were found with the exception of significance between LW and CW treatments and LW and CM treatments on extraction day 3 (table 23).

*Table 23: A Tukey's comparison showing significant differences in standard error of the mean for treatment types on extraction day 3. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.*

Treatment one	SEM (mg/kg)	Treatment two	SEM (mg/kg)	Significance level
LW	5.252	CW	0.1362	**
LW	5.252	CM	0.6236	*

Another two-way ANOVA with a no-matching experiment design comparing the SEM for different extraction days within treatment type found statistically significant differences in the wet pellet treatments (table 24). Both LW and CW treatment had statistically significant decreases in SEM between days 7 and 30 however all other differences were individual to the soil type. In the loam soil, SEM significantly increased between days 0 and 3, and significantly decreased between days 3 and 12, 19 and 30, and between days 7 and 30. For the clay soil, SEM significantly decreased between days 0 and 3, 3 and 7, 7 and 19, and 7 and 30.

*Table 24: A Tukey's comparison showing significant differences in wet-pellet treatment standard error of the mean between extraction days. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.*

Treatment	Day number A	SEM (mg/kg)	Day number B	SEM (mg/kg)	Significance level
LW	0	1.027	3	5.252	*
	3	5.252	12	0.6908	*
	3	5.252	19	0.8700	*
	3	5.252	30	0.01794	**
	7	4.357	30	0.01794	
CW	0	0.7456	3	0.1362	*
	3	0.1362	7	4.705	*
	7	4.705	19	0.5887	*
	7	4.705	30	0.1257	*

3.5.5 Coefficient of variation

The coefficient of variation (CV) was higher in the loam soil treatments than the clay soil treatments for days 0 and 3, with the highest CV at 55.62 % (LM, day 3) and 88.95 % (LD, day 0). For day 0 and day 3 the clay CV was much lower with the highest CV 52.14 % (CM, day 0). The day 7 CV was similar for both soils, averaging 28.97% in the loam soil and 29.01% in the clay soil. On days 12 and 19 each clay soil treatment had higher CV than the loam soil treatments, averaging 57.33% and 52.46 % in comparison to 22.80% and 44.65%. The day 30 CVs had similar averages although there was large variation within treatment types. In the loam soil CV ranged from 29.21% (LW) to 76.13% (LD), whereas in the clay soil CV ranged from 34.66% (CM) to 76.82% (CD).

No statistical significance was found between soil type, treatment type or extraction day for CV.

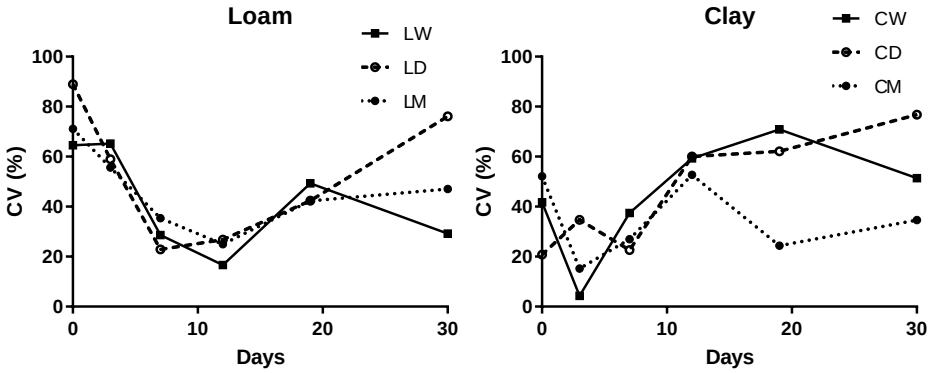


Figure 24: Coefficient of variance of metaldehyde recovered for loam (left) and clay (right) soils from incubation day 0 to day 30.

3.5.6 Degradation model

Using first order degradation, a model was made to show expected decay rate from day 7 to 30 based on the average metaldehyde concentration extracted per treatment type.

Taking the average concentrations (mg/kg) for each treatment type for days 7 to 30, the rate constant, k, was calculated using the solve function on excel, making the root mean square error as close to 0 as possible. Using k, the expected concentration from the model could be calculated and plotted alongside recorded concentration per treatment type (figure 25)

Modelled half-life could also be calculated, shown next to treatment type. The highest half-life was 3.98 days for the LD treatment type, followed by the LW treatment type, $t^{1/2}$ = 3.43 days. For both soil types, dry pellet treatments had the highest half-life, followed by wet pellet treatment, then laboratory-grade metaldehyde. In comparing the same metaldehyde treatment between soil types, loam soil always had the higher half-life.

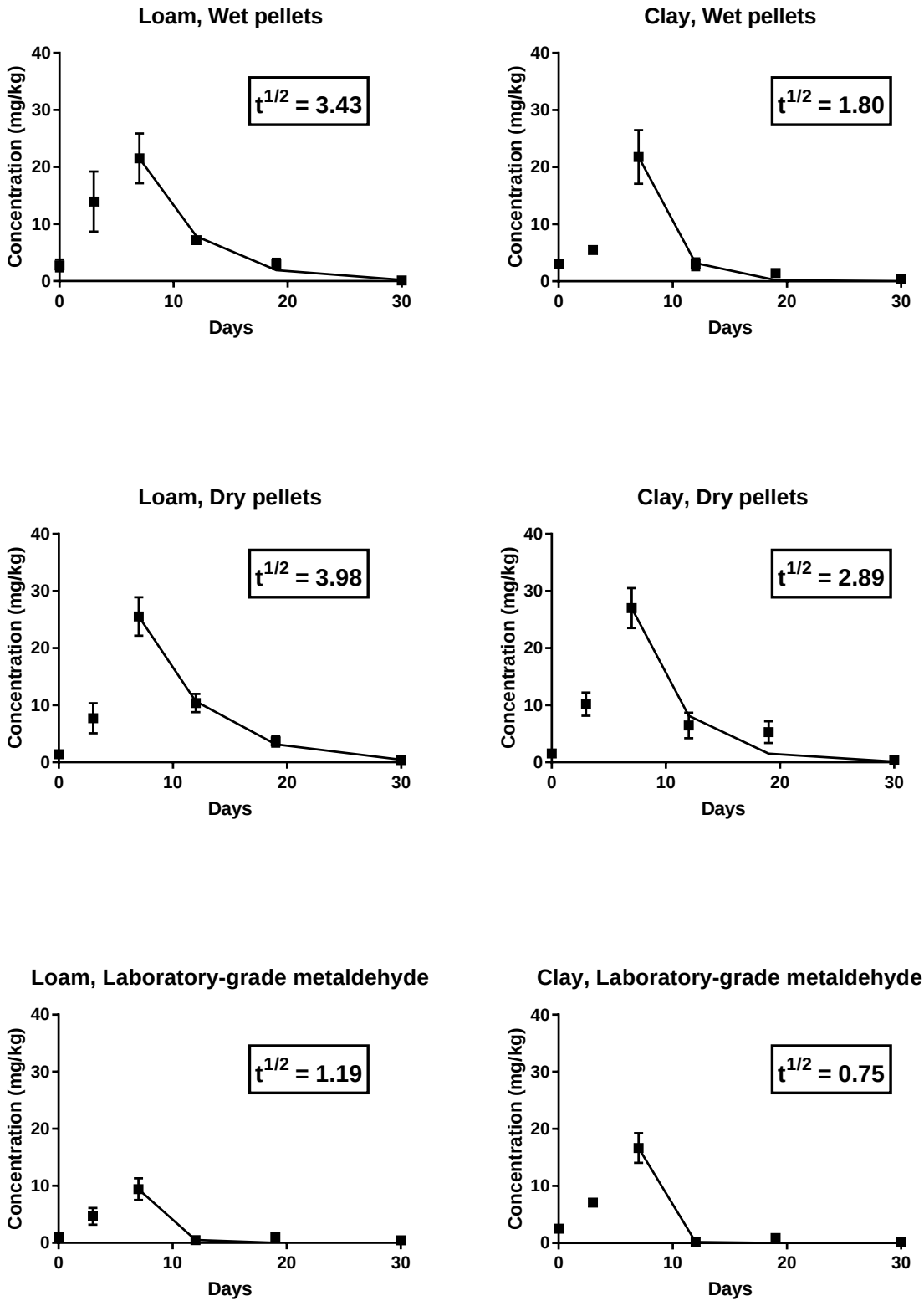


Figure 25: Concentration of metaldehyde recovered from incubation vessels between day 0 and 30, mg/kg. Standard error of the mean is shown by error bars and the model of degradation has been applied from day 7 to day 30. The calculated half-life, $t^{1/2}$, is shown for each treatment type.

3.6 Results Summary

Table 25: Results summary for the in vitro *incubation experiment*

Average concentration	• Average concentration increased between day 0 and 7, then decreased until day 30
	• Significant differences between treatment types on day 7 but no other days
	• Significant differences between average concentrations on different extraction days within treatment types
	• Loam soils
	  ○ Wet pellet had higher concentration until day 7, then dry pellets had higher concentrations
	  ○ Highest recorded concentration 25.56 mg/kg, dry pellets, day 7
	• Clay soils
	  ○ Dry pellets had higher concentrations on all extraction days except day 0
	  ○ Highest recorded concentration 27.00 mg/kg, dry pellets, day 7

SEM	• SEM was highest on day 7, except for the LW treatment which was highest on day 3
	• Lowest SEM was day 30
	• Significant differences between LW and CW, and LW and CM, treatments on extraction day 3.
	• Significant differences between SEM on different extraction days within treatment types
	• Loam soil
	  ○ Wet pellet SEM highest until day 7, then dry pellet SEM highest
	  ○ Highest SEM was 5.252 mg/kg for wet pellets on day 3
	• Clay soil
	  ○ Dry pellets SEM consistently above 1.9 mg/kg
	  ○ All other treatments had SEM <1 mg/kg except on day 7

CV	• Loam soils:
	  ○ High initial CV, up to 88.95% on day 0, dropping to an average 28.97% on day 7.
	  ○ Day 30 CV ranged between 29.21% to 76.13%
	• Clay soils:
	  ○ Lower initial CV, up to 52.14% on day 0, dropping to an average 29.01% on day 7.
	  ○ Day 30 CV ranged between 34.66% to 76.82%
	• No significant differences between soil types, treatment types or extraction days

Recovery	• Overall recovery increased between day 0 and day 7, then decreased until day 30 • Maximum recovery of the applied nominal metaldehyde was 66.9% for loam soils and 59.7% for clay soils • After day 19 recovery for both soils was below 19% • Loam soils had higher wet pellet recovery until day 7, then higher dry pellet recovery • Clay soils had higher dry pellet recovery except for on day 0
Half-life model	• For both soil types, dry pellet treatments had the highest half-life, followed by wet pellet treatment, then laboratory-grade metaldehyde. • $T^{1/2}$ LD: 3.98 days; LW: 3.43 days; LM: 1.19 days CD: 2.89 days; CW: 1.80 days; CM: 0.75 days

3.7 Discussion

The incubation experiment was designed to explore whether there were any marked differences between pelletised and laboratory-grade metaldehyde forms in terms of degradation rates and half-lives. The experiment was not designed to be environmentally realistic, but instead to set a benchmark for slug pellet degradation in comparison to traditional degradation testing. Specifically, the following research question and hypotheses were tested:

Research question:

2. Does the physical casing of the two pellet types influence metaldehyde half-life, particularly in comparison to powdered metaldehyde?

Research hypothesis:

3. Metaldehyde will have the highest longevity in the wet-processed pellets, followed by dry-processed pellets and then powdered metaldehyde, which will breakdown fastest
4. Metaldehyde longevity will be highest in the clay soil, which has lower organic matter content (therefore an expected lower biological activity to degrade metaldehyde)

Metaldehyde recovery was generally low (up to 66.9% for the loam soil and 59.7% for the clay soil). For the majority of the 30 day incubation period, recovery was <30% for all treatments. This is likely due to the extraction method, where mechanical extraction can have low recoveries (Ma et al., 2012).

With pellet casing it could be argued that low recovery might be expected, because metaldehyde may have to leach out of the pellet casing before becoming recoverable. However, if this were the case it could be argued that the laboratory-grade metaldehyde should have a much higher recovery than the pelletized forms. This was not the case. In fact, laboratory-grade metaldehyde had and even lower recovery. This could be because the pellet casing prevented the metaldehyde from binding to the soil as a bound residue (also known as a non-extractable residue).

Extraction processes are often unable to extract all of the pesticide from the soil, even with more exhaustive, repeated, extraction procedures. However, the environmental significance of a pesticide does not depend on its non-extractability, but on its bioavailability. Bound residues can be influenced by biological activity (Gevao et al., 2000). Metaldehyde, which is primarily degraded through bacterial action (Thomas et al., 2017) needs to be biologically available for degradation to occur. Since recovery using mechanical extraction was low, metaldehyde binding to the soil could be one reason for low recorded concentrations and recoveries.

Interestingly, recoveries were lowest immediately after spiking. Concentrations of metaldehyde increased between days 0 and 7 in all treatments, before decreasing monotonically until day 30. This was broadly reflected in the recovery of metaldehyde, which was highest on day 7 for all treatments. This suggests that the apparent increase in concentration was the result of improved recovery. Similarly, as concentration decreases, so does the recovery, therefore absolute concentration cannot be speculated upon accurately. However, the relative changes in concentration between treatments may still have some merit, as all treatments were subject to the same extraction conditions.

On day 7, which had the highest recoveries, the highest concentrations and SEM were also recorded. This was also the only extraction day which produced significant differences in concentration, possibly due to having the highest recovery rate. Clay vessels had slightly higher recorded concentrations than the loam soil, which may have been due to slightly higher recoveries for the clay soil. However, there differences were not statistically significant.

There appeared to be a relationship between SEM and recovery. When SEM (variability) was low, recovery was also low. Coefficient of variance was highest on day 30, further suggesting that recovery rates limited accurate quantification of metaldehyde.

Literature half-life values for metaldehyde extracted from soils in-field (table 3) range between 0.75 and 2.4 (Zhang et al., 2011, Dong et al., 2017) for a powder solution and 3.17 to 4 for granules (Ma et al., 2012, Zhang and Dai, 2006). *In vitro* the range is much

larger, up to 223 days (Kay and Grayson, 2014) and was typically derived using powdered laboratory-grade metaldehyde solutions. In this incubation study, half-lives ranged from 0.75 to 1.19 days for laboratory-grade (powdered) metaldehyde and between 1.80 and 3.98 for metaldehyde pellets. The pelletised half-lives recorded in this study were lower than expected, although not as low as those (0.27d) reported in the research by Calumpang et al. (1995). However, these values cannot be expressed with much certainty due to low recovery and a lack of internal standard. However, half-life values did reflect the differences between half-lives for powdered and granular metaldehyde reported in the literature from in-field experiments. Here, our data show that powdered metaldehyde has the highest degradation rate in either soil type. However, no significant differences in half-life were found between wet or dry pellet treatments, or between soil types. This corresponds to metaldehyde half-life comparison between metaldehyde forms by Zhang et al. (2011).

In regard to research question 1, pellet casing does appear to influence the longevity of metaldehyde, however, due to the many above-described difficulties in metaldehyde recovery, the precise quantification of pellet influence was impossible. From the incubation experiment results, it appears that dry pellet treatments had the highest half-life (highest longevity), followed by wet pellet treatment, then laboratory-grade metaldehyde, for both soil types. This suggests that hypothesis 1 is incorrect regarding the longevity of wet pellets in comparison to dry pellets, however, hypothesis 1 cannot be confirmed without further research using a more accurate extraction method.

Hypothesis 2 theorised that metaldehyde longevity would relate to the bacterial activity of the soil, in which clay soils would have the lowest biological activity and therefore have the slowest metaldehyde degradation rate. Bacterial activity within soils is believed to be the leading cause of metaldehyde degradation. In this study methanol was added to all vessels, because it was required as a matrix for laboratory-grade metaldehyde. Vessels were also maintained in the dark. Although photolysis does not probably impact metaldehyde fate directly (European Food Safety Authority, 2010), light can affect bioactivity by changing the carbon balance and by encouraging

phototrophs to inhabit the soil surface. The effect of methanol addition may have had a toxic effect on the soil microbes. Alternatively, it may also have stimulated microbes by adding a carbon source. As all treatment types received methanol and were incubated without light, half-lives are comparable within this study; however the difference in environmental conditions between this study, laboratory and field studies make meaningful half-life comparisons difficult.

In response to research hypothesis 2, half-life was shown to be slightly higher in the loam soil. This was unexpected because the loam soil had a higher organic matter content. Higher organic matter content is often associated with higher biological activity, although sorption will also be higher which could have reduced bioavailability. As discussed above, biological activity could also have been influence by methanol addition.

Chapter Four

Experiment Three: Physical Degradation

4.1 Introduction

There is relatively little information or understanding in the literature about the environmental behaviour of slug pellets and associated metaldehyde. Studying the physical disintegration of slug pellets will help determine the physical properties of pellets under varying environmental conditions. Specifically, rainfall intensity and soil moisture are believed to control physical pellet breakdown. In this study, physical breakdown was tracked via changes in visible surface area, mass and colour.

Soil moisture pellet degradation experiments and integrated degradation experiments were conducted in conjunction with Leah Beerman as part of her undergraduate project. Data collection using photography and weighing was jointly undertaken and photographs were processed into raw data by Leah. Statistical analysis and discussions were completed independently. Rainfall tower kinetic energy experiments were undertaken entirely independently.

The physical disintegration study was split into three sub-experiments looking at soil moisture, kinetic energy and a combination of both (integrated disintegration experiment) on slug pellet disintegration. All studies used the same soil type, a loam soil, collected from Lyndon farms (figure 2). The behaviour of both wet- and dry-processed pellets were examined.

Throughout this chapter, Red-Green-Blue values are referred to as RGB values, with individual colours Red, Green and Blue referred to as R, G and B. Irrigation regimes are referred to based on their relation to mean daily rainfall, where ‘50% irrigation’ is equivalent to ‘50% of the mean daily rainfall irrigation’.

This chapter has been separated by sub-experiment and addresses the methods, results and discussion for each sub-experiment individually. A final discussion which concludes between all physical degradation experiments is included at the end of the

chapter. This final discussion will also refer back to the original research questions and hypotheses outlined in Chapter One.

4.2 Sub-experiment one: Soil moisture in physical degradation

4.2.1 Methodology

The soil moisture experiments were conducted under controlled laboratory conditions. Twenty-four plant pots, each measuring 51mm x 48mm x 47mm, were filled with 50.00 g ± 1.00 g of loam soil and two pellets, either wet- or dry-processed, were placed on the soil surface. Wet- and dry- processed pellets were distributed equally between each soil irrigation volume and randomised block sampling was used to ensure unbiased environmental conditions between plant pots. Pots were irrigated daily with their assigned volume of water.

Of the 24 plant pots, six were not irrigated, six were given mean rainfall, six mean rainfall minus 50% and six mean rainfall plus 50% (Table 26). Irrigation volumes were based on average daily rainfall between April 2014 and April 2017 in the Gwash catchment, Rutland, the catchment next to the Chater catchment in which the soil was extracted from. Average daily rainfall was chosen as representative of the whole year where only April mean rainfall was statistically significantly different from the population mean. However, April had been much drier between 2014 and 2017 in comparison to the April mean rainfall recorded between 1981 to 2010 at Oakham climate station, the closest station to the Gwash catchment (Met Office, 2017). The Oakham climate station April rainfall mean was not significantly different from the Gwash population rainfall average and therefore the Gwash catchment whole population daily rainfall average was considered to be representative. Intensity of rainfall changes with the season were not taken into account as only soil moisture, not kinetic energy, varied between treatments.

Average whole population rainfall was converted from mm to ml, appropriate to the area of the plant pots, where 1 mm rainfall was considered equal to 1 litre of water per square metre. Therefore 1 mm rain was equal to 0.002448 litres (2.448 ml) per plant pot.

Table 26: Gwash catchment rainfall (mm) and soil moisture plant point irrigation equivalents (ml)

	Not irrigated	Mean rainfall minus 50%	Mean rainfall	Mean rainfall plus 50%
Gwash catchment rainfall whole population (mm)	0	0.88	1.77	2.65
Soil moisture plant pots irrigation equivalent (ml)	0	2.17	4.33	6.50

Pellets, plant pots and soil were all weighed individually to monitor soil moisture. Although soil moisture could not be controlled exactly, mass of water in the soil matrix could be monitored daily and controlled relative to irrigation between individual plant pots. The following formula was used to calculate soil water mass:

$$Msw = Mw - Mp - Md$$

Where: $Md = Mw/(1 + \theta)$

Where *Msw* is the soil water mass, used as a proxy for soil moisture, *Mw* is the wet soil mass, *Mp* is the plant pot mass, *Md* is the dried soil mass and θ is the gravimetric water content. Pellet weight was not calculated within this equation as pellet mass was expected to change over time, pellets were too fragile to remove from the plant pots whilst weighing them and the weight was insignificant in comparison to the other variables. Pellet weight averaged 22.22 mg for dry pellets and 7.498 mg for wet pellets.

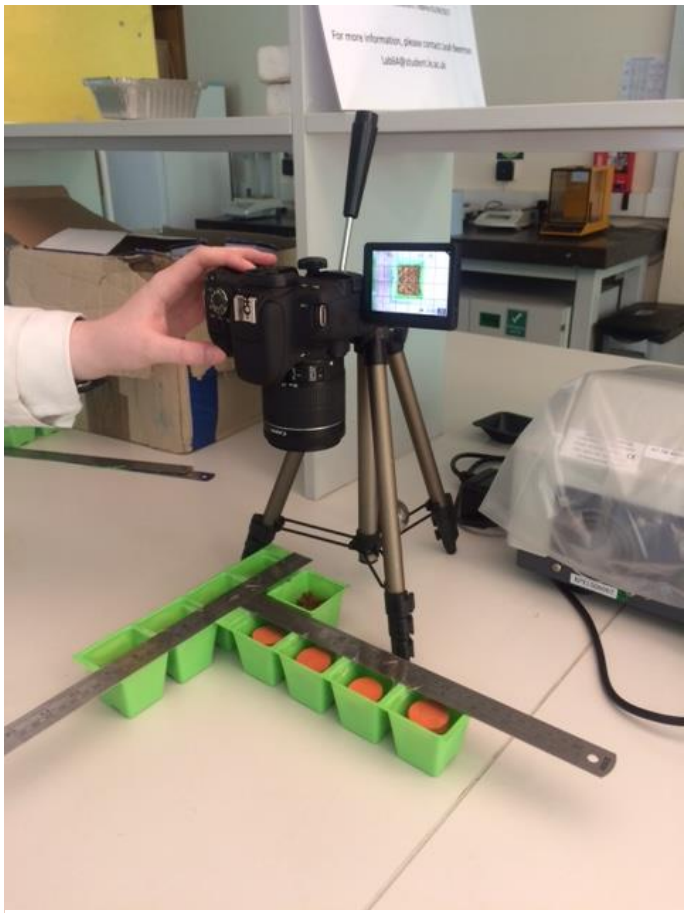


Figure 26: Photography method set-up

To monitor the 2-dimensional physical degradation of slug pellets based on soil moisture, photographs of the pellets were taken daily during the working week from the same position. To minimise difference between pellet photos a tripod was used to take the photographs, set up with the camera facing directly downwards, and rulers were inserted into the photograph for scale (Figure 26). The camera grid-square function was used to line up the internal plant pot square parallel to the rulers and lights were also used to minimise shadows and lighting changes between images.

Using *ImageJ* software, each image was assigned a scale in millimetres via the 'set scale' function, using the in-photo rulers for reference. The scale applied did not account for depth of the pellet, Z, only accounting for the XY 2-dimensional nature of the image. Using the *Polygons selection* tool to draw around each pellet, pellets could be measured to determine their individual visible surface area and perimeter through the *Analyse →Measure* tool. The Red-Green-Blue (RGB) value for each pellet could also be determined via *Plugins → Analyse → RGB Measure*. Normalised RGB values were

used during analysis instead of absolute RGB values to further minimise lighting difference having an influence on analysis, and to allow comparison between pellet types where wet pellets are a lighter blue colour than dry pellets. Over time, 2D changes in pellet dimension and colour, therefore physical degradation, could be monitored. It should be noted that this method only monitored the visible surface area of the pellets and was unable to account for the 3-dimensional nature of the pellets as only 2D imagery was used.

Each R, G and B value is an integer between 0 and 255, which when combined produce one of 16, 777,216 possible colours. Something that is completely Red would have an RGB of (255, 0, 0), whereas Blue would have (0, 0, 255). Since pellets are blue in colour, it is expected that the blue value will be highest before any colour change, although it will be a combination of R, G and B values which produce that blue colour.

4.2.2 Results

4.2.2.1 Soil moisture

Soil moisture visibly differed between treatment types with all irrigated treatments showing visible weekly patterns of soil moisture decrease corresponding to non-irrigation days when access to the experiment was restricted (figure 27). These methodology-induced variations in soil moisture were included in analysis as they may have had an impact on physical pellet degradation. The no irrigation treatment appeared to drop in soil moisture initially and remain and a constant soil moisture throughout.

A two-way ANOVA with respect to time showed that 59.32% of total variation occurred due to the irrigation regime, and 27.63% occurred due to time. Both of these factors were significant to the 99% confidence level ($p<0.0001$). A Tukey's comparison test taking the mean soil moisture content for each irrigation regime irrespective of time showed that there was a statistically significant difference ($p<0.0001$) in soil moisture between all regimes except between the 100% irrigation and 150% irrigation regimes. Therefore, pellets were subject to significantly different soil moisture environments.

Another Tukey's comparison test also showed significant differences over time within treatments, particularly the beginning of the experiment. The 'no irrigation' treatment soil moisture statistically significantly decreased in moisture each day for the first 6 days of the experiment ($p < 0.0001$) and then remained at a non-significant constant soil moisture of approximately 5.3g.

The 50% irrigation treatment also statistically significantly changed, dropping significantly in soil moisture every 3 days for the first 14 days of treatment. After 14 days, with some variations in soil moisture, soil moisture remained at approximately 5.6g. The 100% and 150% irrigation treatments statistically significantly changed only between the first and second day of treatments, averaging 6.3g and 6.2g respectively.

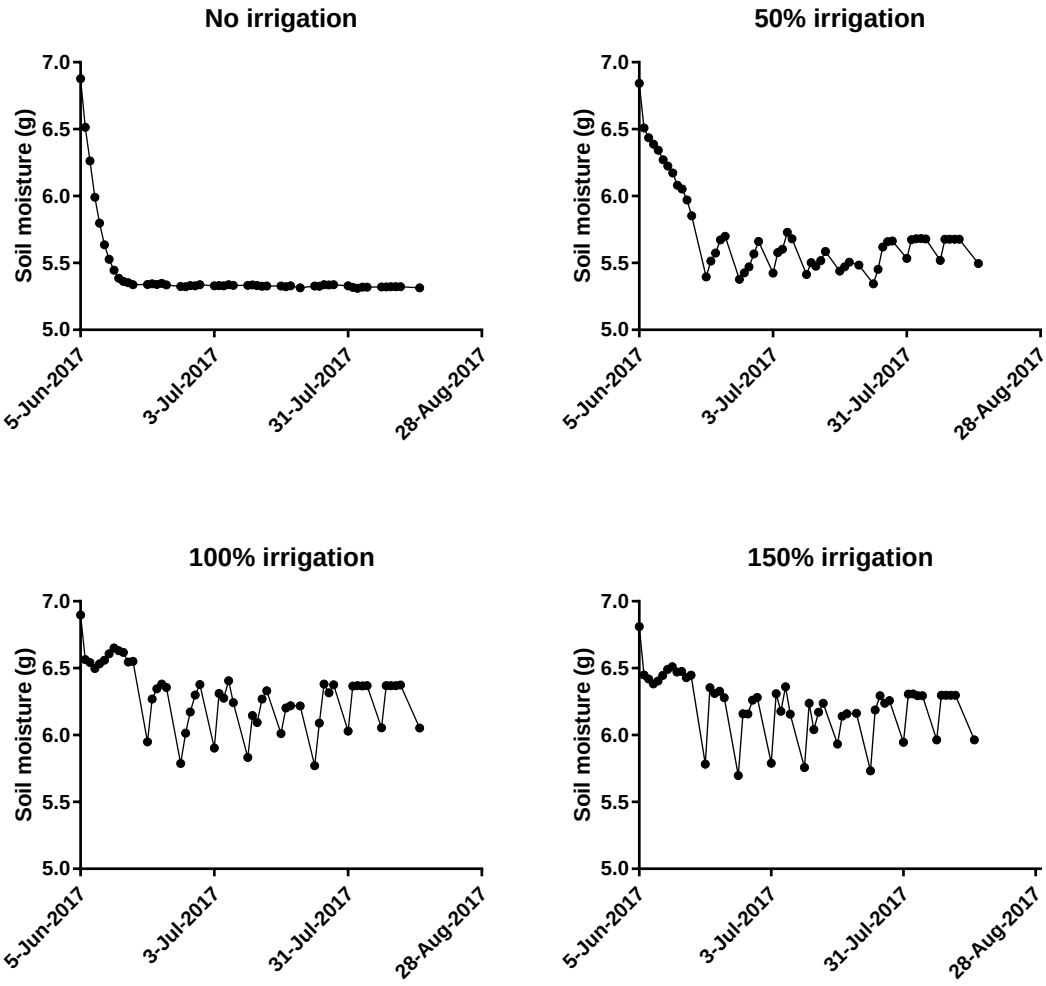


Figure 27: Soil moisture over time for the soil moisture irrigation experiment

4.2.2.2 RGB analysis

RGB values were converted to ratios to eliminate differences in lighting between days which may have affected absolute RGB value. For all treatments, green values appeared to remain similar throughout the experiment duration, whereas blue values decreased over time and red values increased, with the exception of the 'no irrigation, dry pellet' treatment (figure 28). Higher irrigation treatments appeared to produce a faster change in red and blue values.

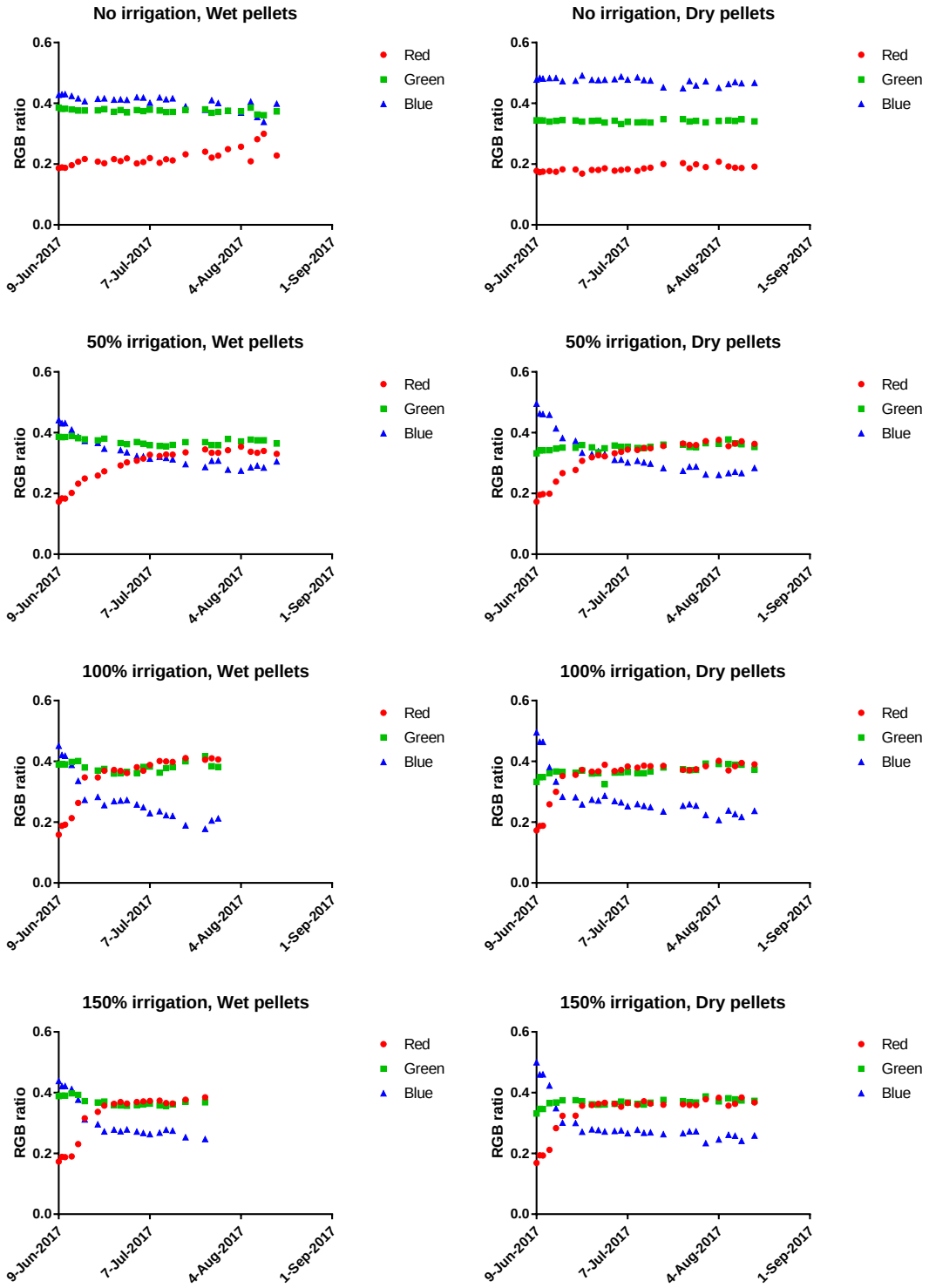


Figure 28: RGB ratio colour change over time for all treatment types within the soil moisture irrigation experiment

RGB absolute values were split by colour, and normalised so wet- and dry- pellets could be compared. As means are used during an ANOVA, analysis was conducted for values recorded between 9th June (day 0) and 3rd July (day 24), when some pellets from the 150% irrigation experiment had fully degraded and therefore RGB values equalled zero. This prevented skewness within the dataset without compromising analysis for pellet colour change, where the colour visually changes before 3rd July.

Red:

A two-way ANOVA with respect to time showed Red values to significantly vary by time, 22.98% of variation, and treatment type, 22.34% of variation, to the 99.99% and 99.90% confidence intervals respectively ($p<0.0001$; $p<0.001$).

A Tukey's comparison test of mean R value for each treatment type showed some statistically significant differences (table 27).

*Table 27: Tukey's comparison showing significant differences in mean R value between treatment types within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	**Treatment Type Two**	**Significance**
No irrigation, Wet pellets	50% irrigation, Dry pellets	*
No irrigation, Wet pellets	100% irrigation, Dry pellets	*
No irrigation, Dry pellets	50% irrigation, Dry pellets	**
No irrigation, Dry pellets	100% irrigation, Dry pellets	**

Another Tukey's multiple comparison test showed that no statistical differences in R value between treatment types were found until 8 days into the experiment. On day 17, there were statistical differences between treatment types (table 28).

*Table 28: Tukey's comparison showing significant difference in R value between treatment types on day 17 within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	**Treatment Type Two**	**Significance**
No irrigation, Wet pellets	50% irrigation, Dry pellets	****
No irrigation, Wet pellets	100% irrigation, Dry pellets	****
No irrigation, Dry pellets	50% irrigation, Wet pellets	*
No irrigation, Dry pellets	50% irrigation, Dry pellets	****
No irrigation, Dry pellets	100% irrigation, Dry pellets	****
50% irrigation, Dry pellets	100% irrigation, Wet pellets	***
50% irrigation, Dry pellets	150% irrigation, Wet pellets	**
50% irrigation, Dry pellets	150% irrigation, Dry pellets	*
100% irrigation, Dry pellets	100% irrigation, Wet pellets	***
100% irrigation, Dry pellets	150% irrigation, Wet pellets	**
100% irrigation, Dry pellets	150% irrigation, Dry pellets	*

Green:

A two-way ANOVA with respect to time showed green values to significantly vary by time, 9.8% of variation, and treatment type, 34.91% of variation, to the 99.99% confidence interval ($p<0.0001$).

A Tukey's comparison test of mean G value for each treatment type showed some statistically significant differences (table 29).

*Table 29: Tukey's comparison showing significant difference in mean G value between treatment types within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	**Treatment Type Two**	**Significance**
No irrigation, Wet pellets	50% irrigation, Dry pellets	*
No irrigation, Dry pellets	50% irrigation, Dry pellets	**
50% irrigation, Dry pellets	100% irrigation, Wet pellets	****
50% irrigation, Dry pellets	150% irrigation, Wet pellets	***
50% irrigation, Dry pellets	150% irrigation, Dry pellets	**
100% irrigation, Wet pellets	100% irrigation, Dry pellets	**
100% irrigation, Dry pellets	150% irrigation, Wet pellets	**

Another Tukey's multiple comparison test showed that no statistical differences in G value between treatment types were found until 8 days into the experiment. On day 17, there were statistical differences between treatment types (table 30)

*Table 30: Tukey's comparison showing significant difference in G value between treatment types on day 17 within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	Treatment Type Two	Significance
No irrigation, Wet pellets	50% irrigation, Dry pellets	****
No irrigation, Wet pellets	100% irrigation, Dry pellets	**
No irrigation, Dry pellets	50% irrigation, Wet pellets	****
No irrigation, Dry pellets	100% irrigation, Dry pellets	***
50% irrigation, Dry pellets	50% irrigation, Wet pellets	**
50% irrigation, Wet pellets	100% irrigation, Wet pellets	*
50% irrigation, Dry pellets	100% irrigation, Wet pellets	****
50% irrigation, Dry pellets	150% irrigation, Wet pellets	****
50% irrigation, Dry pellets	150% irrigation, Dry pellets	****
100% irrigation, Dry pellets	100% irrigation, Wet pellets	****
100% irrigation, Dry pellets	150% irrigation, Wet pellets	****
100% irrigation, Dry pellets	150% irrigation, Dry pellets	**

Blue:

A two-way ANOVA with respect to time did not show significant changes in blue values ($p>0.05$). However, a Tukey's comparison test for mean blue values showed some significant differences between treatment types (table 31).

*Table 31: Tukey's comparison showing significant difference in mean B value between treatment types within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	Treatment Type Two	Significance
50% irrigation, Wet pellets	100% irrigation, Wet pellets	*
50% irrigation, Wet pellets	100% irrigation, Dry pellets	*
50% irrigation, Wet pellets	150% irrigation, Wet pellets	*
50% irrigation, Wet pellets	150% irrigation, Dry pellets	*

Modelling change:

Using the normalised average blue values over time, a model could be fitted to express rate of change (figure 29). Blue was analysed as the primary dye colour of pellets, where R, G and B values produce that colour in combination, although B would contribute the highest value to produce the blue pellet colour.

The model fitted to the data was designed to show relative change only, allowing the data to be compared between treatments. It is noted that the fitting is NOT appropriate for the data and alternative approaches would be needed if the kinetics of dissipation are to be accounted properly and half-lives to be reliably established.

Calculated half-life was highest for the 'no irrigation' regimes, falling with increasing irrigation (table 32).

Table 32: Modelled B value rate constant, half-life and root mean square error for the soil moisture irrigation experiment

Treatment type	Rate constant, k	Half-life (days)	Root mean square error
No irrigation, Wet pellets	0.001727	401.3	0.02004
No irrigation, Dry pellets	0.0003527	1965	0.01962
50% irrigation, Wet pellets	0.008232	84.20	0.2790
50% irrigation, Dry pellets	0.017310085	40.04	0.05749
100% irrigation, Wet pellets	0.02506	27.66	0.07920
100% irrigation, Dry pellets	0.02547	27.22	0.1163
150% irrigation, Wet pellets	0.01808	38.35	0.08181
150% irrigation, Dry pellets	0.02367	29.28	0.1123

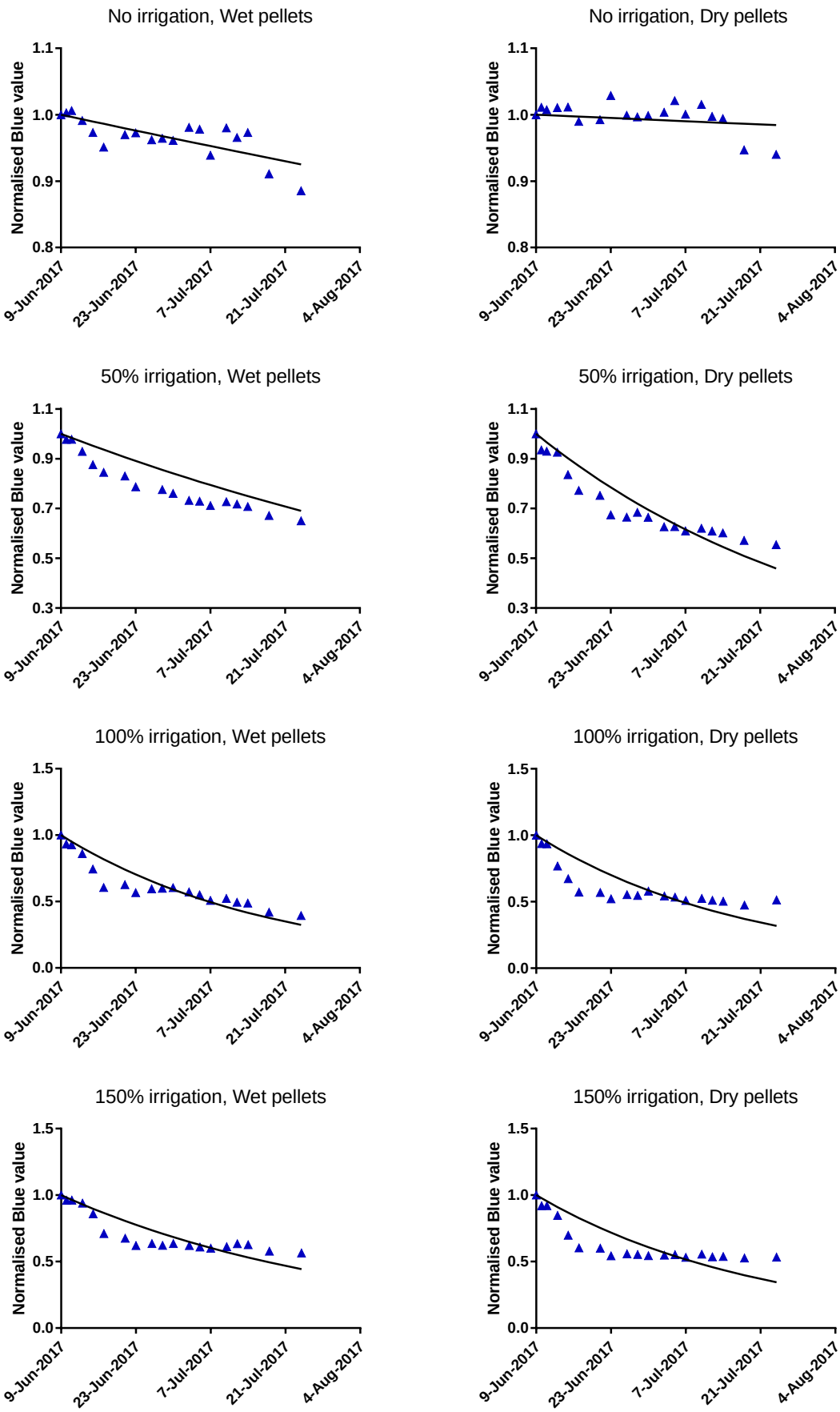


Figure 29: Modelled and measured B value change with time for the soil moisture irrigation experiment

4.2.2.3 Pellet visible surface area

Absolute pellet area was normalised to allow direct pellet comparison. Over time, higher irrigation regimes visually showed the largest pellet change, with wet pellets fully degrading by 26th July for the 100% and 150% irrigation regimes, after 45 days (figures 30 and 31). At the beginning of the experiment, wet pellets had a mean visible surface area of 4.124 mm^2 and dry pellets of 9.844 mm^2. Error was also visibly large, with an average standard deviation of 0.7990 mm^2 for wet pellets and 2.257 mm^2 for dry pellets.

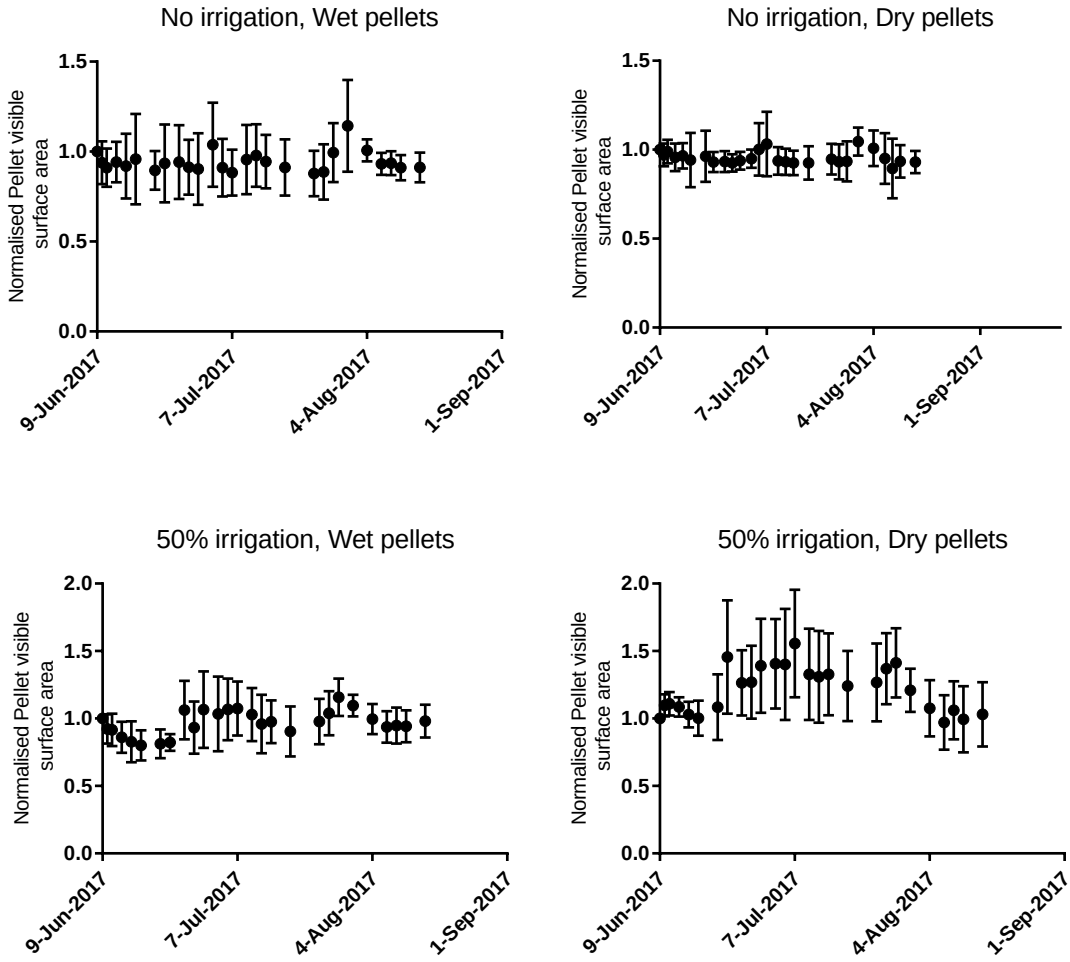


Figure 30: Wet- and Dry-processed pellet visible surface area change for 'no irrigation' and '50% irrigation' treatment types within the soil moisture irrigation experiment. Error bars shown are standard deviation.

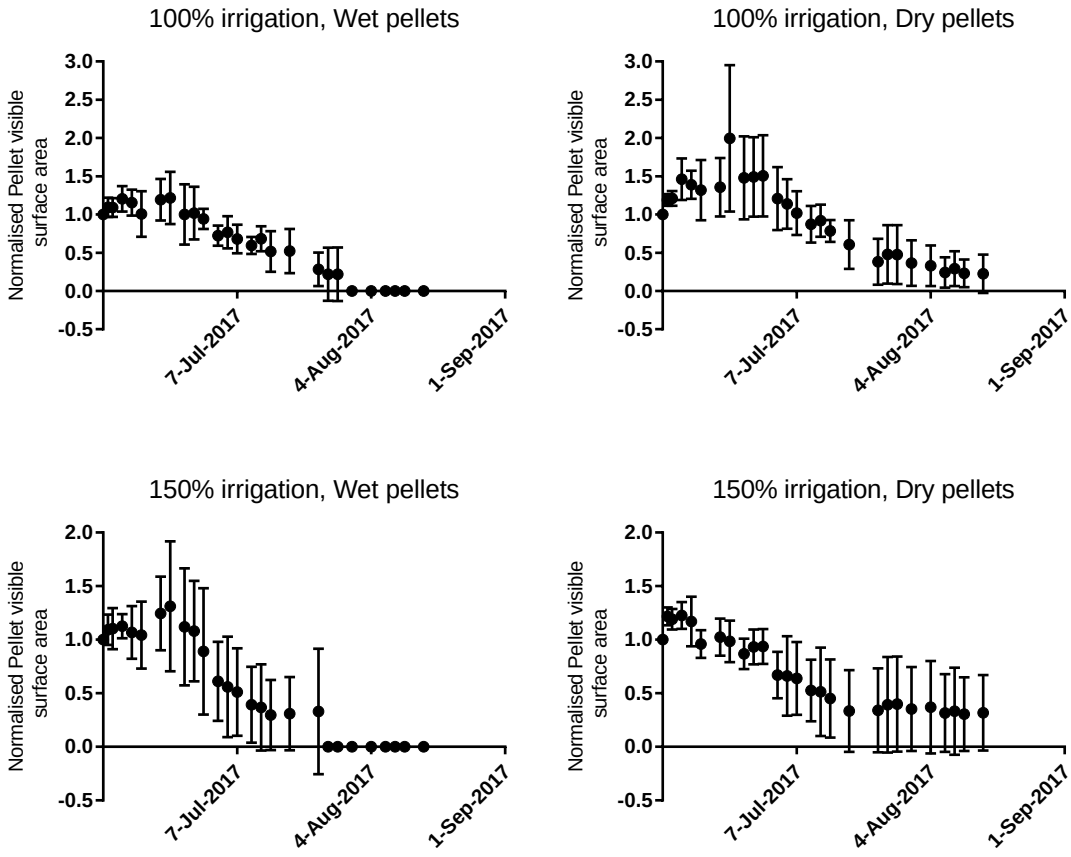


Figure 31: Wet- and Dry-processed pellet visible surface area change for '100% irrigation' and '150% irrigation' treatment types within the soil moisture irrigation experiment. Error bars shown are standard deviation

Taking the normalised visible surface area data for wet-processed pellets, a two-way ANOVA with respect to time showed that time accounted for 25.27% of variation in pellet visible surface area and irrigation regime by pellet type accounted for 17.34%. Both of these were significant to the 99.99% confidence interval ($p<0.0001$).

A Tukey's multiple comparisons test showed that overall mean visible surface area by irrigation regime was significantly different between all regimes except from 'No irrigation, wet pellets' and '50% irrigation, wet pellets', and between '100% irrigation, wet pellets' and '150% irrigation, wet pellets' (table 33).

*Table 33: Tukey's comparison showing significant differences in normalised mean visible surface area between wet-pellet treatment types within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	Treatment Type Two	Significance
No irrigation, Wet pellets	100% irrigation, Wet pellets	**
No irrigation, Wet pellets	150% irrigation, Wet pellets	***
50% irrigation, Wet pellets	100% irrigation, Wet pellets	***
50% irrigation, Wet pellets	150% irrigation, Wet pellets	***

Taking the normalised visible surface area data for dry-processed pellets, a two-way ANOVA with respect to time showed that time accounted for 22.39% of variation in pellet visible surface area ($p<0.0001$) and irrigation regime by pellet type accounted for 18.28% ($p<0.001$).

A Tukey's multiple comparisons test showed that overall mean visible surface area by irrigation regime was significantly different between half of the irrigation regimes (table 34).

*Table 34: Tukey's comparison showing significant differences in normalised mean visible surface area between dry-pellet treatment types within the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	Treatment Type Two	Significance
No irrigation, Dry pellets	150% irrigation, Dry pellets	*
50% irrigation, Dry pellets	100% irrigation, Dry pellets	*
50% irrigation, Dry pellets	150% irrigation, Dry pellets	****

In comparing pellet types, normalised visible surface areas were compared using a two-way ANOVA with respect to time.

Comparing 'no irrigation' wet and dry pellets, time was found to be a significant source of variation accounting for 8.729% ($p<0.001$), and pellet type was not significantly different ($p>0.05$). This was corroborated by a Sidak's multiple comparisons test which showed no significant differences in visible surface area between pellet types any one point in time.

Similarly, the '150% irrigation' found that time was a significant source of variation, accounting for 61.67% ($p<0.0001$), however pellet type was not a significantly variation source ($p>0.05$). Again, a Sidak's multiple comparisons test showed not significant differences in visible surface area between pellet types any one point in time.

Opposed to the extreme irrigation regimes, the '50% irrigation' and '100% irrigation' regimes both showed time and pellet type to be significant sources of variation (50% irrigation: time = 17.88 % of variation, $p<0.0001$; Pellet type = 20.49% of variation, $p<0.05$; 100% irrigation: time = 67.82 % of variation, $p<0.0001$; Pellet type = 6.721 % of variation, $p<0.01$).

A Sidak's multiple comparison test showed that within the 50% and 100% irrigation regimes, some dates had significantly different visible surface areas (table 35).

*Table 35: Sidak's comparison showing significant differences in normalised visible surface area within treatment types for the soil moisture irrigation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Irrigation Regime	**Date**	**Wet Pellet average surface area One (mm^2), average normalised area in brackets**	**Dry Pellet average surface area Two (mm^2), average normalised area in brackets**	**Significance**
50%	23rd June	3.434 (0.823)	12.801 (1.456)	****
	7th July	4.445 (1.074)	13.541 (1.556)	**
100%	23rd June	5.490 (1.217)	21.795 (1.995)	***
	30th June	4.263 (0.942)	16.425 (1.505)	*

Modelling change:

Using the normalised average visible surface area over time, a model was fitted to express rate of change (figure 32). **The model fitted to the data was designed to show relative change only, allowing the data to be compared between treatments. It is noted that the fitting is NOT appropriate for the data and alternative approaches would be needed if the kinetics of dissipation are to be accounted properly and half-lives to be reliably established.**

Calculated half-life was lowest for the higher irrigation regimes (table 36).

Table 36: Modelled pellet visible surface area rate constant, half-life and root mean square error for the soil moisture irrigation experiment

Treatment type	Rate constant, k	Half-life (days)	Root mean square error
No irrigation, Wet pellets	0.0006849	1012	0.1577
No irrigation, Dry pellets	0.001470	471.6	0.07730
50% irrigation, Wet pellets	0.0002235	3101	0.1963
50% irrigation, Dry pellets	0	N/A	0.2400
100% irrigation, Wet pellets	0.01850	37.47	0.2372
100% irrigation, Dry pellets	0.006067	114.2	0.3811
150% irrigation, Wet pellets	0.02518	27.53	0.2632
150% irrigation, Dry pellets	0.01615	42.91	0.1344

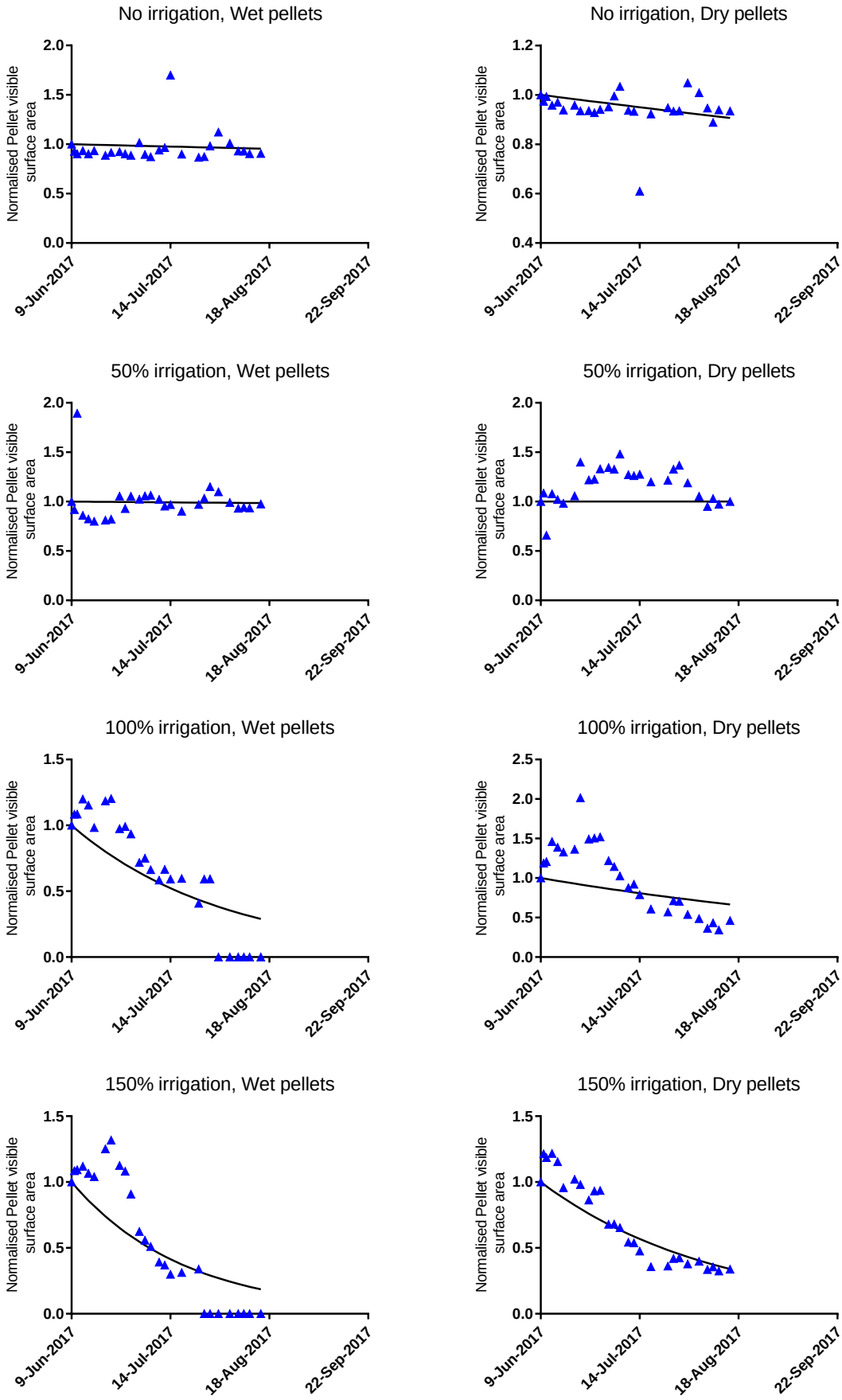


Figure 32: Modelled and measured pellet visible surface area change with time for the soil moisture irrigation experiment

4.2.2.4 Qualitative Analysis of pellet size and colour

Photos were taken of the pellets each time the plant pots containing them were weighed. 09/06/2017 was day 1 of the experiment, and 15/08/2017, the final day, was day 68 of the pellet irrigation duration. Photos shown are of one plant pot from each treatment type over time. In total each irrigation regime had three plant pots for each pellet type.

The no irrigation pellet appear to maintain their colour and visible surface area throughout the experiment duration, although pellets do move within the plant pot, changing rotation in the dry soil (figures 33 and 34).

The 50% irrigation shows stages of pellet colour change. By the 20/06/2017, 12 days into the experiment, pellet appeared mouldy and eventually the pellet took on the same white colour as the mould (figures 35 and 36). This was the case for both pellet types, although the dry pellets, being larger, had more visible mould.

Mould was also visibly present on the 100% irrigation pellets, appearing earlier than for the 50% irrigations, at just 5 days into the experiment (figures 37 and 38). Visible surface area change was also apparent within this irrigation regime, with pellets decreasing in size as the mould reduced. By the 15/08/2017 (day 68), wet pellets had completely degraded and dry pellets were much reduced in size.

The 150% irrigation regime had a similar response to the 100% irrigation regime, with mould appearing after 5 days and pellets gradually decreasing in visible surface area (figures 39 and 40). Wet pellets also completely degraded by then end of the 68 day experiment duration.

For the highest two irrigation regimes, pellets firstly turned white, as with the 50% irrigation regime, but then blended into the soil before physically degrading in size.

No irrigation, Dry pellet treatment 1D

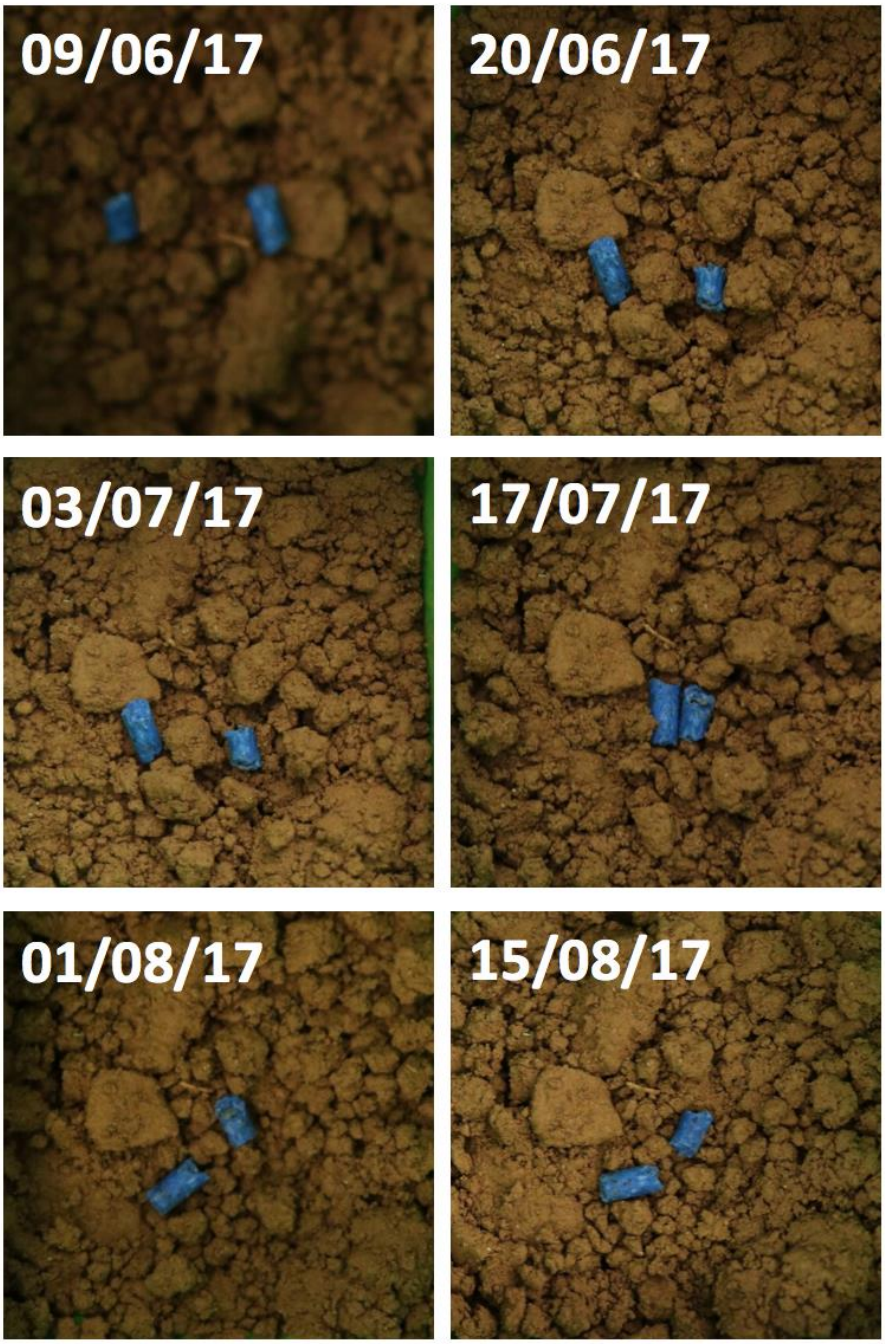


Figure 33: Photographs of the 'no irrigation, dry pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 20/06/2017 = day 20; 03/07/2017 = day 25; 17/07/2017 = day 39; 01/08/2017 = day 54; 15/08/2017 = day 68.

No irrigation, Wet pellet treatment 1A

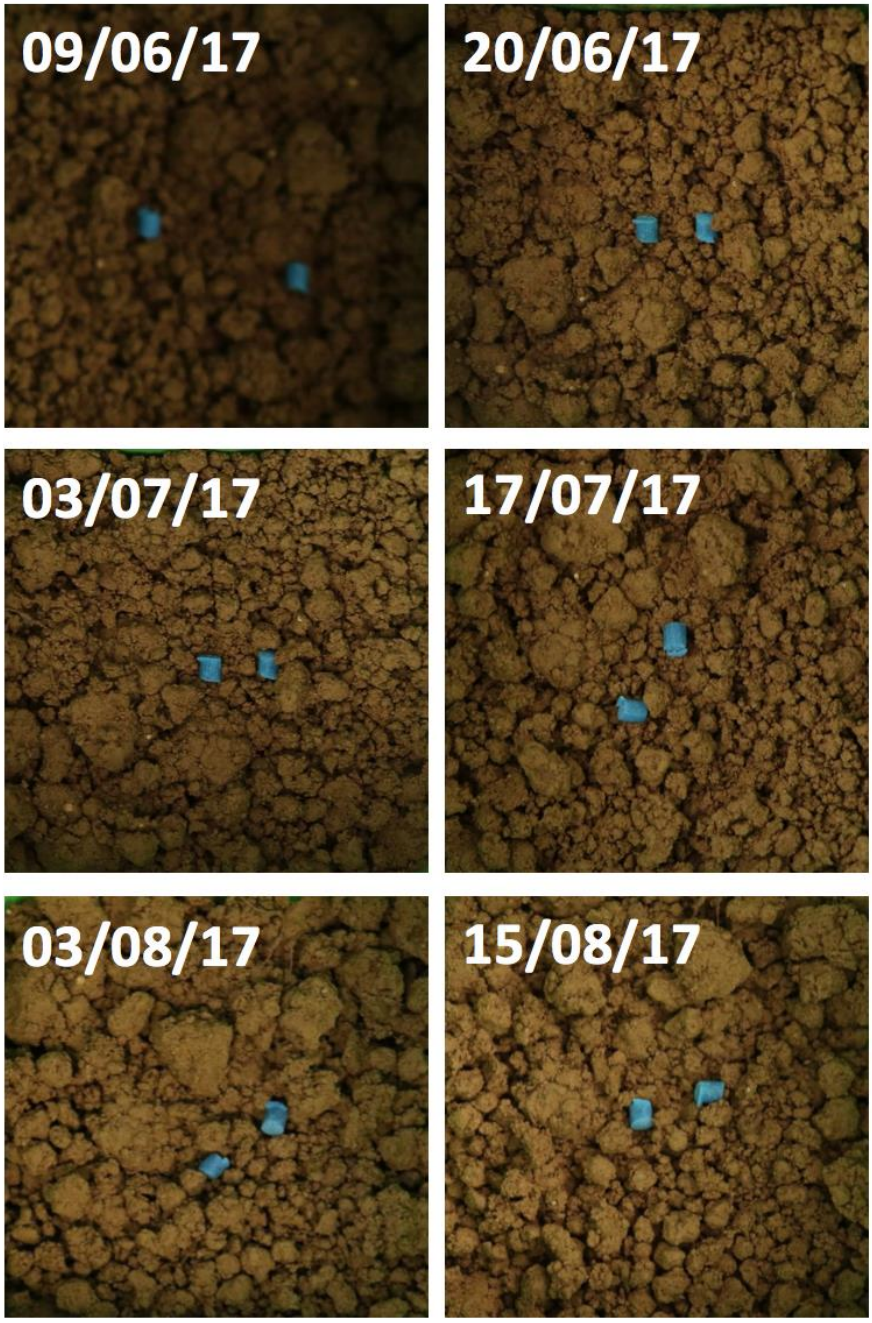


Figure 34: Photographs of the 'no irrigation, wet pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 20/06/2017 = day 20; 03/07/2017 = day 25; 17/07/2017 = day 39; 03/08/2017 = day 56; 15/08/2017 = day 68.

50% irrigation, Dry pellet treatment 2F

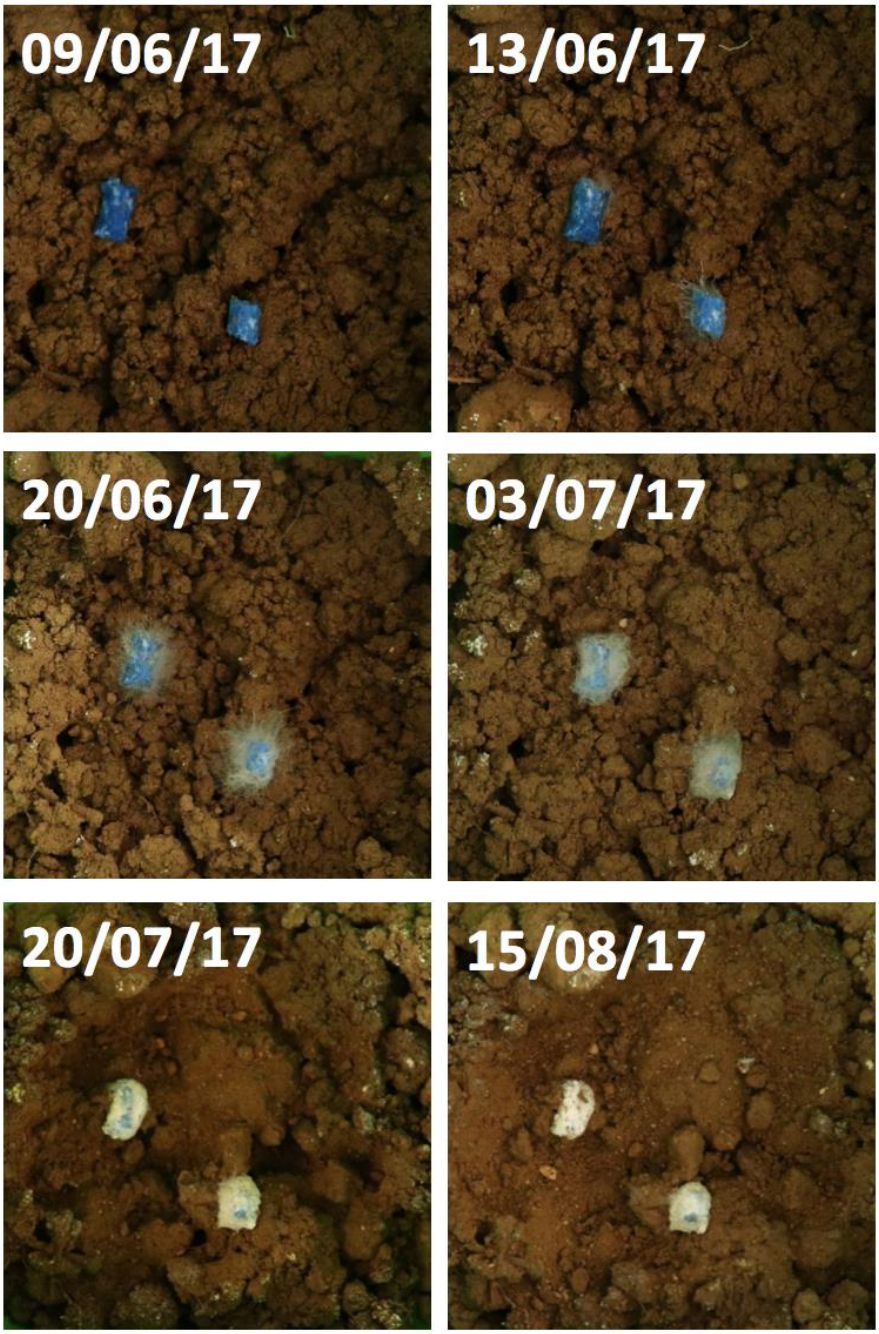


Figure 35: Photographs of the '50% irrigation, dry pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 13/06/2017 = day 5; 20/06/2017 = day 12; 03/07/2017 = day 25; 20/07/2017 = day 42; 15/08/2017 = day 68.

50% irrigation, Wet pellet treatment 2C

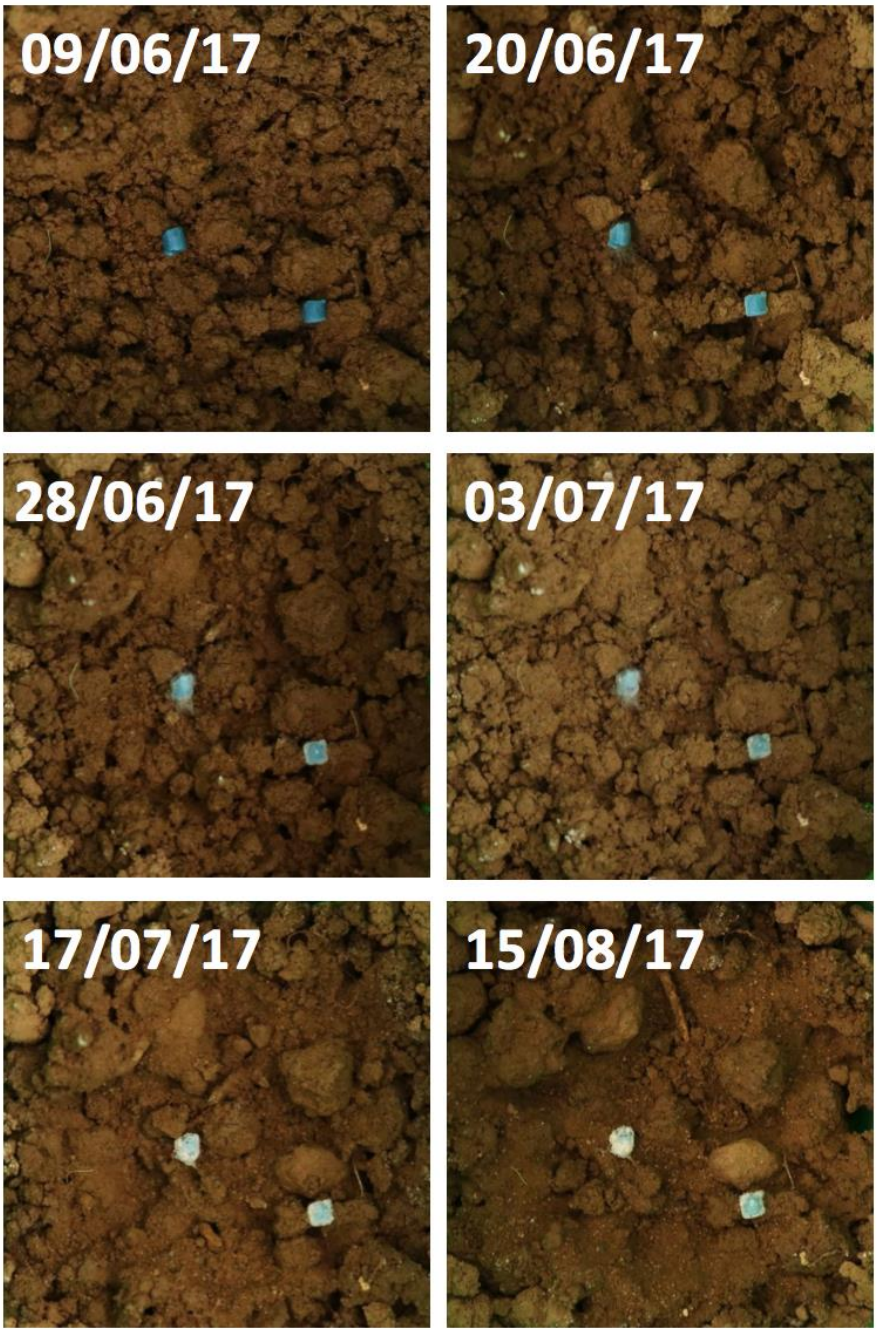


Figure 36: Photographs of the '50% irrigation, wet pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 20/06/2017 = day 20; 28/06/2017 = day 20; 03/07/2017 = day 25; 17/07/2017 = day 39; 15/08/2017 = day 68.

100% irrigation, Dry pellet treatment 3F

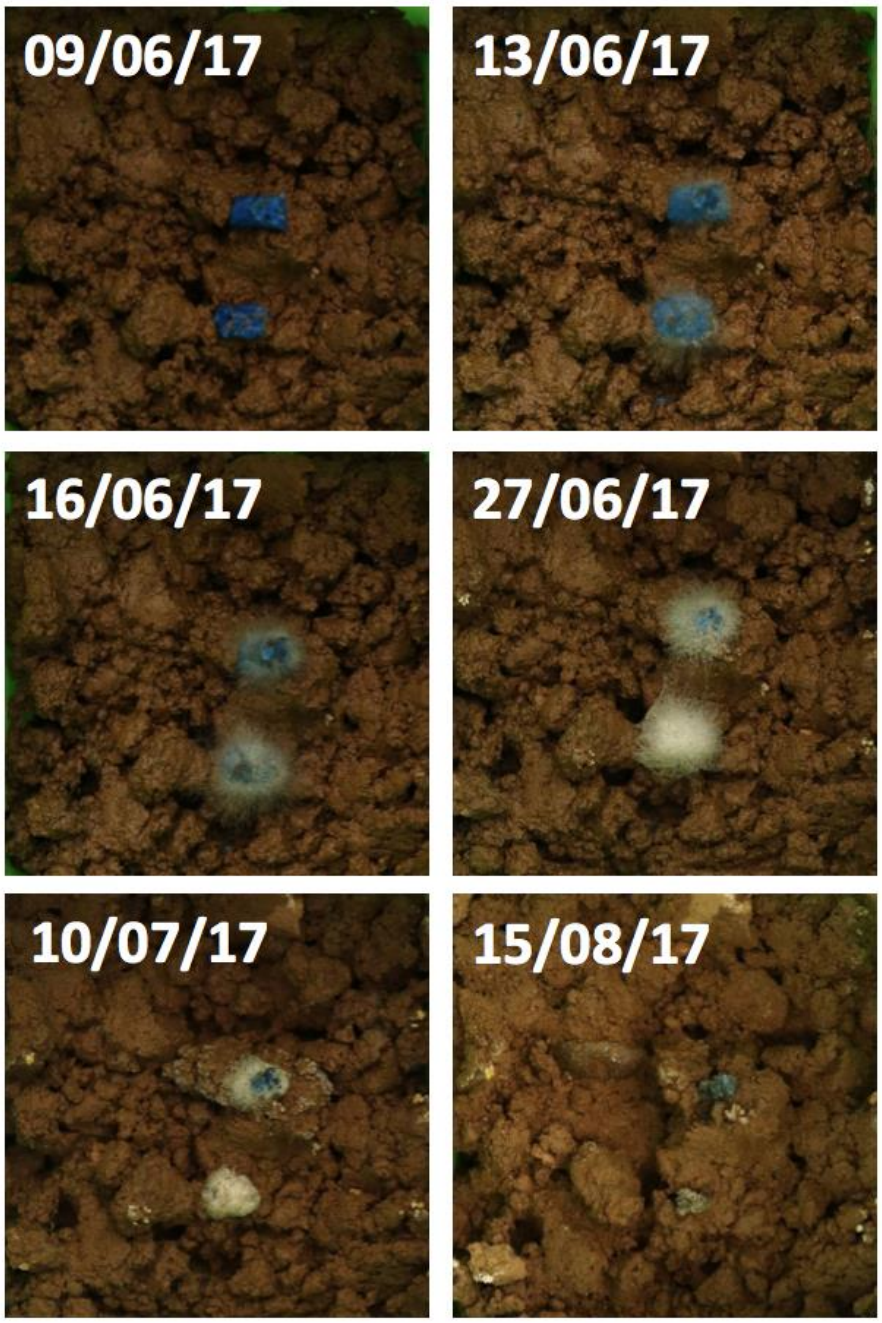


Figure 37: Photographs of the '100% irrigation, dry pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 13/06/2017 = day 5; 16/06/2017 = day 8;
27/06/2017 = day 19; 10/07/2017 = day 32; 15/08/2017 = day 68.

100% irrigation, Wet pellet treatment 3B

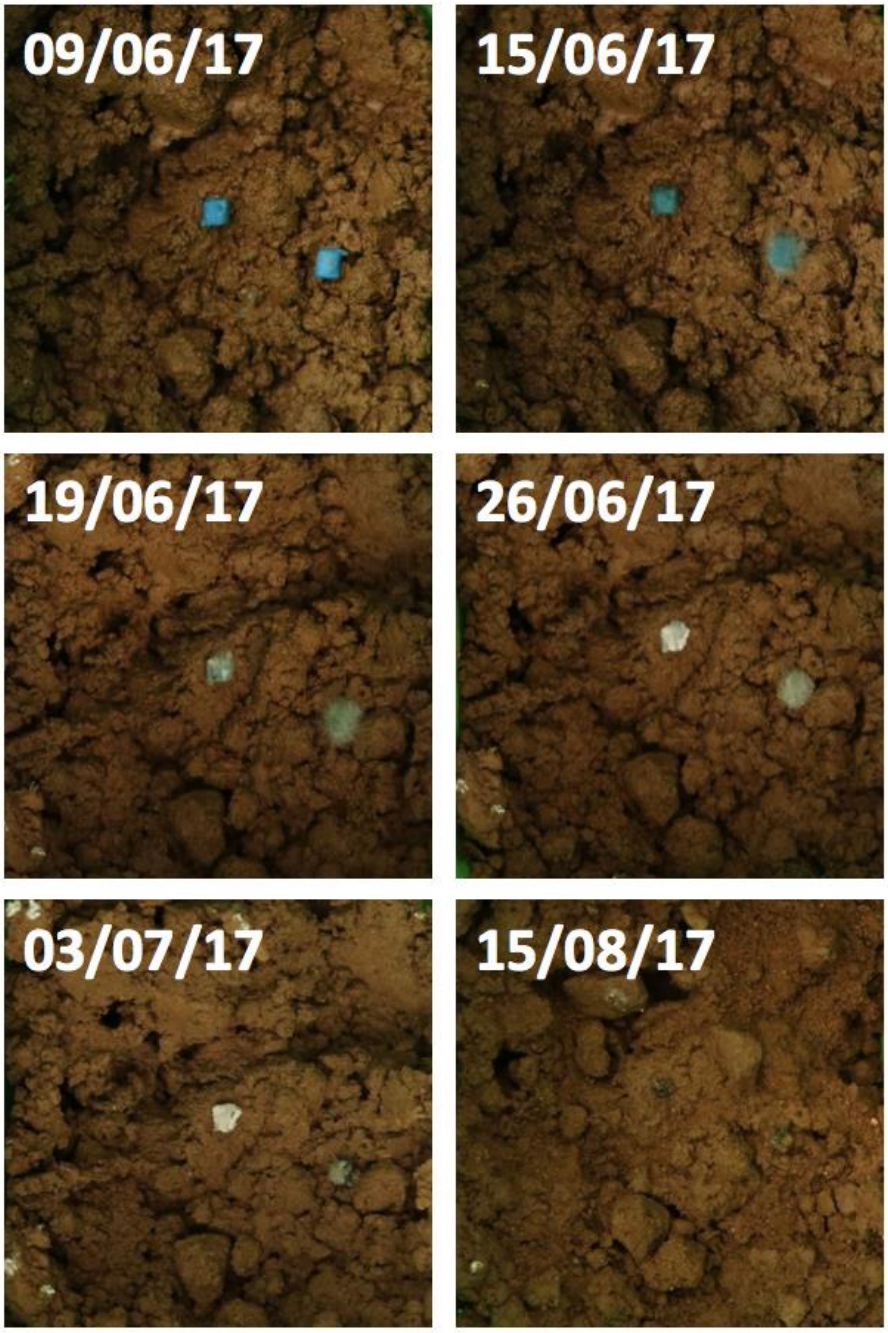


Figure 38: Photographs of the '100% irrigation, wet pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 15/06/2017 = day 7; 19/06/2017 = day 11; 26/06/2017 = day 18; 03/07/2017 = day 25; 15/08/2017 = day 68.

150% irrigation, Dry pellet treatment 4D

Figure 39: Photographs of the '150% irrigation, dry pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 15/06/2017 = day 7; 23/06/2017 = day 15;
03/07/2017 = day 25; 18/07/2017 = day 40; 15/08/2017 = day 68.

150% irrigation, Wet pellet treatment 4C

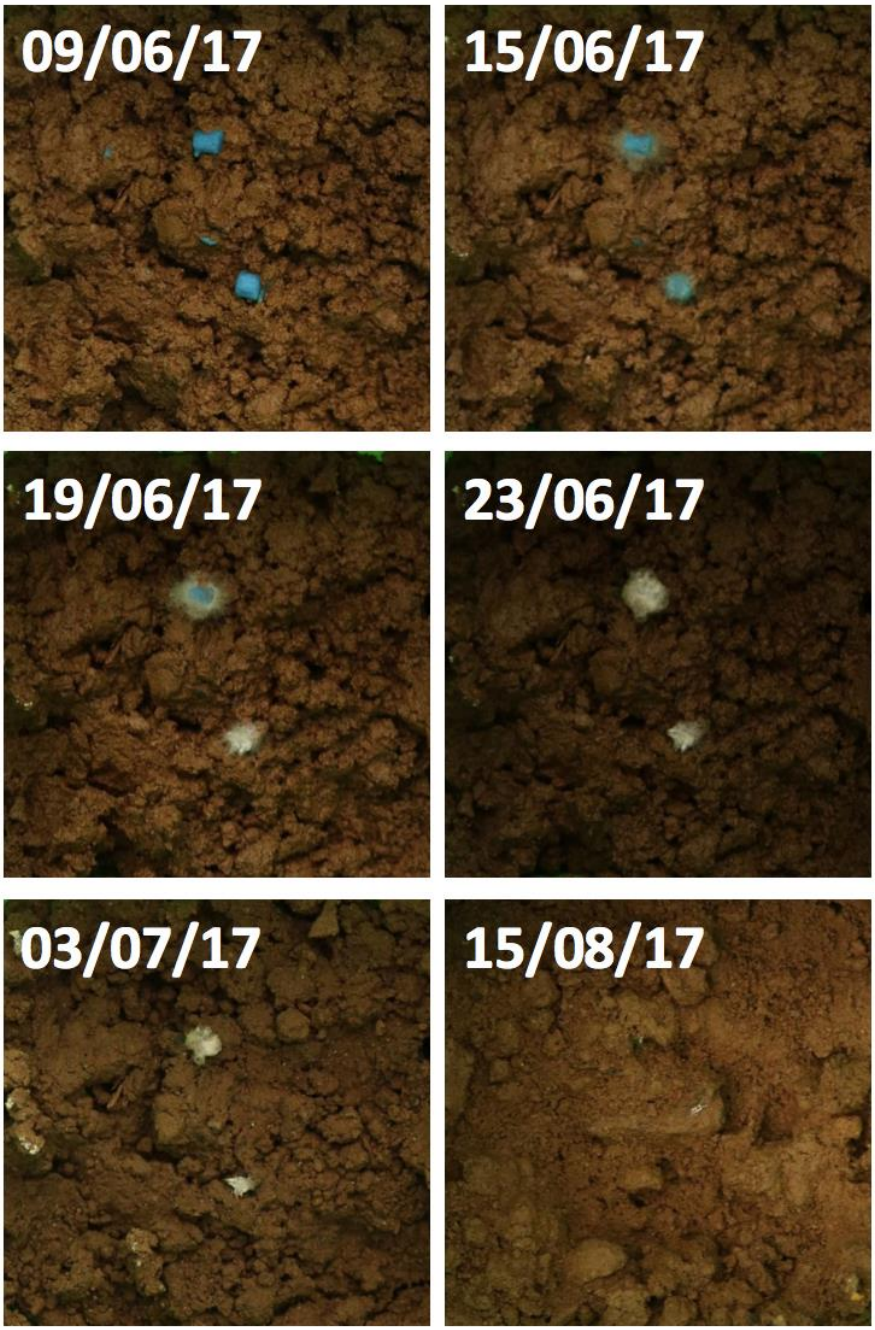


Figure 40: Photographs of the '150% irrigation, wet pellet' treatment for the soil moisture irrigation experiment, over time.

09/06/2017 = day 1, 15/06/2017 = day 7; 19/06/2017 = day 11; 23/06/2017 = day 15; 03/07/2017 = day 25; 15/08/2017 = day 68.

4.2.3 Soil moisture experiment: Results summary

Table 37: Soil moisture irrigation experiment results summary

Irrigation experiment	Soil moisture	○ No irrigation regime remained at a constant soil moisture of 5.3 g. Irrigation regimes had higher average soil moisture contents of 5.6g (50%), 6.3g (100%) and 6.2g (150%). ○ Significant differences between all irrigation regimes except from between '100%' and '150%' irrigations. ○ All irrigation regimes significantly changed in soil moisture during the initial stages of the experiment
	Visible Surface Area	○ All wet pellets degraded fully for the '100%' and '150%' irrigation schemes by day 45 ○ Higher irrigations had visually faster decreases in visible surface area ○ At the beginning of the experiment, wet pellets had a mean visible surface area of 4.124 mm^2 and dry pellets of 9.844 mm^2. ○ Standard deviation was visually high throughout, averaging 0.7990 mm^2 for wet pellets and 2.257 mm^2 for dry pellets ○ Significant differences were found in normalised mean visible surface area between irrigation regimes for both pellet types, although not all regimes had significant differences. ○ The 'No irrigation' and '50% irrigation' regimes had no statistical difference in normalised visible surface area between pellet types; however, '100%' and '150%' regimes did show that pellet type was a significant source of variation. ○ Normalised visible surface area degradation model did not visually fit the measured data well, however showed a relative difference in area change over time. ○ Higher irrigation regimes had lower calculated half-lives.
	RGB Values	○ Visually, for non-irrigation dry pellets, all RGB values remained the same throughout the experiment duration, whereas for all other treatments green values remained the same, blue values decreased over time and red values increased. ○ Higher irrigation visually produced faster colour change ○ R, G and B values all had significant normalised mean value differences between treatment types ○ Normalised blue value degradation model did not fit the measured data appropriately, however showed a relative difference in blue change over time.

4.2.4 Soil moisture experiment: Discussion

4.2.4.1 Soil moisture

Soil moisture was significantly different between all regimes except for between the '100%' and '150%' irrigations. In these high input treatments water content was close to saturation and excess water (over the field capacity) drained away. Soil moisture content at the beginning of the experiment rapidly changed to pseudo steady state levels in each irrigation regime.

4.2.4.2 RGB analysis and photographs

RGB analysis was important because the pellet visibility in-field may influence the likelihood of re-application rate. This could unnecessarily exacerbate the pollution problem if metaldehyde is still available in the field soil. In the soil moisture experiment, higher irrigation rates produced faster colour changes in the pellets, where pellets became mouldy and then white (also indicative of fungal colonisation), before blending into the soil surface with a darker colour. This suggests that soil moisture is the driver of colour change. This could be seen in both the quantitative RGB analysis and in the qualitative photographs over time.

Interestingly, changes in blue values had the least significant differences between treatment types for the irrigation experiment, although it should be noted that the R, G and B values relate to one colour. Since the pellets eventually changed to a brown colour, the relative disaggregation of R and G would change more than blue. Although brown colours have lower B values, the colour change in R appeared to be most significant in changing the overall pellet colour. The white stage of pellet discolouration in the irrigation experiments may also have influenced this, because white is produced from high R, G and B values. The white colour may have prevented the B value from reducing as much as expected.

4.2.4.3 Visible surface area and photographs

The visible surface area of pellets gave a 2-dimensional indication of pellet size change over time. For the irrigation experiment, wet-processed pellets fully degraded after <45 days for the '100%' and '150%' irrigation regimes, with a gradual size decrease over time. For the 'no irrigation' and '50%' regimes, pellet surface area did not change so obviously. These treatments were shown to have significant differences in surface area compared with the higher irrigation regimes. This suggests that moisture availability is the driver of size change. Enough moisture is needed to initiate disintegration.

Pellet type was only a significant source of variation for the two higher irrigation regimes. Results of a Tukey's comparison test suggest that normalised area was significantly different between pellet types for only four days in the 72 day experiment. We can, thus, conclude that there is no clear difference in disintegration rate between pellet types, and that irrigation regime is the only factor responsible for differences.

Visible surface area only records the part of the pellet not in direct contact with the soil surface. The rate of physical change recorded may be slower than the actual rate. It is possible that the non-visible side of the pellet therefore physically disintegrates faster than the apparent change from photographic analysis. Another issue with the visible surface area analysis was that pellets moved over time, particularly in dry soils, becoming partially covered with soil particles. The orientation of pellets on the soil surface also changed, exposing different angles and sides of the pellet which would have had difference surface areas. Orientation and sediment changes were clear from photographs taken of the pellets over time. Changes recorded, therefore, may not necessarily be representative of all dimensional changes. Another potential issue is the possibility that the *ImageJ* software analysis may not capture all the apparent physical degradation over time. It is possible that as pellets become less vibrantly blue they blend into the soil surface and become more difficult to identify.

4.2.4.4 Modelled data

Both the blue value and visible surface area data were normalised using initial values and first order models were fitted. The models did not fit the measured data well, because changes did not typically follow an exponential pattern. Although the long-term pellet area and B values may ultimately decay, pseudo-exponentially, these phenomena were not well captured here. The models fits did facilitate a relative comparison between treatments, via fitted half-life values, although these values should not be taken absolutely.

For B value modelled changes, B value decreased fastest under higher irrigation regimes. Although half-lives calculated are relative only (and not absolute or accurate values), the 100% irrigation treatments were shown to have the lowest half-lives and therefore degrade fastest.

Half-life values calculated for the no irrigation treatments particularly display the inaccuracy of the model, calculating a half-life of 401.3 days for the wet pellets and 1965 days for the dry pellets. Clearly the experiment duration was shorter than both of these time periods, and the graphical representations of the model do not suggest such a clear difference in potential half-life. The difference calculated is primarily suggested to be due to poor model fit appropriateness, where colour did not change according to an exponential function.

For visible surface area modelled changes, the lowest half-lives were derived for wet pellets in the high-irrigation treatments in the soil moisture study. As above, half-lives were highly variable and inaccurate due to poor model fit.

4.3 Sub experiment two: Integrated physical degradation

4.3.1 Methodology

To show the combined effect of soil moisture and kinetic energy of physical disintegration, twelve plant pots, each measuring 51mm x 48mm x 47mm, were filled with 50.00 g ± 1.00g loam soil and placed outside under natural conditions, subject to changes in evapotranspiration and rainfall, therefore soil moisture and kinetic energy. Using randomised block sampling, two pellets were put on the soil surface in each plant pot, with half of the pots receiving dry-processed pellets and half wet-processed pellets.

As a control, twelve more plants pots, with the same set-up as above, were also put outside but under an open cover which prevented direct rainfall input and therefore restricted soil moisture increase. Environmental conditions between the covered and exposed pots were organised to be as similar as possible, excluding rainfall input, although direct sunlight, and therefore temperature and evapotranspiration effects on soil moisture, may have had more influence on the exposed pots than those in shadow.

Plant pots were analysed using the same processes involved in the soil moisture study. Plant pots were weighed daily to record soil water mass, using the same equation as above, and photographs were taken regularly, although not daily, to monitor change over time. Images were analysed using *Image J* and the visible surface area, perimeter and RGB values of pellets were recorded. From this data, the 2D changes in pellet size and colour could be recorded.

The experiment was conducted over 50 days, from 09/06/2017 (day 1) to 28/07/2017 (day 50).

4.3.2 Results

4.3.2.1 Rainfall, temperature and soil moisture

There was more rainfall towards the end of the experiment duration, with temperature fluctuating throughout (figure 41), however a one-way ANOVA showed that there were no significant differences in rainfall or temperature between months during the experiment duration ($p>0.05$).

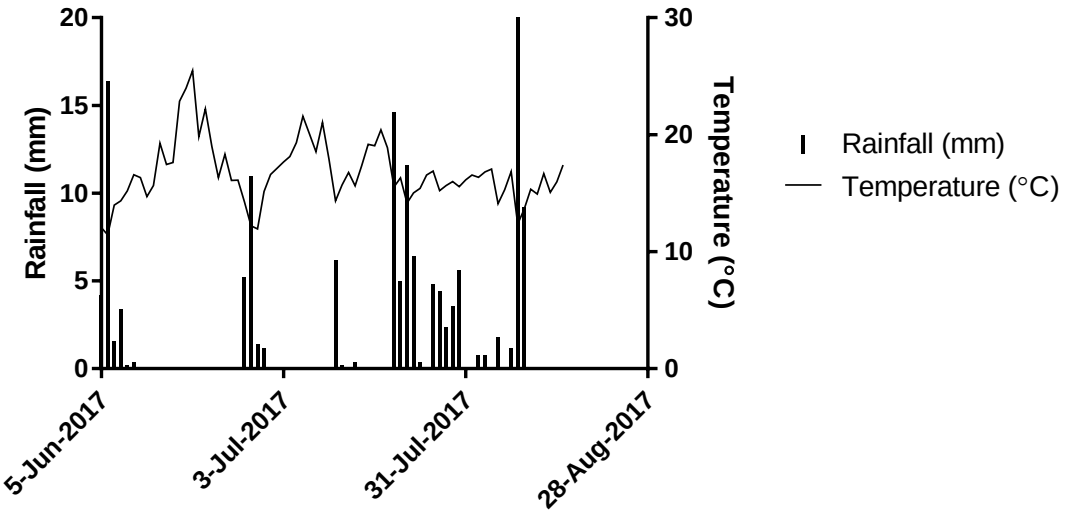


Figure 41: Rainfall and temperature for the integrated physical degradation experiment

Soil moisture content was visually different between the exposed and covered treatments (figure 42). Covered treatments decreased in soil moisture and remained relatively constant throughout, whereas the exposed treatment had periods of higher soil moisture. A two-way ANOVA with respect to time showed that 37.25% of soil moisture variation occurred due to time and 13.29% of the variation in soil moisture was between the treatment types. Both sources of variation were significant to the 99.99% confidence interval ($p<0.0001$).

A Tukey's comparison test showed that there was a statistically significant difference to the 99% confidence level between soil moisture in the exposed experiments in comparison to the covered experiments ($p<0.0001$). No significant difference was found between pellet treatments within the same environmental exposure ($p>0.05$).

Another Tukey's comparisons test showed that the covered, wet-pellet treatment and covered, dry pellet treatment both significantly decreased in soil moisture each day for the first 5 days of the experiment ($p<0.0001$) and then remained at a non-significant constant soil moisture of approximately 6.3g and 6.4g respectively. Exposed treatments varied significantly in soil moisture with each 'peak' throughout the experiment duration.

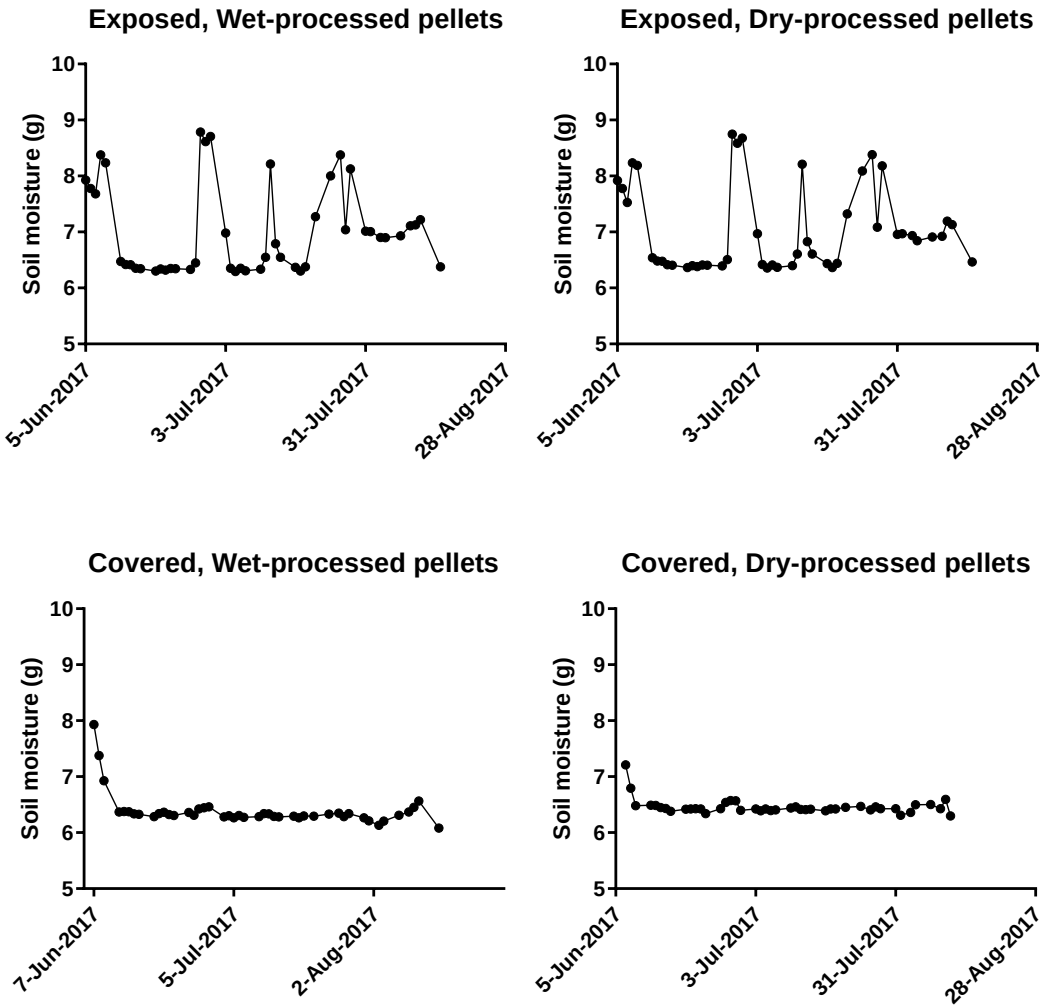


Figure 42: Soil moisture over time for the integrated physical degradation experiment

The exposed treatments' soil moisture visually responded to rainfall events (figure 43), increasing after rainfall and falling to approximately 6.3g, the same as the covered treatment which had no external moisture input, during periods of little or no rainfall. Soil moisture peaked at 8.8g after an 11mm rainfall event on 28/06/2017 (day 20). Soil moisture content also visually reacted to temperature, where the highest temperatures had the lowest soil moisture.

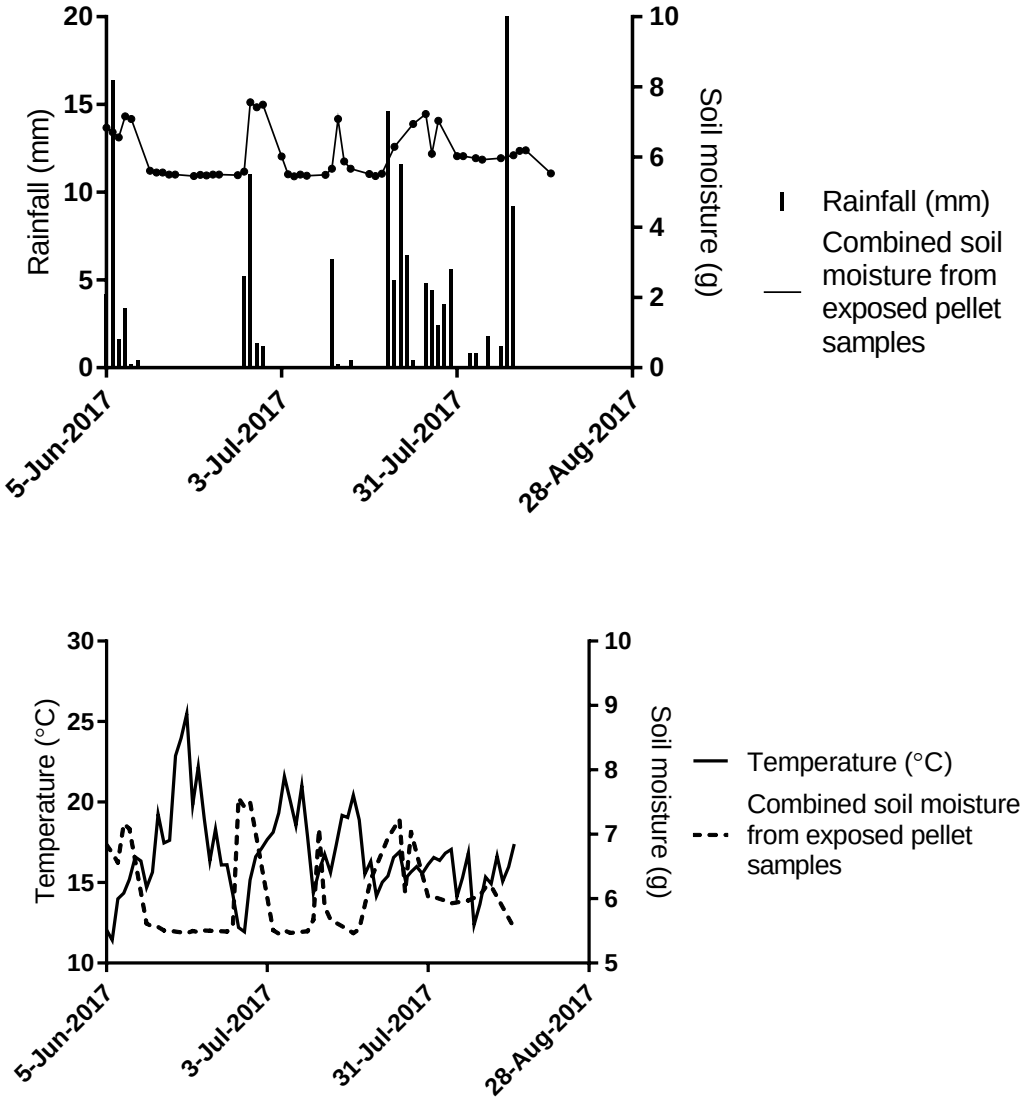


Figure 43: Rainfall and temperature change with exposed treatment soil moisture over time

4.3.2.2 RGB analysis

Using the average percentage red, green and blue over time, RGB ratios were plotted to show colour change. Both covered treatment did not appear to change in colour for the duration of the experiment, whereas red and blue values for the exposed treatments did visually change (figure 44).

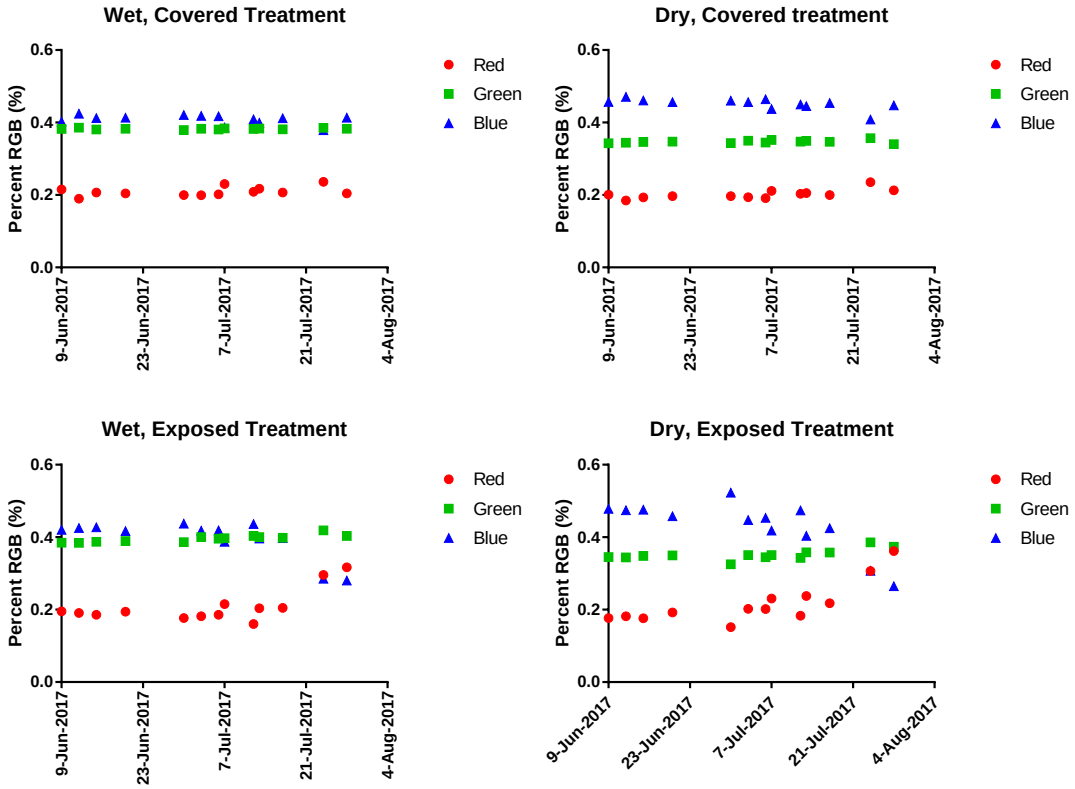


Figure 44: RGB ratio colour change for all treatment types within the integrated physical degradation experiment over time

RGB analysis was split by colour, and the data was normalised so wet- and dry- pellets could be compared. As means are used during an ANOVA, and pellets did not fully degrade over time, row means were used to fill in any blanks within the dataset. Blanks were always the result of pellets falling between cracks in the soil. Sometimes pellets could be recovered and sometimes not, therefore the dataset was not continuous for all pellet RGB values over time. ANOVA analysis requires a full dataset.

Three ANOVAs with respect to time were conducted for each of the normalised R, G and B data. Each ANOVA had statistically significant variation sources in time and treatment type. For R, time accounted for 14.16% of variation ($p<0.0001$) and

treatment type 12.33% ($p<0.01$). For G, time accounted for 8.714% of variation ($p<0.0001$) and treatment type for 33.62% ($p<0.0001$); and for B, time accounted for 12.69% of variation ($p<0.0001$) and treatment type accounted for 34.32% ($p<0.0001$).

For each ANOVA, a Tukey's comparison test found significant differences in overall mean R, G and B values between treatment types (table 38).

*Table 378: Tukey's comparison showing significant differences in mean R, G and B values between treatment types for the integrated physical degradation experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Treatment Type One	**Treatment Type Two**	**Significance**		
		R	**G**	**B**
Wet Pellets, Covered	Dry Pellets, Covered	NS	NS	NS
Wet Pellets, Covered	Wet Pellets, Exposed	*	****	****
Wet Pellets, Covered	Dry Pellets, Exposed	*	****	****
Dry Pellets, Covered	Wet Pellets, Exposed	*	**	***
Dry Pellets, Covered	Dry Pellets, Exposed	*	****	****
Wet Pellets, Exposed	Dry Pellets, Exposed	NS	NS	NS

Colour degradation:

For the integrated physical degradation, the half-life of the B-value could not be modelled. However, the exposed treatment B values were measured to approximately half in value after 24/07/2017, day 45 of the experiment for both the wet and dry pellet treatments. Although it cannot be statistically proved for colour analysis, the similarity in B value decrease between pellet types suggests a similar rate of colour change in an environmentally realistic environment.

4.3.2.3 Pellet visible surface area

At the beginning of the experiment, wet pellets had a mean visible surface area of 4.569 mm^2 the covered treatment and 5.096 mm^2 for the exposed treatment. Dry pellets had a mean visible surface area of 9.117 mm^2 the covered treatment and 10.41 mm^2 for the exposed treatment. Error was also visibly large, with an average standard deviation of 0.5155 mm^2 and 0.5626 mm^2 for covered and exposed wet pellet treatments respectively; and 1.249 mm^2 and 1.694 mm^2 respectively for dry pellets.

Pellet visible surface area visually changed most for the exposed dry pellet treatment, which also had the largest standard deviation. Covered pellets did not appear to change over time, however exposed pellets increased in visible surface area periodically (figure 45).

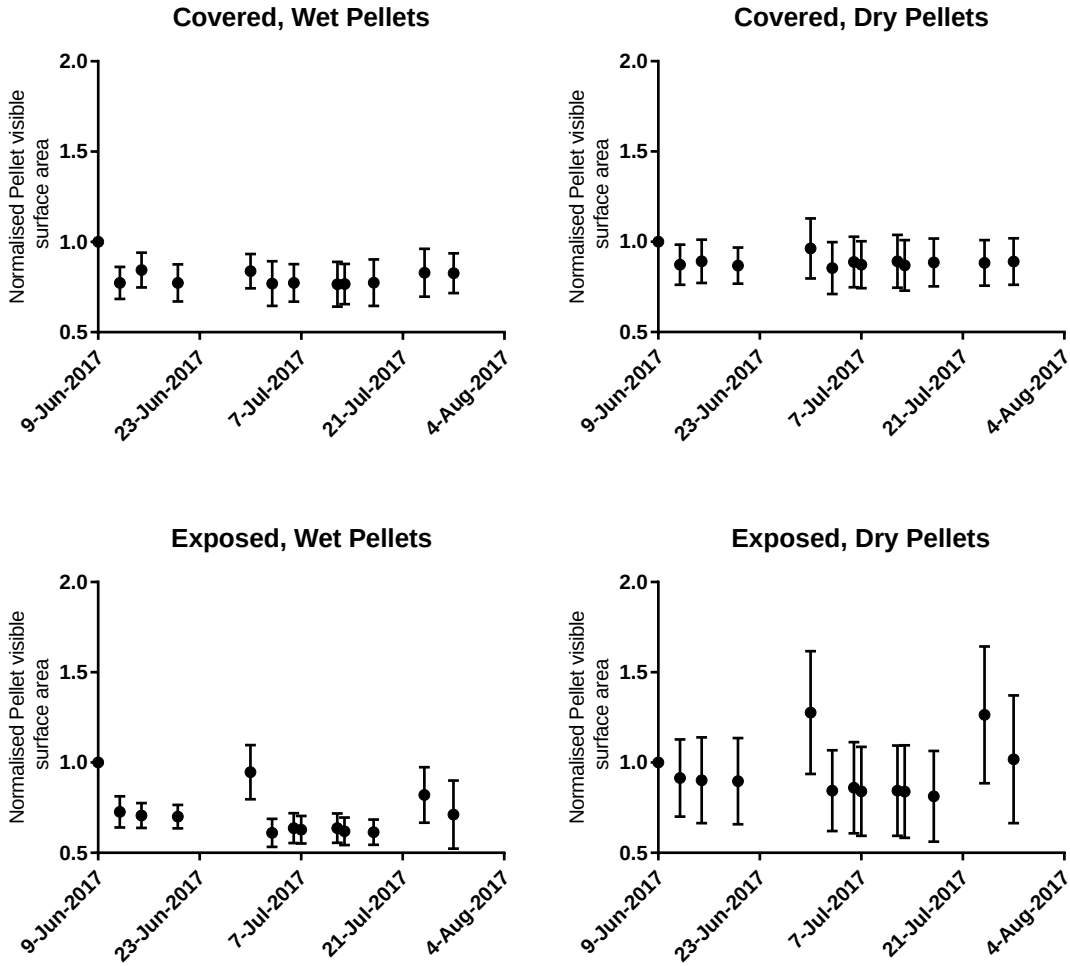


Figure 45: Pellet visible surface area change over time for all treatments within the integrated physical degradation experiment

A two-way ANOVA with respect to time showed that 24.16% of exposed pellet variation occurred due to time ($p<0.0001$) and 18.98% of variation occurred due to pellet type ($p<0.01$). A Sidak's multiple comparison test showed significant differences in exposed pellet visible surface area on 30th June, day 22 ($p<0.001$), 24th July, day 46 ($p<0.0001$) and 28th July, day 50 ($p<0.01$).

Another two-way ANOVA with respect to time for the covered pellet treatment showed that time was a significant source of variation ($p<0.0001$), however pellet type was not ($p>0.05$). A Sidak's multiple comparison test for the covered pellets showed significant differences in visible surface area between wet and dry pellets on 30th June, day 22 ($p<0.05$) and 12th July, day 34 ($p<0.05$).

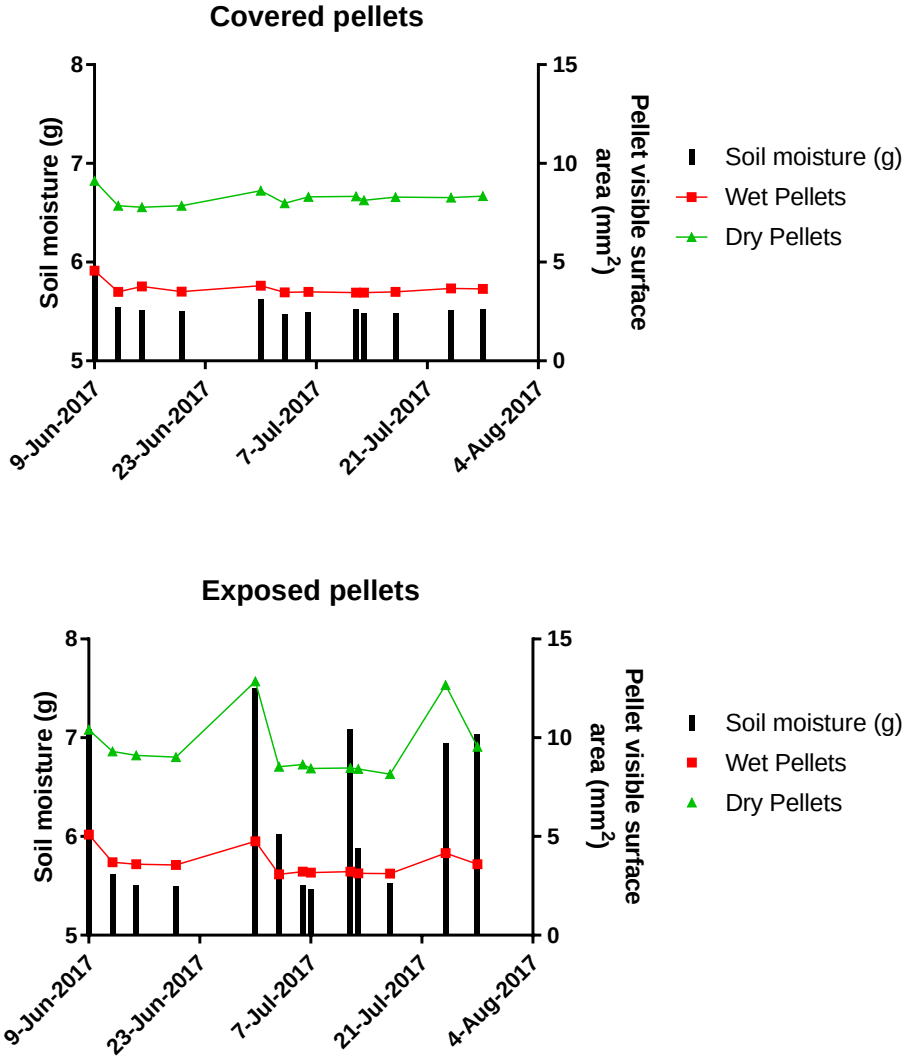


Figure 46: Pellet visible surface area change with soil moisture for all treatments within the integrated physical degradation experiment

Pellet visible surface area visually changed with soil moisture content (figure 46), however Spearman's rank correlation was not significant between soil moisture and pellet type for either of the exposed treatments or the wet pellet covered treatment ($p>0.05$). Spearman's rank was significant for the wet pellet, covered treatment ($p<0.01$), showing wet-processed pellet visible surface area to significantly correlate with soil moisture.

Modelling change:

Using the normalised average visible surface area over time, a model could be fitted to express rate of change (figure 47). The models fitted to the data were designed to show relative change from the normalised initial pellet visible surface area only, allowing the data to be compared between treatments.

Covered pellets were not predicted to change over time (hence the modelled intercept was locked for y=1), however dry-processed pellets perceptibly appeared to increase in normalised pellet surface area against the locked modelled intercept. A discussion of this is included in the 'integrated physical degradation' sub-experiment discussion section.

Exposed dry pellets had a lower predicted half-life (table 39), however both exposed pellet surface areas did not fit the model well, having increases in surface area over time instead of a consistent reduction in area.

Table 38: Modelled pellet visible surface area rate constant, half-life and root mean square error for the integrated physical degradation experiment

Treatment type	Rate constant, k	Half-life (days)	Root mean square error
Exposed, Wet Pellets	0.002753	251.7845	0.2247
Exposed, Dry Pellets	0.01057	65.59984	0.2143
Covered, Wet pellets	0	N/A	0.4654
Covered, Dry pellets	0	N/A	0.2955

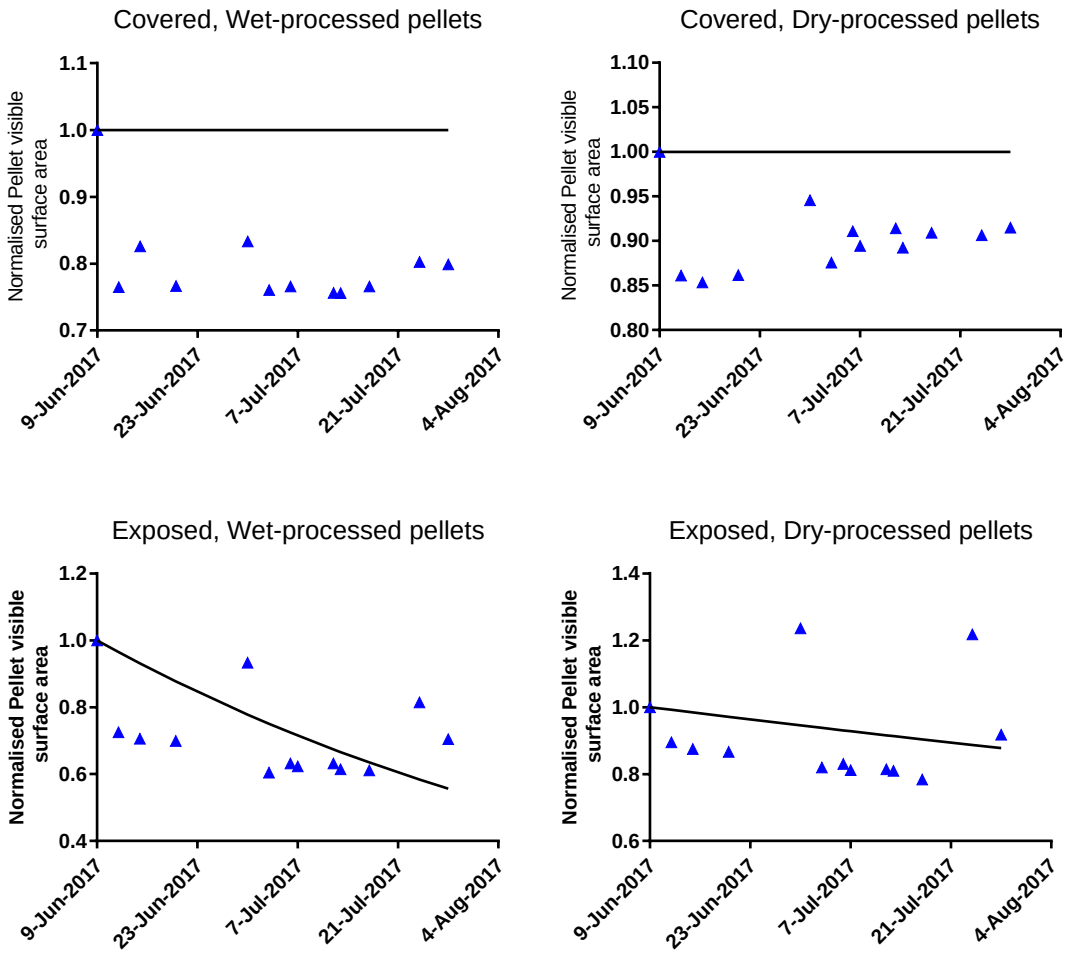


Figure 47: Modelled and measured normalised pellet visible surface area change with time for the integrated physical degradation experiment

4.3.2.4 Qualitative Analysis of pellet size and colour

Photos were taken of the pellets each time the plant pots containing them were weighed. 09/06/2017 was day 1 of the experiment, and 15/08/2017, the final day, was day 68 of the pellet field duration. Photos shown are of one plant pot from each treatment type over time (figures 48 – 51). In total each treatment had six plant pots.

Over time the covered pellets moved across the soil surface, sometimes moving beneath the soil surface or changing rotation, although vibrancy of colour and pellet size did not appear to change over the 68 days. In comparison, the exposed pellets did appear to reduce in both size and colour vibrancy over time for these specific plant pots. There was no clear visual distinction in degradation between wet and dry pellet treatment types.

Covered, Dry pellet treatment 2D

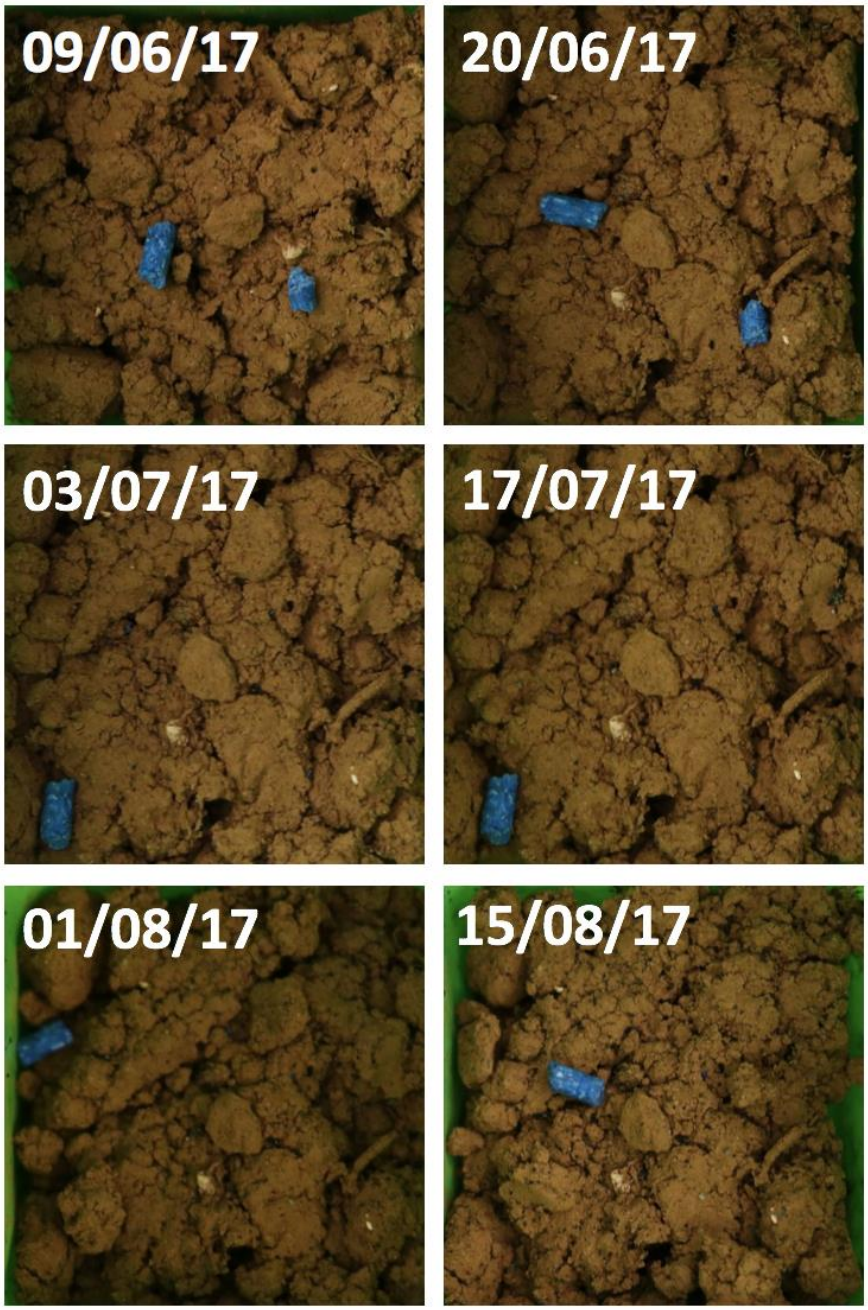


Figure 48: Photographs of the 'Covered, dry pellet' treatment for the integrated physical degradation experiment.

09/06/2017 = day 1, 20/06/2017 = day 12; 03/07/2017 = day 25; 17/07/2017 = day 39; 01/08/2017 = day 54; 15/08/2017 = day 68.

Covered, Wet pellet treatment 2W

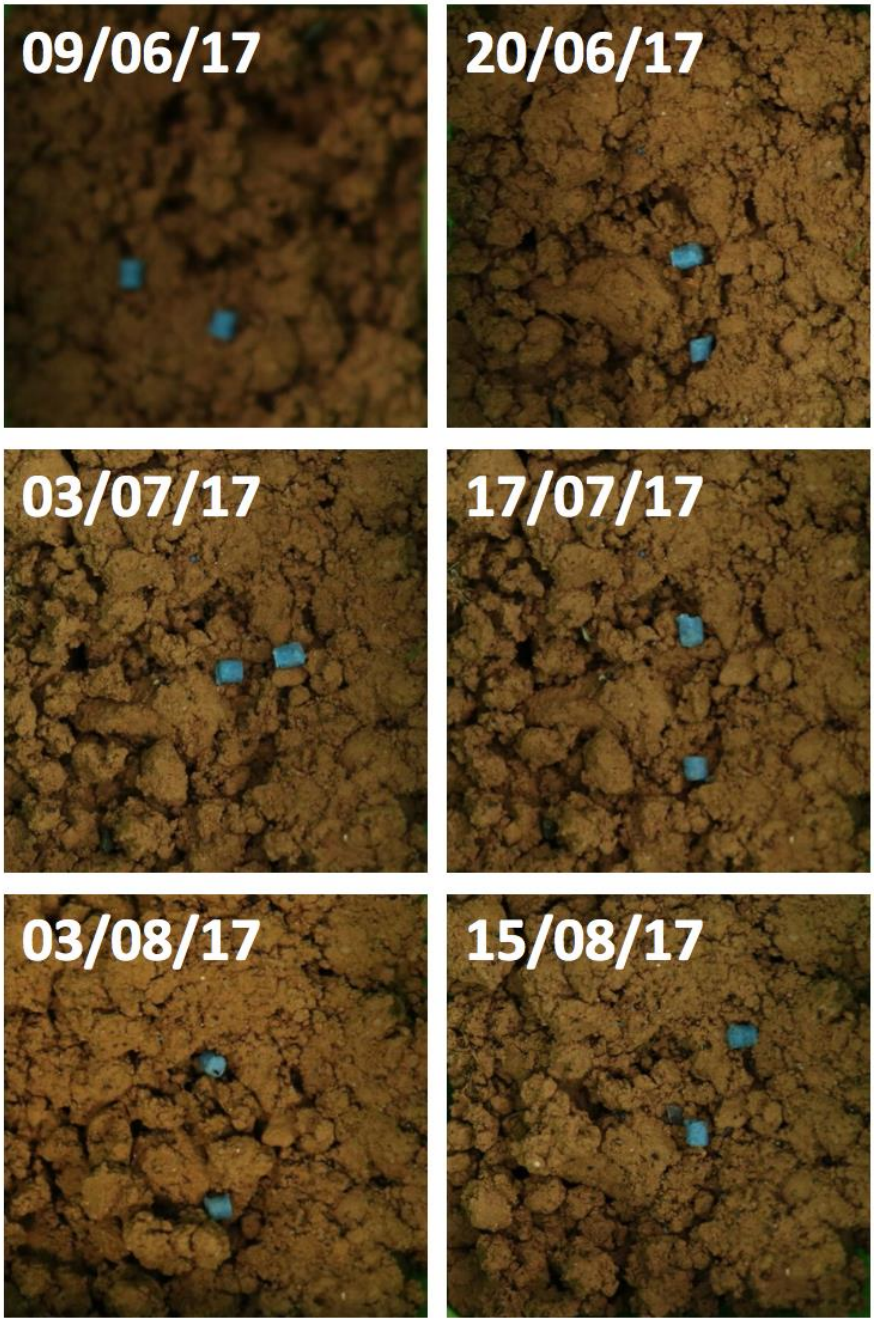


Figure 49 Photographs of the 'Covered, wet pellet' treatment for the integrated physical degradation experiment.

09/06/2017 = day 1, 20/06/2017 = day 12; 03/07/2017 = day 25; 17/07/2017 = day 39; 03/08/2017 = day 56; 15/08/2017 = day 68.

Exposed, Dry pellet treatment 5D

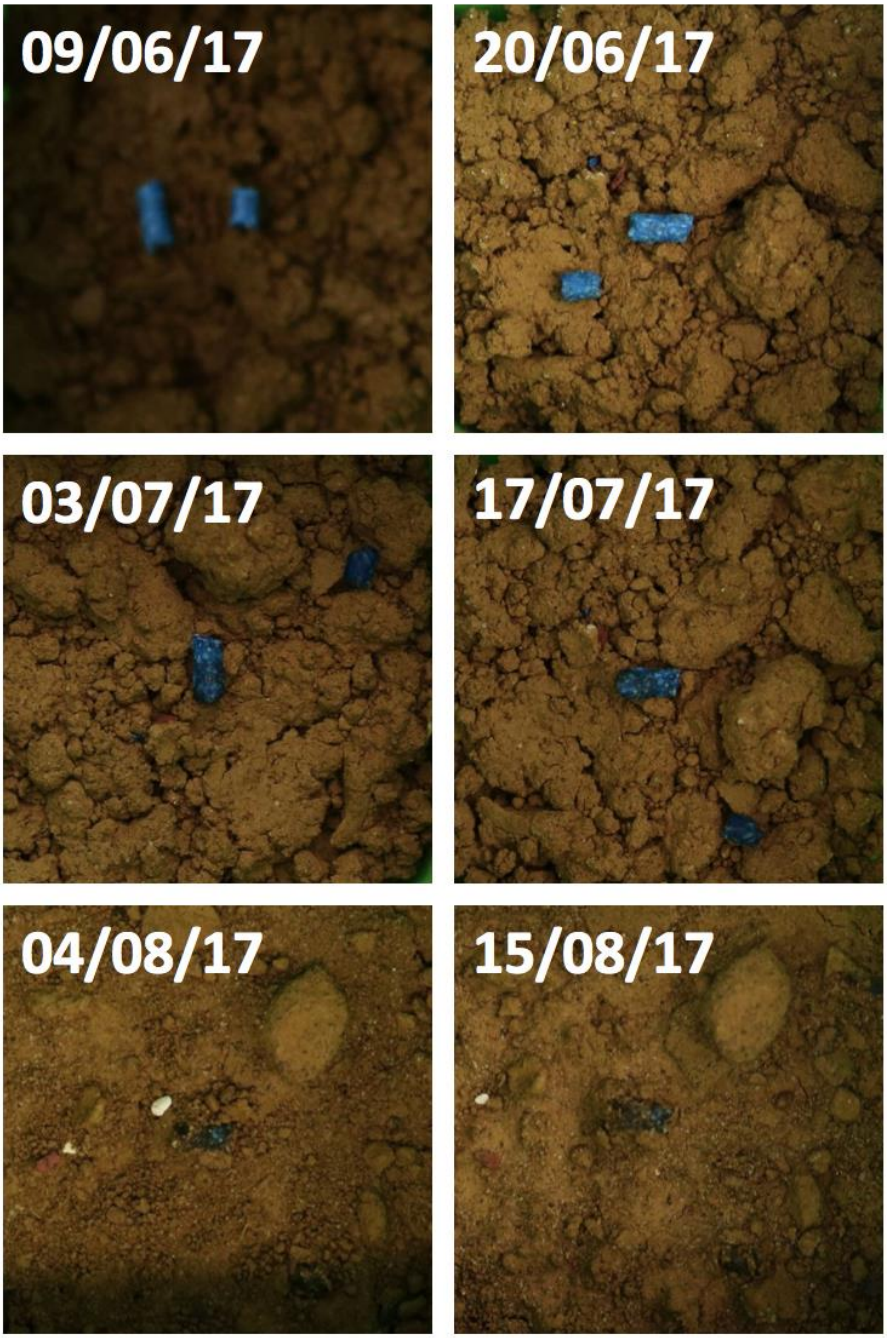


Figure 50: Photographs of the 'Exposed, dry pellet' treatment for the integrated physical degradation experiment.

09/06/2017 = day 1, 20/06/2017 = day 12; 03/07/2017 = day 25; 17/07/2017 = day 39; 04/08/2017 = day 57; 15/08/2017 = day 68.

Exposed, Wet pellet treatment 6W

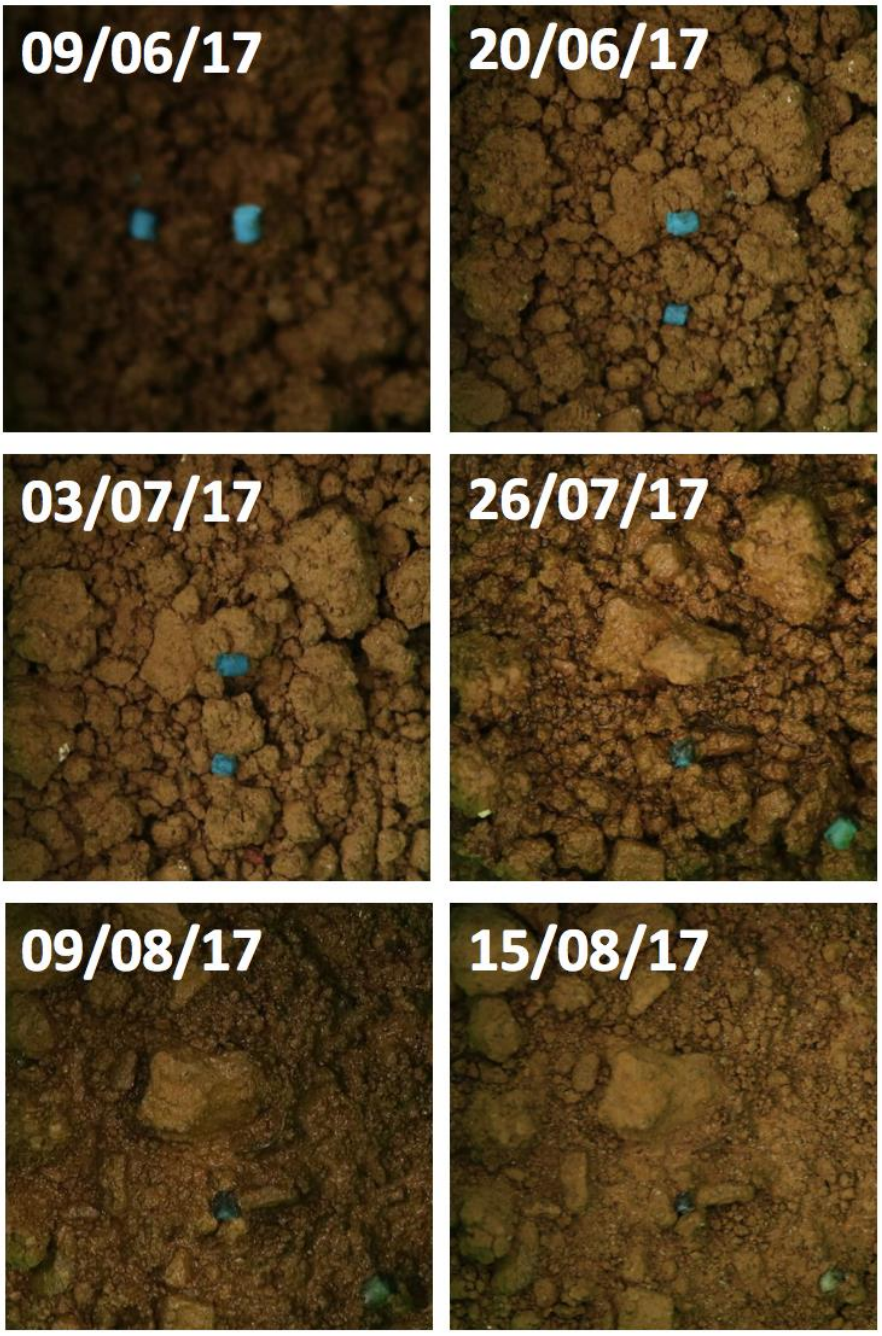


Figure 51: Photographs of the 'Exposed, Wet pellet' treatment for the integrated physical degradation experiment.

09/06/2017 = day 1, 20/06/2017 = day 12; 03/07/2017 = day 25; 26/07/2017 = day 48; 09/08/2017 = day 62; 15/08/2017 = day 68.

4.3.3 Results summary

4.3.3.1 Exposed pellet treatment

Table 390: Integrated physical degradation experiment, exposed pellets, results summary

Integrated physical degradation (Exposed pellets)	Visible Surface Area	○ Dry pellets had visually larger standard deviation than wet pellets, with the mean visually remaining closer to the original pellet visible surface area. ○ Significant differences between normalised wet and dry pellet visible surface areas only on 3 days out of 50. Dry pellets increased in size more than wet pellets. ○ Spearman's rank showed no correlation between soil moisture and visible pellet area ○ Normalised visible surface area degradation model did not visually fit the measured data well, however showed a relative difference in area change over time. ○ Wet pellets had a lower calculated half-life ○ Photos show pellets decreasing in visible surface area over time
	RGB Values	○ Visually, G values remained the same, B values decreased over time and R values increased ○ R, G and B values all significantly varied by time and treatment type within ANOVA analysis ○ All exposed treatments were significantly different to covered treatments for their respective R, G and B normalised mean values ○ No significant differences in normalised mean R, G and B values between wet and dry pellets within the exposed treatment ○ Photos show decrease in colour vibrancy over time, with the pellets blending into the soil surface colour
	Soil moisture	○ No significant soil moisture difference between pellet treatments ○ Significantly different soil moisture between the covered and exposed integrated physical degradation experiments ○ Visually responded to rainfall events, increasing after rainfall and falling to approximately 6.3 g, the same as the covered treatment which had no external moisture input. ○ Visually responded to temperature where higher temperatures had lower soil moisture
	Rainfall/ Temperature	○ No significant differences in rainfall or temperature between months during the experiment duration ($p<0.05$)

4.3.3.2 Covered pellet treatment

Table 41: Integrated physical degradation experiment, covered pellets, results summary

<table>
<tr><td rowspan="4">Integrated physical degradation (Covered pellets)</td><td>Visible Surface Area</td><td colspan="2">○ No visual change in visible surface area over time
○ Significant differences between normalised wet and dry pellet visible surface areas only on 2 days out of 50. Dry pellets increased in size more than wet pellets.
○ Wet pellet treatment had a significant spearman’s rank correlation to soil moisture
○ Normalised visible surface area degradation model predicted no change in normalised area over time
○ Although the model did not visually fit the measured data well it showed the same visual trend in a lack of degradation
○ Photos show no visual change in visible surface area, although pellets did rotate and were moved beneath the soil surface</td></tr>
<tr><td>RGB Values</td><td colspan="2">○ No visible changes to R, G, or B values throughout the experiment
○ R, G and B values all significantly varied by time and treatment type within ANOVA analysis
○ All covered treatments were significantly different to exposed treatments for their respective R, G and B normalised mean values
○ No significant differences in normalised mean R, G and B values between wet and dry pellets within the covered treatment
○ Photos show no visual change in colour or colour vibrancy over time</td></tr>
<tr><td rowspan="2">Soil moisture</td><td>○ Significant decrease in soil moisture for the first 5 days, then non-significant constant soil moisture of 6.3 g</td><td>○ Significant decrease in soil moisture for the first 5 days, then non-significant constant soil moisture of 6.4 g</td></tr>
<tr><td colspan="2">○ No significant soil moisture difference between pellet treatments
○ Significantly different soil moisture between the covered and exposed integrated physical degradation experiments</td></tr>
<tr><td>Rainfall/ Temperature</td><td colspan="2">○ No significant differences in rainfall or temperature between months during the experiment duration ($p<0.05$).</td></tr>
</table>

4.3.4 Discussion

4.3.4.1 Soil moisture

Soil moisture fluctuated with rainfall input. There was a clear response of soil moisture content changes to rainfall and temperature, which controls evapotranspiration. This was reflected in a statistical difference in soil moisture content between the exposed and covered treatments.

4.3.4.2 RGB analysis and photographs

RGB analysis was important because the visibility of the pellets in the field may influence the likelihood of re-application rate. This could unnecessarily exacerbate the pollution problem if metaldehyde is still available in the field soil.

A relationship between soil moisture content and RGB change was observed in the exposed pellets during the integrated outdoor experiment. Pellet colour visually changed over time after exposure to rainfall. In the covered treatment, which had no external moisture input, apparent difference in R, G or B values was observed. R, G and B values all significantly differed between covered and exposed treatments for both pellet types. However, there was no significant difference between pellet types exposed to the same environmental conditions.

Colour change for both exposed and covered treatments and pellet type was documented in both the RGB analysis and in photographs. The photographs also provided evidence of pellet longevity. Exposed pellets, although largely physically degraded, were still partially present after 68 days in exposed conditions. Colour reduced in vibrancy during this time, showing that the pellet casing may discolour but remain intact under some conditions.

4.3.4.3 Visible surface area and photographs

The visible surface area of pellets gave a 2-dimensional indication of pellet size change over time. Significant differences in visible surface area between pellet types were found for the outdoor experiments, related to rainfall events. Pellets did not measurably decrease in visible surface area over time. Rather, normalised area increased with soil moisture, particularly for the exposed treatments. These differences may have been due to variable pellet swelling with rainfall. Differences were also found between wet and dry pellets within both exposed and covered treatments, where the normalised area for dry pellets was significantly higher than for wet pellets, suggesting that dry pellets expand more when wet.

Pellet size in the covered experiment treatments also increased, suggesting that there may have been some moisture input to the pellets, despite being under cover. This may have been the result of other sources of moisture including absorption from the soil or from the atmosphere, or from rain splash off the ground during heavy rain.

Visible surface area only records the part of the pellet not in direct contact with the soil surface. The rate of physical change recorded may be slower than the actual rate. It is possible that the non-visible side of the pellet therefore physically disintegrates faster than the apparent change from photographic analysis. Another issue with the visible surface area analysis was that pellets moved over time, particularly in dry soils, becoming partially covered with soil particles. This was particularly clear in the exposed, dry pellet treatment photographs. Particle attachment altered the visible surface area, sometimes increasing as the pellets became more exposed. The orientation of pellets on the soil surface also changed, exposing different angles and sides of the pellet which would have had difference surface areas. Orientation and sediment changes were clear from photographs taken of the pellets over time. Changes recorded, therefore, may not necessarily be representative of all dimensional changes. Another potential issue is the possibility that the *ImageJ* software analysis may not capture all the apparent physical degradation over time. It is possible that as pellets become less vibrantly blue they blend into the soil surface and become more difficult to identify.

4.3.4.4 Modelled data

The visible surface area data were normalised, and first order models were fitted. The models did not fit the measured data well, because changes did not typically follow an exponential pattern. This was particularly true of the exposed pellet visible surface area changes, which increased as the pellets expanded after moisture input.

In addition to poor model fit due to the non-exponential nature of the data, the covered pellet treatment modelled degradation was fixed at y=1, as no change was expected from the initial normalised visible surface area. It is recognised that this approach further limited the model applicableness. By locking the intercept, the model assumed that moisture is obtained from rainfall only, and that in covering the plant pots, no external moisture could influence the pellets. It also assumed that moisture is the only driving factor behind pellet disintegration.

Interestingly for both the exposed and covered pellet treatments, pellet visible area was noticeably lower than the initial normalised pellet surface area (area = 1), with the exception of a few measured exposed treatment instances which were linked to pellet expansion in rainfall. The lower-than-expected surface area values could be the result of pellet shrinkage from an initial reaction to outdoor conditions (having previously been kept indoors). However, it is also possible that the difference in pellet area is the result of an initial pellet measurement error.

The models fits did facilitate a relative comparison between treatments, via fitted half-life values, although these values should not be taken absolutely. Half-lives were only calculated for the exposed pellet treatments since the model intercept was locked for the covered treatments. In addition to the poor model fit, half-live calculated were considered to be inappropriate as their values were higher than the experiment duration, therefore were an extrapolation of the data.

4.4 Sub-experiment three: Kinetic energy

4.4.1 Methodology

To measure physical degradation based on kinetic energy, the University of Leicester Rainfall Tower was used to simulate multiple storm events and the effect on pellets. 30 pellets were placed on each of six equal areas on a sandbox. Sand was used as a fast-draining material which would minise soil-moisture impact on the pellets. Three of the measured areas contained dry pellets and three, wet pellets and randomised block sampling was used to assign pellets to pellet area.

For both sub-experiments simulated rainfall was collected in bottles with 10.2 cm funnels for a set duration of time in order to quatify storm intensity. Bottles were placed in ever pellet area to assess rainfall distribution.

4.4.1.1 Pellet size analysis methodology

Rulers were placed alongside each of the pellet areas so scale could be applied during analysis. Again, *ImageJ* was used to quanitfy pellet visible surface area, perimeter and RGB values, averageing the pellet outcome per pellet area instead of taking into account pellets individually. This was due to pellet movement and loss during the simulated storm events. Photographs of the pellets were taken before and after each simulated rainfall event (figure 52).

Two storm events were simulated. The first event lasted 5 minutes and had an average intensity of 235.47 mm/hour. The second event lasted 10 minutes and had an average intensity of 203.64 mm/hour.

Figure 52: Rainfall tower set-up

4.4.1.2 Pellet weight analysis methodology

Pellet change by kinetic energy was also investigated through pellet weight change, which incorporated '3D' changes where image analysis could not. 30 pellets were weighed out, taking note of their collective weight, and systematically placed on the sand surface within their allocated plots.

After simulating rainfall for 10 minutes at an intesity of 218.33 mm/hour, remaining pellets were carefully removed, keeping pellets from within the same plot together. Pellets were then dried for 24 hours at 105°C, before being re-weighed. More pellets were also weighed and dried in the oven for 24 hours at 105°C to assess average original water content. Average weight for a single pellet, taking into account original water content, was used to quatify change because of some pellet losses during the experiment duration.

4.4.2 Results

4.4.2.1 Rainfall intensity

Rainfall was simulated three times across two sub-experiments. Intensity varied between 203.6 and 235.5 mm/hour, averaging 219.1 mm/hour, and there was no measurable significant difference in rainfall between simulated events (one-way ANOVA, $p>0.05$).

4.4.2.2 Pellet visible surface area

Wet pellets increased in 2D visible size by 40.46% on average between pre-storm and storm event one, and by 12.40% between storm events one and two. Dry pellets had a smaller percentage change, increasing by 31.36% on average between pre-storm and storm event one, and by 11.79% between storm events one and two (figure 53).

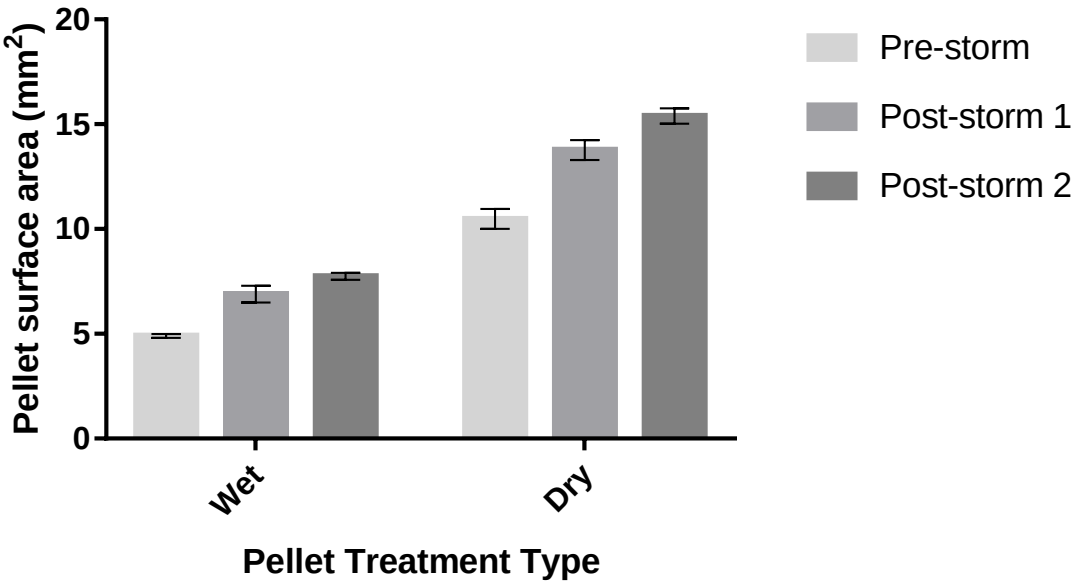


Figure 53: Pellet area change with simulated storm event by treatment type for the rainfall tower experiment

A two-way ANOVA with respect to time showed a significant difference in pellet surface area, from the *ImageJ* analysis, between storm events. Of the total variation in pellet size, 78.63% was attributed to pellet type and 18.28% to pellet visible surface area by storm event number, and both were significant to the 99.99% confidence level ($p<0.0001$). A Tukey's multiple comparisons test showed that both wet and dry pellets statistically significantly increased in visible surface area between storm events (table 42).

*Table 42: Significant difference in average pellet weight within treatment type between storm events for the rainfall tower experiment. * = p<0.05, ** =p <0.01, *** = p<0.001, **** = p<0.0001.*

Pellet treatment type	Storm event one	Average pellet visible surface area one (mm^2)	Storm event two	Average pellet visible surface area two (mm^2)	Significance
Wet	Pre-storm	4.90	Post-storm 1	6.89	**
	Pre-storm	4.90	Post-storm 2	7.74	***
	Post-storm 1	6.89	Post-storm 2	7.74	Not significant
Dry	Pre-storm	10.5	Post-storm 1	13.8	***
	Pre-storm	10.5	Post-storm 2	15.4	****
	Post-storm 1	13.8	Post-storm 2	15.4	*

After normalising visible surface pellet area for both pellet types, another two-way ANOVA with respect to time showed that 86.68% of variation in pellet size occurred with time, significant to the 99.99% confidence level ($p<0.0001$). Pellet type was not a significant source of variation and a Sidak's multiple comparison test showed that there were no significant differences in pellet area between pellet types after being subject to the same storm event ($p>0.05$).

4.4.2.3 Pellet mass

Average pellet mass changed between storm events, differing by pellet type (figure 54). Wet pellets increased in weight by 16% on average across 134 total pellets weighed in 6 replicate groups of up to 30 pellets each. Dry pellets decreased in weight by 10% on average across 140 total pellets weighed in 6 replicate groups of up to 30 pellets each. In weight terms, average wet pellet weight changed from 0.006926 g to 0.007932 g and average dry pellet weight changed from 0.02001 g to 0.01777 g.

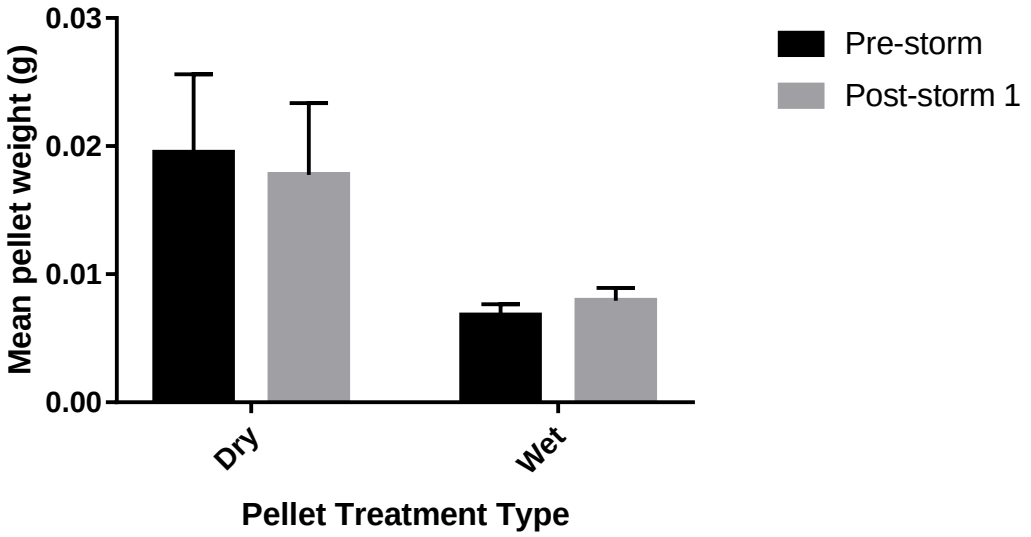


Figure 54: Pellet weight change with simulated storm event by treatment type for the rainfall tower experiment

In combining pellet weights for replicate simulated storm events, for which there was no significant difference in storm intensity, a two-way ANOVA, taking into account mean pellet weight, %CV and n, showed 67.57% of variation was due to pellet type, significant to the 99.99% confidence level ($p<0.0001$), where dry pellets were significantly heavier than wet pellets. A Sidak's multiple comparisons test showed that neither wet- nor dry- pellet average weight statistically significantly changed between storm events ($p>0.05$).

4.4.3 Results summary

Table 403: Rainfall tower experiment results summary

		Wet Pellets	Dry Pellets
Rainfall Tower	Rainfall intensity	○ No significant differences in rainfall between simulate events ○ Intensity ranged between 203.6 – 235.5 mm/hr	
	Visible Surface Area	○ Increased in visible size by 40.46% between pre-storm and post-storm one, then by a further 12.40% after post-storm two ○ Pellet area change was significantly different between pre-storm and post-storm events	○ Increased in visible size by 31.36% between pre-storm and post-storm one, then by a further 11.79% after post-storm two ○ Pellet area change was significantly different between all storm events
		○ No significant difference between normalised wet and dry pellet areas after being subject to the same storm event	
	Pellet mass	○ Weight increased by 16% on average ○ Not a statistically significant change	○ Weight decreased by 10% on average ○ Not a statistically significant change

4.4.4.4 Discussion

The intense rainfall simulation experiment, which was conducted in the rainfall tower, showed that even in two intense simulated storm events of 5 minutes (with intensity averaging 219.1 mm/hour) pellets did not noticeably physically disintegrate. Hewson-Fisher (2015) stated that dry pellets should last 3 to 7 days under rainfall and wet pellets 21 days. Wet pellets are believed to last longer because they have greater elasticity, enabling them to expand. Under simulated rainfall conditions, both pellet types significantly expanded between rainfall events, and, although wet pellets did expand to a higher percentage, there was no significant difference in the change in normalised pellet area post storm event. This suggests that, under direct rainfall there is little quantifiable difference between the wet and dry pellets, as both expand.

In the second simulated storm, some ponding occurred on the sand surface which may have lessened the impact of the rainfall on the pellets. However, pellets still did not disintegrate, even when submerged in the ponded water. Some pellets were also lost from the confines of the six pellet areas due to overland flow. Although both pellet types do sink in standing water, they can move in heavy rainfall, suggesting that they could be transported to surface waters and down macropores towards field drains.

Pellet mass change was statistically insignificant between simulated storm events, although dry pellets measurably lost weight and wet pellets appeared to gain weight. The apparent weight gain could be the result of pellet movement during the storm event, leading to pellets being weighed for the second time as part of a different set of 30 pellets. Given the high variability for pellet mass (CV is 31.45% for dry pellets and 12.74% for wet pellets), weight errors can be large over an average of 30 pellets. Small particles may also have been adhered to the pellets which may also account for apparent mass increase, even though pellets were washed.

4.5 Concluding discussion for all physical degradation experiments

The physical degradation experiments were designed to explore the extent to which pellet integrity is controlled by soil moisture regime and rainfall. This was tracked using visible surface area and colour changes of the pellet casing over time. Since farmers rely on pellet visibility to assess whether re-application is necessary, these factors are essential to metaldehyde management. The extent to which they are related to metaldehyde mobility in the environment is still unknown. The following research questions and hypotheses were outlined in Chapter One:

Research questions:

3. How quickly do the different pellet casings physically breakdown under realistic environmental conditions, under artificially-irrigated conditions and under simulated rainfall conditions?
4. Does the colour of the different pellet types change over time under irrigated and environmentally realistic conditions, and to what extent?

Research hypothesis:

6. Soil moisture will be a driver for physical degradation where increased soil moisture will result in faster degradation
7. Kinetic energy from rainfall impact will be a driver for physical degradation where prolonged exposure to high intensity rainfall will cause faster pellet degradation
8. Pellets will expand in visible surface area immediately following storm events, before decreasing in visible surface area. Wet pellets will expand more than dry pellets.
9. Dry-processed pellets will reduce in visible surface area faster under all experiment conditions in comparison to wet-processed pellets
10. Colour for both pellet types will change over time

In response to research question 1, the rate of degradation for different pellet types was not possible to absolutely quantify in all sub-experiments except for the high-

irrigation regimes in the soil moisture experiment. For the soil moisture irrigation experiment and the integrated outdoor experiment, models were applied to the data for visible surface area. However, these models were universally inappropriate for the data as the data did not follow an exponential decay. In addition, half-lives calculated were frequently longer than the experiment duration, therefore did not necessarily reflect the actual degradation pattern of the pellets. As the physical world reality of the pellet degradation was so different from the modelled degradation, an accurate half-life value or quantified difference between the two pellet casing types not possible from the modelled data.

In the soil moisture irrigation experiment, the higher irrigation regime pellets (100% and 150% irrigation) did fully disintegrate within the experiment duration, completely degrading in <45 days. This experiment provided the only accurately quantifiable answer to research question 1. However, the soil moisture irrigation experiment was not environmentally realistic and therefore cannot be used as an indicator of in-field degradation. Instead the soil moisture experiment proved only a connection between soil moisture and degradation rate, where higher irrigation regime caused a faster degradation rate for both pellet types on loam soil. This confirmed research hypothesis 1 as correct.

In the rainfall tower experiment, pellet disintegration was not observed, where no significant differences were recorded in pellet mass or visible surface area. This was unexpected, as research hypothesis 2 stated that kinetic energy would impact pellet degradation by weakening the pellet casing. Instead, no physical degradation was observed.

Although no measurable breakdown of pellets was recorded during the kinetic energy sub-experiment, both pellet types did significantly expand in visible surface area post-storm event. Wet pellets were shown to increase in visible surface area more than dry pellets, however not statistically significantly so. This partially confirmed hypothesis 3, which stated that pellets will expand in visible surface area immediately following storm events and that wet pellets will expand more. Hypotheses 3 was based on pellet manufacturing guides which suggest that wet-processed pellets would last longer because they have greater elasticity, enabling them to expand. Pellet expansion

following storm events was also confirmed as part of the integrated outdoor experiment in which exposed pellets significantly increased in visible surface area following storm events. However, in this sub-experiment, dry pellets expanded more than wet pellets.

Hypothesis 4 was based on Hewson-Fisher (2015), who stated that dry pellets should last 3 to 7 days under rainfall and wet pellets 21 days. As part of the soil moisture irrigation experiment, wet pellets fully degraded in <45 days whereas dry pellets remained partially intact throughout the experiment. However, this may be the result of pellet size, where dry pellets are much larger than the wet pellets. On normalising the pellet size, significant differences in visible surface area between the wet and dry pellet types occurred during just four days over the 72-day experiment, and only for the 100% and 150% irrigation regimes. This suggests that, overall, there is no overwhelming statistical evidence for a difference in degradation between pellets types, particularly given the unrealistic nature of the irrigation regime.

In addition to the lack of concluding evidence from the soil moisture experiment, all modelled data was inappropriate for quantifying degradation rate, as discussed above. Similarly, the integrated outdoor experiment and kinetic energy experiment had no quantifiable degradation recorded. Therefore, a quantifiable difference between wet and dry processed pellets was not possible in this research.

RGB analysis and qualitative photograph analysis was important because the visibility of the pellets in the field may influence the likelihood of re-application rate. This could unnecessarily exacerbate the pollution problem if metaldehyde is still available in the field soil.

In response to research question 2 and research hypothesis 5, colour change was recorded in both the soil moisture irrigation and integrated outdoor experiments. For the indoor irrigations, pellets became mouldy and then white (also indicative of fungal colonisation), before blending into the soil surface with a darker colour. In the exposed integrated outdoor experiment pellets did not visibly colonise with mould. Instead, the faded to a brown colour without any white. This may be due to the different environmental conditions in the two experiments. In the irrigation experiment pots

were kept indoors with a different temperature, light and moisture regime which may have affected microbial colonisation. The exposed outdoor experiments are more environmentally realistic, and hence, may better represent the colour change expected in the field.

Since modelled data for RGB colour analysis had the same issues are those of the modelled visible surface area data, absolute degradation for pellet Blue-values cannot be accurately recounted. Using just the graphical data, a time-frame of degradation can loosely be attached to the pellet colour change for B-values in the integrated outdoor exposed treatment, although this is not statistically supported. For the integrated experiment, B-values were measured to approximately half in value after 24/07/2017, day 45 of the experiment, for both the wet and dry pellets. For the soil moisture irrigation experiment, blue values did not reach 50% of their value in the experiment duration, which may have been due to the white fungal impact on RGB analysis.

Chapter Five

Overall discussion and conclusion

5.1 Introduction

This thesis describes a set of experiments designed to improve our understanding of the behaviour of pelletised metaldehyde in soil environments. This is important because metaldehyde pollution of drinking water supplies is a major problem for the UK water industry. Information on metaldehyde properties derived from standard laboratory tests (e.g. K_{OC} and DT_{50}) have uncertain roles in predicting the propensity of the chemical to leach if it is applied as a component of a pellet matrix. It is therefore imperative better understand the behaviour of pellets themselves as well as the fate of metaldehyde in soils receiving slug pellets.

The following chapter will discuss the following overarching research questions, one question per section, drawing conclusions from all three experiments in this thesis. Concluding remarks will also comment on recommendations for metaldehyde management.

1. What is the overall influence of soil type on metaldehyde pellet degradation (physical casing and chemical metaldehyde degradation)
2. What is the overall influence of metaldehyde pellet type (wet-processed verses dry-processed pellets) on degradation?
3. Is metaldehyde effected by residuality?

5.2 The overall influence of soil type on pellet degradation

In the incubation study, no significant differences were found between soil types in the degradation of metaldehyde slug pellets and that of laboratory-grade metaldehyde. However, in the soil cores experiment, a significant difference in leaching was found between soil types where leaching from the clay soils was significantly higher than from the loam soil. This was most likely due to a combination of different degradation rates and drainage rates in the core experiment.

The clay soils visibly cracked in the dry weather. This allowed pellets to be washed below the soil surface and enhanced metaldehyde transfers to lower layers of the soil profile. This not only would have reduced the distance to the base of each core, but also would have reduced degradation due to lower biological activity at depth, increased by the dryness of the soil (Kookana et al., 2005). Furthermore, organic matter content tends to decrease with depth, reducing sorption. As shown in the physical degradation study on soil moisture, pellets are significantly influenced by the soil water content and, although metaldehyde was shown to leach out of the pellets before complete physical degradation, water appears to be most important factor in pellet degradation, transport and metaldehyde flux from soils. After one rainfall event onto the dry clay soils, metaldehyde was quickly transported into the base of the cores as leachate. The loam soils had a higher organic matter content (with potentially higher biological activity) and no obvious macropores. In different environmental circumstances, for example in which the clays have higher water content due to more rainfall and lower evapotranspiration. Here, clays expand, closing cracks and reducing propensity for leaching. However, under those circumstances Metaldehyde Stewardship guidelines recommend no pellet application. Nineham et al. (Retrieved December 2017), currently recommend no metaldehyde application to clay soils.

In comparison to the soil cores, the vessels used in the incubation study were under controlled temperature and moisture conditions, and contained only 15g moist soil. This would have minimised differences in the biological activity in soils of the same type. The addition of methanol may have influenced biological activity although both soil types were subject to the same conditions with no measurable difference between them.

It is likely that hydrological processes were influenced by soil type in the cores, which affected the concentrations of metaldehyde found. Clay soils had significantly higher concentrations of metaldehyde in leachate than the loam soils, even though the incubation study suggested that loam soils tend to have longer metaldehyde half-life (although no significant difference was found between soil types). With a higher half-life, metaldehyde would remain in the loam soil for longer, making it eligible for transport for a longer period. This also demonstrates the importance of using realistic

data for understanding environmental fate. The *in vitro* experiments showed no significant differences between soil types and suggested that loam soil might even have slower degradation rates.

Soil moisture does not only affect clay cracking and pesticide leaching through preferential flow; it was also shown to influence physical degradation of pellets. Higher soil moisture caused faster degradation in visible surface area and "blue" colour. Although only the loam soil was used during physical degradation experiment in the rainfall simulator, the capacity of a soil to hold moisture could be an important factor in pellet movement. When soils were dry, as in the 'no irrigation' and covered outdoor experiments, pellets often fell between cracks in the soil. Moisture tended to increase pellet 'stickiness', keeping pellets on the surface.

With increased soil moisture, pellets were found to expand rather than disintegrate from the outset. This indicates an environmental endurance in the field. During the soil core and outdoor physical degradation experiments, photos show pellets lasting on the soil surface for much longer than indicated in the literature by Hewson-Fisher (2015), who suggested that under rainfall conditions dry pellets could last 3 to 7 days and wet pellets up to 21 days. Although not under constant rainfall, pellets could clearly be seen after rainfall events in the soil cores (figures 12, 13, 20 and 21) and in the exposed outdoor experiments (figure 50 and 51). Although intense rainfall was shown to not directly cause pellet disintegration, the application of moisture to pellets caused pellet expansion and wetted the soil. The latter would initiate biodegradation by stimulating microbial communities within the soil, and possibly within the pellets themselves. With multiple rainfall events, pellet integrity may become weaker, although it was not observed after the two simulated rainfall events in this study. Further investigations are required to determine whether intense rainfall could disintegrate pre-wetted pellets.

5.3 The overall influence of pellet type on degradation

No significant differences were found by pellet type between metaldehyde concentrations in either the integrated fate assessment experiment or in the

incubation study. Dry pellet soil cores did produce higher metaldehyde concentrations in leachate than the equivalent wet pellet cores, but this difference was not statistically significant. In the incubation study, dry pellets produced higher extracted concentrations than the wet pellets, and modelled half-lives were higher for dry pellets in both soil types. Physically however, wet pellets appeared to disintegrate faster in the irrigation experiment, although none of the visible surface area or RGB colour values were statistically significant between pellet types. The only statistical differences recorded were for pellet expansion which was likely due to soil moisture changes.

Higher soil moisture caused faster visible surface area decreases and faster change in colour. Wet pellets completely degraded under high irrigation regimes. This suggests that dry pellets may physically last longer due to their size. However, neither pellet type completely degraded during the integrated outdoor experiment. The intensive soil moisture irrigation regime was also not environmentally realistic (the same volume of water was added regularly over its 72 day duration).

In general, dry pellets were more fragile and 'dusty' than the wet pellets, both before and after rainfall. Both pellet types became 'sticky' to handle after being in contact with water. As wet pellets were smaller they were more easily transported into soil cracks. However, there will be a higher coverage of wet pellets per hectare when applied due to their smaller size. Therefore, for pest control, this means that there is a higher contact for number of pellets per mollusc. If molluscs are more likely to come across pellets then they are more likely to consume a lethal dose of metaldehyde.

Significant leaching from the soil cores was observed on the 18/05/2017 (day 55, sampling phase 1), 29/09/2017 (day 3, sampling phase 2) and 09/10/2017 (day 13, sampling phase 2) even when some pellets could still be seen on the soil surface of both soil types. This supports Calumpang et al. (1995), who suggested that pellets 'constantly release' metaldehyde. This indicates that metaldehyde leaching is not strictly linked to physical pellet integrity. This may have implications for pest control: if pellets lose metaldehyde via leaching before they physically break up then molluscs may consume the pellet without receiving a lethal dose.

Leaching from the soil cores on the 22/05/2017 (day 59, sampling phase 1) occurred when pellets were much less visible and some of the pellets may have degraded fully or have been moved below the soil surface. High concentrations were recorded after physical breakdown, therefore if farmers reapply based on lack of visible pellet presence, they may be applying metaldehyde onto soils already containing high metaldehyde concentrations.

5.4 Residual Metaldehyde in soils

The soils used for all three experiments had not previously had metaldehyde applied since November 2014 (loam soil) and November 2015 (clay soil). However, some residual metaldehyde was observed in leachate from the control soil cores in both soil types, run as part of the integrated fate assessment. Concentrations of up to 3.835 ug/l in leached water were observed, which contradicts the low soil half-lives implied in this study and other reports. Although literature soil half-life for metaldehyde is up to 223 days (Kay and Grayson, 2014), if metaldehyde degraded with a $t^{1/2}$=3.98 days (the lowest degradation rate recorded by this study), metaldehyde should not be detectable in control cores. The concentrations recorded in the control cores were also much higher than the drinking water directive limit of 0.1 ug/l, although expected leachate concentrations would be higher than in surface waters due to dilution and dispersion.

In direct contradiction to the soil cores, control incubation vessels found no residual metaldehyde over six repeats. The incubation vessels were subject to controlled conditions, and therefore contamination was highly unlikely between vessels. That said, recovery of metaldehyde was very low and without an internal standard, half-life values calculated must be seen only in relative terms as an indicator of differences between treatment types. In other words, the lack of metaldehyde in the incubation control vessels may be the result of low recovery as opposed to metaldehyde absence.

The presence of metaldehyde in soils after up to two years after last application could be due to the formation of a slowly reversible bound residue. Indeed, data from lysimeters for other pesticides have also show the potential for leaching of chemicals

as bound residues, having previously been believed to have been permanently removed from the soil. Reversing of the bound residue may have occurred due to changes to biochemical or physiochemical environmental conditions (Gevao et al., 2000). Pesticide may also have been occluded from degrading microorganisms by diffusing into small pores which are too small to act as habitats for the microbial biomass. Pesticides are relatively large molecules, but are still many orders of magnitude smaller than the typical microbial cell. Pesticides present in the pure water of small pores are also known to be much less mobile than in large pores because the water in small pores is held more tightly by capillary forces (Pullan et al., 2016). Finally, it is possible that metaldehyde which was leached below the near surface horizons (and therefore subject to reduced microbial degradation in the relatively poor subsoil) was retained in the top 30cm of soil by inversion under ploughing.

The soil cores were placed far enough apart to prevent pellet saltation between cores. No pellet 'jumping' was encountered in the simulated heavy rainfall experiment as part of the physical degradation study.

5.5 Conclusion

The primary conclusions of this study are as follows: no statistical difference was found between the behaviour of metaldehyde in 'Trigger 3' wet-processed pellets and 'Carakol 3' dry-processed pellets. However, dry processed pellets did produce slightly higher (but not significantly so) metaldehyde concentrations in leachate. Wet pellets also physically degraded faster under the same (environmentally unrealistic) soil moisture conditions.

Clay soils had significantly higher metaldehyde concentrations in leachate in the integrated fate assessment. This contradicted longer apparent half-life values for loam soils calculated in the incubation experiment. This was hypothesised to be the result of differences in hydrological processes operating in the two soil types. The clay soils developed cracks during dry weather, which are known to act as preferential flow pathways for pesticides and nutrients. Although physical disintegration analysis under high-intensity rainfall simulation was only carried out on loam soil, clay soils have the

potential to hold more soil moisture in wet conditions and, therefore, may have a higher impact on physical breakdown in the longer term. Soil moisture was proven to have a statistically significant effect on pellet breakdown in the other experiments.

Biodegradation of metaldehyde probably has a greater impact in metaldehyde fate than physical disintegration of the pellet, because metaldehyde appears to leach from the pellets before they break down. The main factor influencing this is water, which initiates both physical and chemical changes, including transport out of the pellet and through soils to water resources.

In terms of metaldehyde management, the results of this study suggest that metaldehyde application to clay soils should be restricted, particularly for any applications after a prolonged period of drying when soils are cracked, because higher leachate concentrations are likely. Clay soils are also likely to be under-drained, further decreasing the lag time from application to transport in surface waters. Since 90% of metaldehyde is believed to enter surface waters through field drains (Hewson-Fisher, 2015), this could greatly reduce surface water concentrations.

Since no measurable difference was found between leached or extracted metaldehyde concentrations by pellet type, either pellet appear to be equally suitable for application. Differences were also not statistically significant, although dry pellets did physically last slightly longer when subject to high soil moisture over a long period of time. Wet-processed pellets have a higher number of bait-points per hectare and are less fragile than dry pellets before application. They also appeared to generate lower concentrations in both the incubation and soil core experiments. Wet-processed pellets may therefore be a more suitable for pest control and for water quality management.

Appendix A

LoD

The LoD is the limit of detection. It is calculated using the equation:

$LoD = 3.3*\sigma$

3.3 is a constant taken from statistical tables where the z distribution has a 90% confidence interval. At the 90% confidence interval z = 1.645. As LoD is a two tailed test, you multiply 1.645 by two to get the constant 3.3.

The constant, represented by k, could be changed based on the statistical confidence you need.

At the 95% confidence interval, z = 1.96, therefore k = 3.92

At the 99% confidence interval, z = 2.576, therefore k = 5.14

By using a z distribution you assume that the sample mean is equivalent to the population mean.

If y = mx + c, where y is the response, x is the concentration, c is the intercept and m is the slope:
Concentration = (response – c)/m
For this study, the response is the ratio: metaldehyde/deuterated metaldehyde.

We want to find the highest response for when the concentration is 0ug/l. This will be the LoD.

Usually, at 0 ug/l you would expect the intercept to be 0. However, this hardly ever happens. Therefore, the highest response for a 90% CI will be '3.3*(the standard error)' because that will give the highest response value that could be for a 0 ug/l concentration. The standard error of the mean (SEM) of the intercept of the calibration curve was used in this study as an estimate of the standard deviation of the blank

response, which is traditionally used, because this study did not have that distribution. This estimate is subject to uncertainty.

Assuming that the uncertainty in the intercept estimate has a normal distribution (an underlying assumption of central limit theorem) the confidence intervals can be calculated from the SE. For a standard normal distribution ($\mu = 0$, $\sigma = 1$), the 90% confidence interval is 1.645*SE. An upper estimate for the intercept of the calibration curve is 2*1.645. We can be 90% certain that the true value for the intercept is less than this value.

LoD = 3.3(SE of the calibration curve intercept/m)*

LoQ

LoQ is the limit of quantification. Here you can say that your analyte is present but you can't give an accurate value for its concentration. This is because the distribution of possible intercepts partially overlays the distribution of intercepts for the LoD.

It is conventional to estimate the LoQ as 10*σ of the blank response. In our case, we do not have a blank response distribution so we have also used SE of the intercept for LoQ.

LoD and LoQ were calculated for each individual calibration curve associated with samples. This represented the LoD and LoQ in dichloromethane, which was converted to a sample LoD and LoQ based on the sample leachate volume.

Appendix B

Method troubleshooting

The best use of DCM to elute solid phase extraction cartridges (part one)

The method used to analyse leachate from soil cores was based on an Anglian Water (AW) method for metaldehyde concentration in rivers, in which 500 ml raw water samples were analysed using solid phase extraction followed by GC-MS. After applying this method directly to leachate samples, it was found that the working internal standard (IS), 50mg/l deuterated metaldehyde in methanol, was not eluting through the cartridge. Therefore, if the known concentration IS was not eluting through the cartridge, it was assumed that the unknown metaldehyde concentration in the leachate samples may also not have been eluted.

The metaldehyde elution problems could have been due to one of three factors:

1) The volume of the sample analysed, as leachate samples are much smaller than the 500 ml raw water samples typically used in this method. With a smaller sample volume, it is possible that metaldehyde was sorbed to the top of the cartridge matrix, rather than being drawn down to the bottom of the cartridge. Therefore, the 2 ml dichloromethane (DCM) used to elute the metaldehyde from the cartridge matrix into GCMS vials was not enough to remove all of the metaldehyde, resulting in part or no metaldehyde being recorded by GCMS. By increasing the volume of DCM used to elute the cartridge, more metaldehyde should be eluted into the GCMS vial.
2) The metaldehyde was being 'lost' somewhere in the method process. The Anglian Water method was completed by an automated machine, whereas this study was manually undertaken. There could have been an unknown influencing factor which impacted metaldehyde behaviour. This was hypothesised to be due to the volatility of metaldehyde and DCM, which are both considered to be highly volatile (Lewis et al., 2016, Kim et al., 2015).
3) The metaldehyde could have been passing through the cartridges into the waste, rather than being caught in the cartridge matrices.

Each of these possibilities was tested individually, beginning with the first, which was thought to be most likely. To test the volume of DCM needed to elute the cartridge, different concentrations of metaldehyde in deionised water were eluted through SPE cartridges, following cartridge preparation as set out in the Anglian Water method. A high concentration, 100 mg/l, was initially chosen as a concentration that would be easily registered by the GCMS, although the concentration was not considered environmentally realistic. Cartridges were prepared with 10 ml methanol followed by 2 ml deionised water, then eluted with 5ul of 50mg/l deuterated metaldehyde in methanol and 20ml of the 100 mg/l sample, followed by a further 2 ml deionised water. After drying the cartridges, different quantities of DCM ranging from 1 to 5 ml at 0.5 intervals were used to elute the cartridges into GCMS vials, which were then processed in the GCMS.

It was found that 100 mg/l overloaded the cartridges, producing irregular chromatograms which could not be integrated easily (figure 55). This suggested that the concentration of metaldehyde was too high for both the cartridge and the GCMS. The impact of DCM on eluting the cartridges could not be speculated.

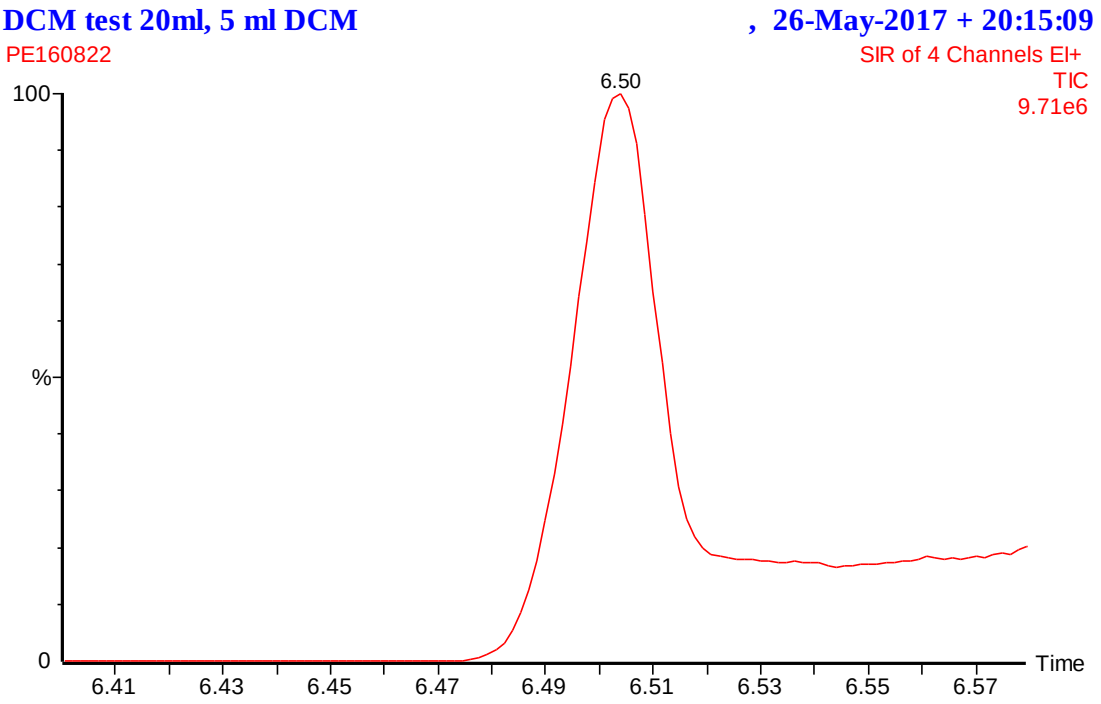


Figure 55: Chromatogram showing an overloaded cartridge metaldehyde peak and no deuterated metaldehyde peak response.

As the deuterated metaldehyde concentration pipetted into the 100 mg/l standard was not altered from the original AW method, the deuterated metaldehyde peak was not visible next to the metaldehyde peak response in most cases, therefore they were not comparable (figure 55). Where the IS peak was visible its response was not consistent between samples, varying with the DCM volume as much as the metaldehyde response varied. Again, this could have been a response to either metaldehyde overloading of the matrices or not enough DCM used to elute the cartridges.

In response to the cartridge metaldehyde overload, a much lower concentration, 1 ug/l metaldehyde in deionised water, was tested as a potentially environmentally realistic metaldehyde concentration, given the current 0.1 ug/l maximum allowable individual pesticide concentration in the EU (Council Directive, 1998). By testing a lower metaldehyde concentration it was hoped that metaldehyde overloading could be avoided, focusing on the volume of DCM needed to elute the cartridges. The cartridges were prepared as above with methanol and deionised water, eluted with 5ul of 50 mg/l deuterated metaldehyde in methanol and 20ml of the 1 ug/l solution, followed by a further 2ml deionised water. The vials were eluted with a greater range of DCM than previously, varying the DCM volume per cartridge between 1 to 12 ml over 8 cartridges.

Following the overload from the 100 mg/l solution, the 1 ug/l samples had no response from the GCMS, with neither the deuterated metaldehyde nor metaldehyde detected. Considering this, it was decided that limit of detection tests would be carried out to find the lowest detectible concentration of metaldehyde, before resuming DCM testing.

Limit of detection analysis

To find the lowest detectable environmentally realistic concentration of metaldehyde for the developed GCMS method, standards were made of known concentration and directly input into the GCMS without use of the cartridges. This 'rough' LoD should not be confused with the sample LoD which is calculated using the calibration curve standard deviation. The 'rough' LoD could be converted to a sample LoD, where the first was metaldehyde in methanol and the second was metaldehyde in water. In finding the rough limit of detection for the GCMS, samples could be made to test the method which were definitely going to be detectable using the developing method.

A new stock calibration standard, 1000 mg/l metaldehyde in methanol, was made by weighing 50mg ± 0.5 mg metaldehyde into a 100 ml Pyrex bottle and adding 50 ml methanol using a glass volumetric pipette.

From the stock calibration standard, a 1 mg/l metaldehyde in DCM solution was made by pipetting 50 ul of the 1000 mg/l metaldehyde in methanol stock calibration standard into a 50 ml volumetric flask and topping up to 50 ml with DCM.

Before dilution further, the 1 mg/l standard was tested in the GCMS alongside deuterated metaldehyde, of which 5ul of a 50mg/l deuterated metaldehyde in methanol solution was pipetted into the GCMS vial with 1 ml of the 1 mg/l standard.

Given;

$$M = VC$$

M_{dm}= 5 ul * 50 mg/l	M_m= 1 ml * 1 mg/l
M_{dm} = 5 ul * 0.05 ug/ul	M_m = 1 ml * 1 ug/ml
M_{dm} = 0.25 ul	M_m = 1 ug

Where *M* is mass, *V* is volume, *C* is concentration, M_{dm} is mass of deuterated metaldehyde and M_m is mass of metaldehyde.

For 5 ul of a 50 mg/l deuterated metaldehyde solution and 1 ml of a 1 mg/l metaldehyde solution, the mass of deuterated metaldehyde in the each GCMS vial should be 0.25 ug and the mass of metaldehyde should be 1 ug. Therefore the response ratio between the two peaks should be approximately four. Using a min-max error calculation, with 5% error, the ratio between the two response peaks would be between 3.3 and 4.9. With 2% error, the ratio would be between 3.7 and 4.3.

In testing the 1 mg/l standard made, the ratio between the two peaks was 3.779, suggesting an error of <2%.

Using the 1 mg/l standard, seven working calibration standards were made; 10 ug/l, 25 ug/l, 50 ug/l, 75 ug/l 100 ug/l, 150 ug/l and 200 ug/l. Response peaks showed for all of the concentrations except 10 ug/l (figure 56).

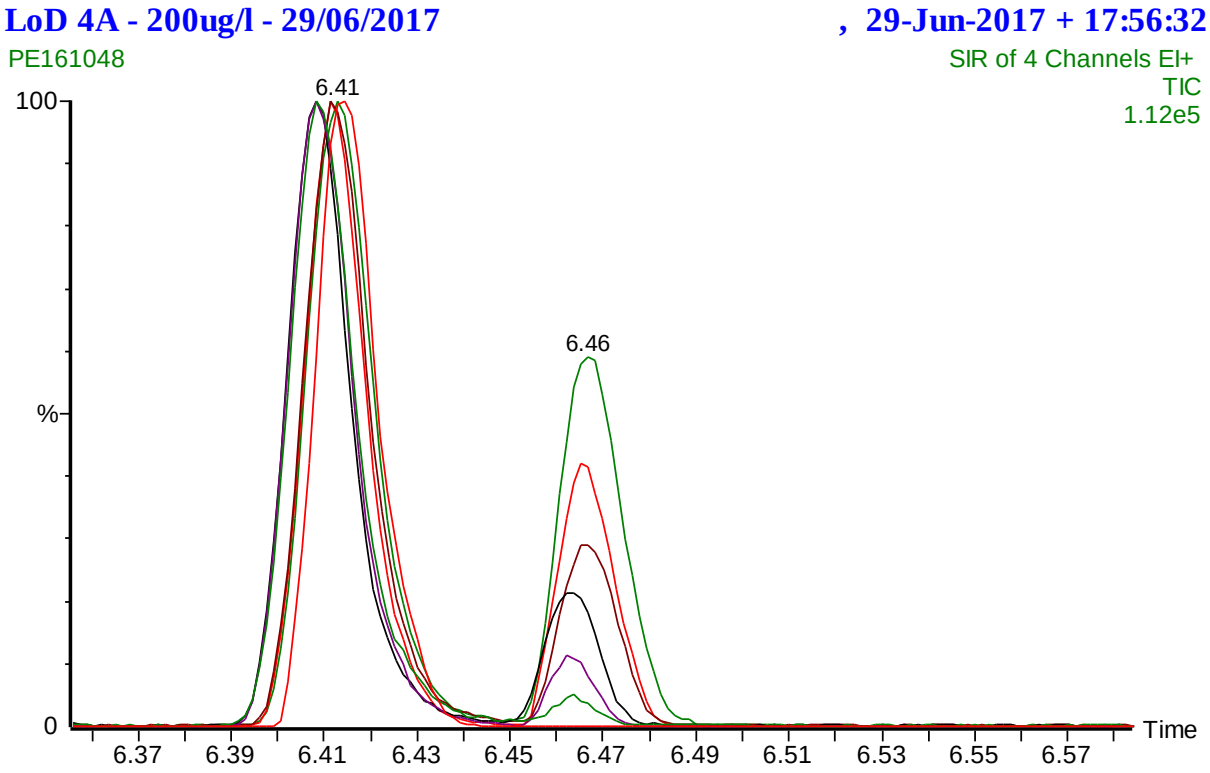


Figure 56: Example chromatogram showing working calibration standards

Metaldehyde concentration responses were evenly spaced, with response areas for individual peaks correlating well with each other, having an R^2 value of 0.9843. However, inconsistent deuterated metaldehyde response was thought to be from a pipetting error, which impacted the deuterated R^2 and ratios between the peak responses. At this time of analysis, the calibration curve was calculated plotting the nominal concentrations directly against area response, and it was expected that metaldehyde area response would increase with increasing nominal concentration and deuterated metaldehyde response would be even across all nominal concentrations. In most instances deuterated metaldehyde decreased with increasing nominal concentration and this was first assumed to be a pipetting error.

To test if the deuterated response was from a pipetting error standards were run in the GCMS again with newly pipetted GCMS vials. More standards were also made between 5ug/l and 25 ug/l at 5ug/l intervals to try to push the limit of detection below 25ug/l. Changes were also made to the GCMS, increasing the multiplier to improve sensitivity, and using a DCM wash instead of hexane to reduce chances of contamination between samples. It was hoped that these machine changes also would push the detection limit lower than 25 ug/l.

In re-running the working calibration standards, including new standards of 5 ug/l, 10 ug/l, 15 ug/l and 20 ug/l, area responses were not able to be consistently detected below 25 ug/l. Therefore, 25 ug/l metaldehyde in DCM was recognised as the lowest limit of detection for this method and GCMS. This is equivalent to approximately 1.25 ug/l in water.

Deuterated response was again found to decrease with increasing metaldehyde response and, therefore, it was decided that pipetting was not the error causing inconsistent deuterated metaldehyde response.

The best use of DCM to elute solid phase extraction cartridges (part two) and reducing the volatility of metaldehyde by changing method processes

From the literature and AW records, a new concentration of 200 ug/l was chosen as the maximum expected environmentally realistic metaldehyde concentration. By testing this concentration through the SPE cartridges at a low volume it was hoped that the volume of DCM needed to elute the cartridges would be the highest required volume, encompassing all metaldehyde concentration possibilities.

To increase test efficiency, the DCM test was combined with making small changes in the method to see if a difference could be found. Samples were prepared in the same way as when testing the volume of DCM, using the same sample concentration, 200 ug/l, and volume, 20 ml, across all SPE cartridges. As before, after drying the cartridges, different quantities of DCM ranging from 1 to 12 ml were used to elute the cartridges into GCMS vials, which were then processed in the GCMS. However, the method was altered in how the samples were eluted into GCMS vials after drying.

Looking at the properties of metaldehyde and DCM it was thought that volatility was the most likely influence on metaldehyde concentration, potentially explaining why metaldehyde and deuterated metaldehyde were 'lost' during sample preparation. There were two steps within the manual method which could affect volatilisation and could be easily changed:

1. Eluting the GCMS vials with DCM using the vacuum manifold
2. Evaporating GCMS vials to 0 ml after elution through cartridges by passing air over the cartridges

To test the first potential solution, cartridges were prepared in the same way as during the previous tests up until cartridge elution into GCMS vials. Instead of attaching the cartridges to the vacuum manifold, cartridges were attached to a clamp and stand with the GCMS vials under the cartridges. Varying volumes of DCM were eluted through each cartridge under gravity. After elution through the cartridges, vials were gently evaporated under a stream of air to 0 ml and the rehydrated with DCM to 1 ml.

Putting the samples through the GCMS, response areas were present for both metaldehyde and deuterated metaldehyde for all of the different volumes of DCM tested (figure 57). This suggested that metaldehyde volatility was the main cause of metaldehyde loss when the vacuum manifold was used to elute and dry cartridges. As a result, 2ml DCM was chosen to elute the cartridges under gravity where more DCM did not elute more metaldehyde through the cartridges.

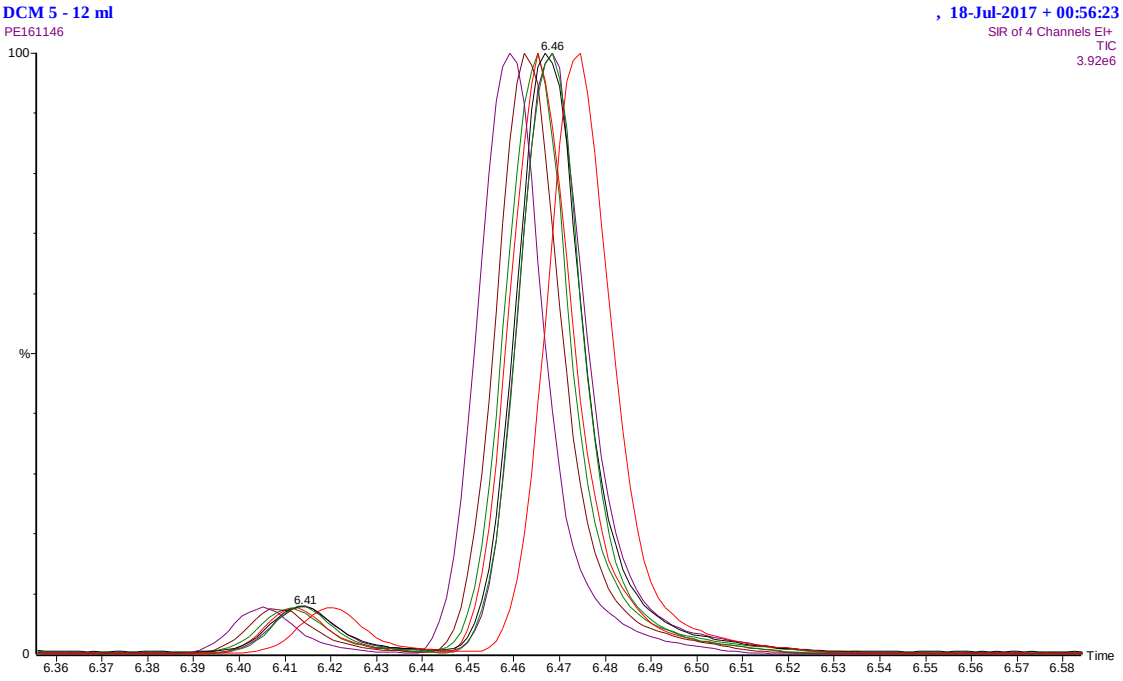


Figure 57: DCM test chromatogram showing all responses of a 200 ug/l metaldehyde in water solution having been eluted through SPE cartridges and then eluted into GCMS vials using differing volumes of DCM. All responses are similar showing that the volume of DCM had no effect on metaldehyde peak response.

However, the decreasing trend in deuterated metaldehyde response area seen in previous tests was also present. It was hypothesised that this trend was due to the GCMS machine response which showed a comparison peak response relating the two analytes to each other, rather than showing absolute responses from both. Therefore the calibration curve calculation method which calculated the concentration of metaldehyde using direct deuterated responses to internally correct any metaldehyde loss was inaccurate.

Metaldehyde concentration calculation changes to improve deuterated metaldehyde response

Originally, metaldehyde concentration was going to be calculated by plotting the working calibration standard nominal concentrations directly against peak response areas for those concentrations, excluding the internal standard, using the following method (figure 58):

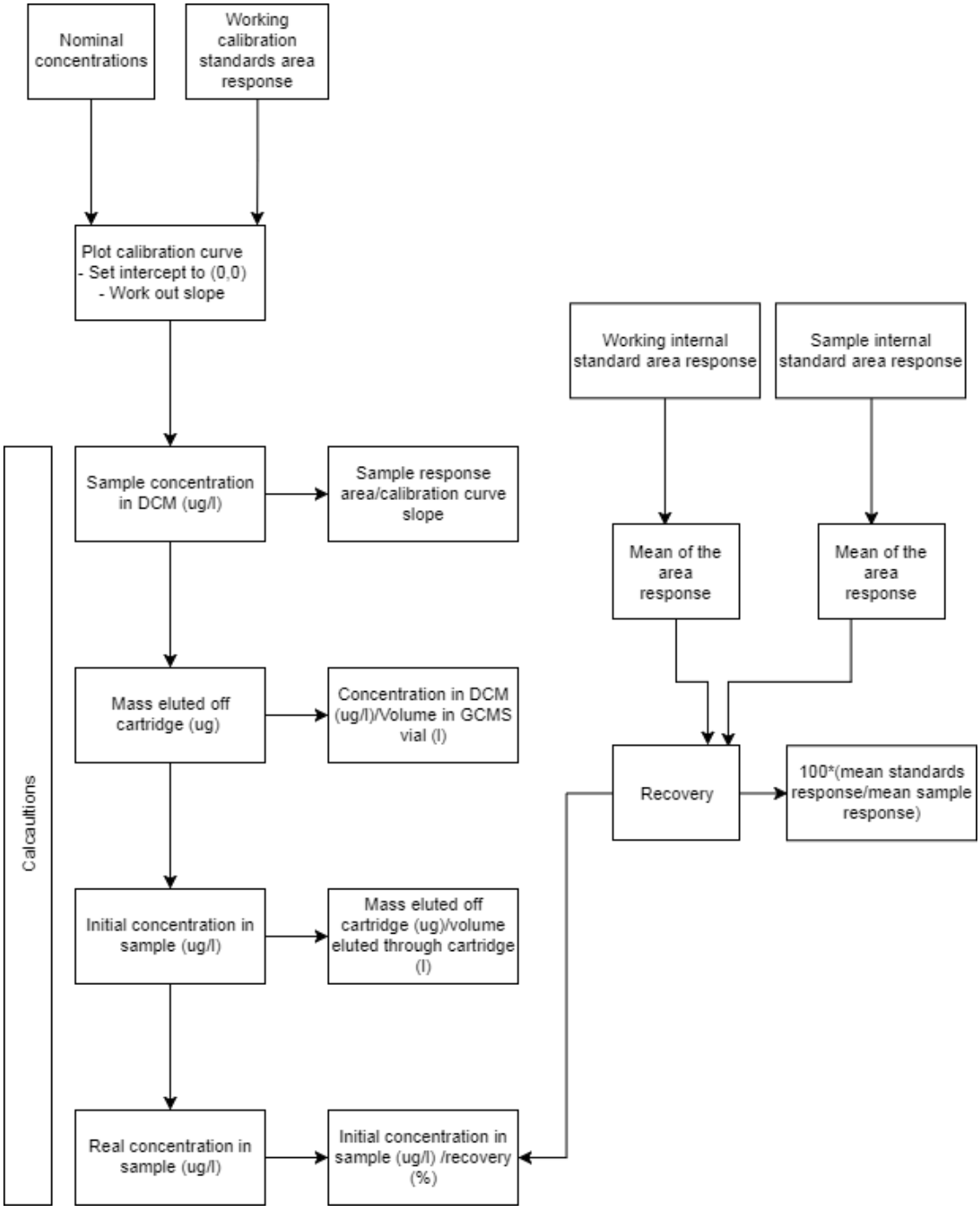


Figure 58: Original calibration curve method for calculating sample metaldehyde concentration

The working internal standard was used to calculate recovery by dividing the mean deuterated metaldehyde response in the samples by the mean deuterated metaldehyde response in the working calibration standards and multiplying by 100. Using this, the 'real' sample concentration could be calculated by dividing the calculated sample concentration by the recovery.

However, on the GCMS, the deuterated metaldehyde response kept dropping as the nominal concentration increased. If the deuterated response was not consistent within the working calibration standards, the average response was not representative.

Therefore, it was decided that the ratio between the two response peaks was a better indicator of the sample concertation. Plotting area response against working calibration standards, as in the original method, the deuterated response is not linear and therefore inappropriate for use as recovery applied to a linear calculation for metaldehyde concentration (figure 59a). When response peak ratios were plotted against nominal concentration the calibration curve was linear and consistent with a high R^2 value (figure 59b). As a linear relationship, this could be used to calculate metaldehyde concentration, incorporating deuterated metaldehyde, and therefore recovery, directly into calculations.

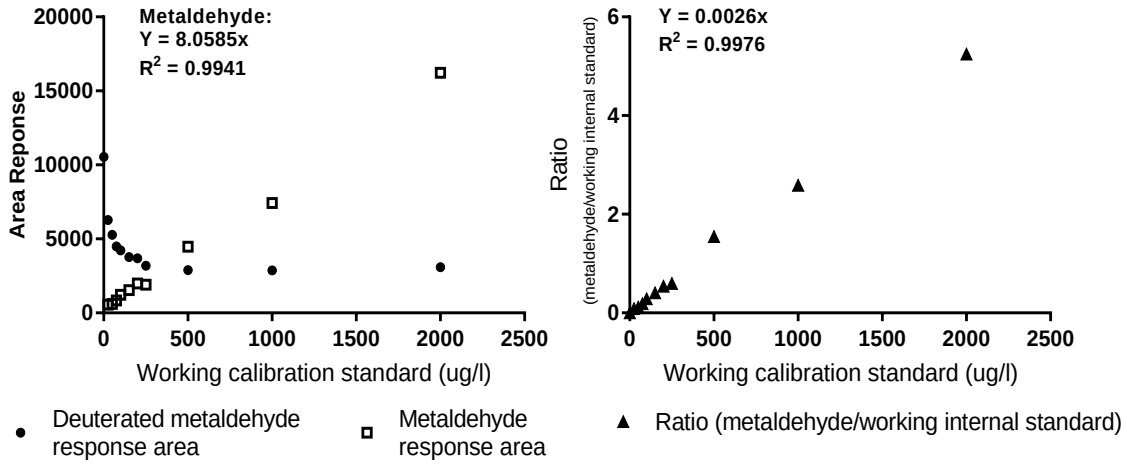


Figure 59: A) Left, nominal concentration plotted against chromatogram area response. Regression equation and R^2 of the metaldehyde area response is displayed. Metaldehyde response is linear, whereas the deuterated response is non-linear. B) Right, nominal concentration plotted against area response ratio (metaldehyde/deuterated metaldehyde). Response is linear.

As a result of changing the methods, deuterated responses were consistent and representative of changes in the working calibration standards nominal concentrations. When the new method was paired with the DCM test for eluting cartridges and changes to the elution process by using gravity to elute DCM instead of the vacuum manifold, it was shown that metaldehyde responses could be made consistent between samples with minimum metaldehyde loss. Only 2ml DCM was needed to elute the cartridges because the predominant loss was originally from metaldehyde volatility due to the method. The finalised method is shown in figure 60.

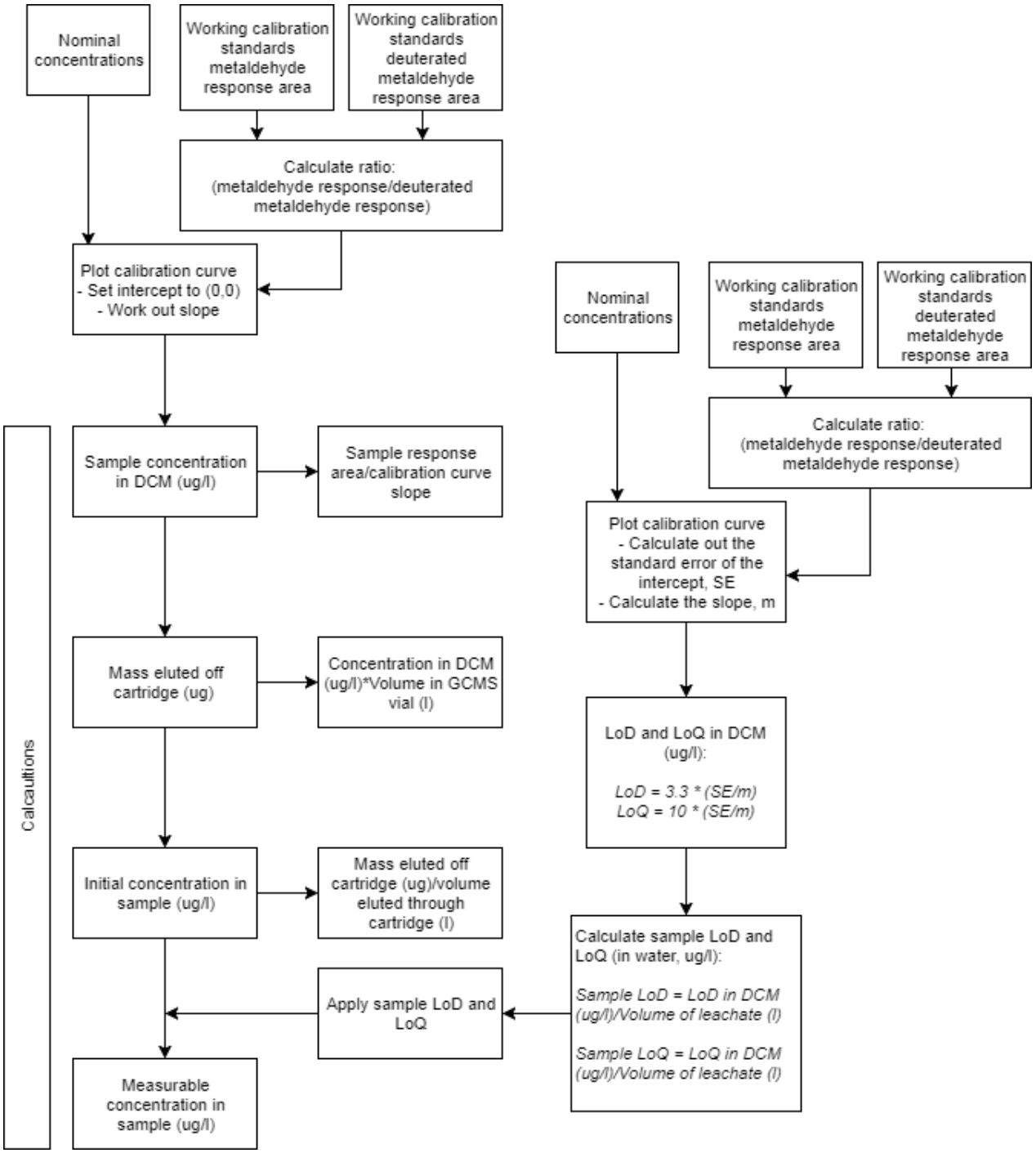


Figure 60: Finalised calibration curve method for calculating metaldehyde concentration

Method development summary

- The volume of DCM used to elute cartridges does not actually make a measurable difference to the sample we used, even with high-concentration and low-volume.
- The issue with metaldehyde being lost in elution appeared to stem from metaldehyde volatility under vacuum. This was solved by eluting cartridges under gravity.
- Deuterated metaldehyde has a non-linear relationship with increasing nominal concentration of metaldehyde in DCM. This meant the method had to be changed to incorporate metaldehyde/d16 metaldehyde ratios to accurately represent concentration.

Appendix C

Method development and justification

Mechanical solvent extraction was chosen because it is applicable to partially volatile compounds, such as metaldehyde. Metaldehyde has a variety of reported K_{OC} values, and previous experiments have often had to use strong solvents to extract metaldehyde from the soil solid phase. Mechanical solvent extraction could be used to reduce DCM and metaldehyde volatility, where extraction methods involving heat, vacuum filtration or strong air flow for evaporation would not have been appropriate.

In the study by Ma et al. (2012), mechanical extraction via centrifuge was used, extracting metaldehyde from soils initially using ethyl acetate, for which recoveries were below 15%, before switching to acetonitrile where recoveries were higher but still below 60%. In their study a purification step was used to increase recovery, however, due to time constraints, this study decided to use a stronger extraction solvent, DCM, which is commonly used for extracting metaldehyde in water industry raw water analysis and was used for integrated fate analysis in another experiment of this study. This allowed GCMS settings to remain the same between the two experiments, allowing for co-analysis.

Because of changes to the solvent used to extract metaldehyde, centrifuging could not be used with the equipment available. DCM was not compatible with the plastic centrifuge tubes and glass tubes would shatter in the centrifuge, even at relatively low rpm. As an alternative to this, mechanical shaking for 24 hours and filtration steps were employed.

Metaldehyde solubility in methanol is 1730 mg/l in comparison to 188 mg/l in water (Lewis et al., 2016), therefore HPLC methanol was used as the solvent for laboratory grade metaldehyde. Metaldehyde solubility in water was too low to make a solution with high enough in concentration to add a minimal volume of that solution to the receiving soil. If too much liquid had been applied it would have created a saturated environment and the freeze-dryer would not be efficient enough to remove 100% of the liquid present. Methanol was also applied to pellet-containing vessels to ensure a

similar bacterial environment between all vessels. Removing the methanol before freeze-drying was an important step to ensure maximum freeze-drying efficiency.

0.667 mg metaldehyde per vessel was selected as an approximate equivalent to one dry-processed pellet per vessel and three wet-processed pellets. OECD test 307 suggests that an environmentally-realistic application rate, often the maximum dose rate per unit area, is used to be representative of the substance tested. However, the maximum total dose of metaldehyde, 700g metaldehyde per hectare, was not appropriate for the 100 ml vessels chosen and would have required the pellets to be broken up pre-application. Therefore the equivalent of metaldehyde dose for one dry-processed pellet was chosen as the smallest applicable dose per vessel, even though it is approximately equivalent to 135 times the maximum total dose per hectare recommended by the '*Get Pelletwise'* campaign (Metaldehyde Stewardship group, 2009). An incubation temperature of 20 °C was chosen as the laboratory standard following OECD method 307 (OECD, 2002).

An internal standard was not used for this experiment because, post-freeze drying, metaldehyde was contained in solvent only and involved few extraction processes. As a method comparing treatment types rather than concentrating on degradation concentration precision, recovery was of lower importance because all vessels were subject to the same extraction and analysis processes. In analysis, response area was averaged across treatment type replicates, showing recovery variation within the method. The external standard, 50 mg/l deuterated metaldehyde in methanol, would have also been required in a much higher volume per vessel than available to be used.

Different filtration methods were tested prior to beginning the incubation. Firstly, soxhlet filtration inserts were used to filter the solvent without application of heat as in soxhlet extraction. However, this did not filter out all the sediment in the sample and therefore could not be input into the GCMS. Filtration using aluminium oxide and steel wool inserted into a glass pipette was also tried, but this method was very time-consuming, increasing the chance of DCM evaporation during the process, and therefore concentrating the metaldehyde in solution. Also, as each pipette was individually filled with aluminium oxide and steel wool, the amount of filtration

material in each pipette was variable and therefore the samples not comparable because some of the metaldehyde may have been retained by the filtration matrix. Without an internal standard this could not be corrected for.

0.45 µm Syringe disc filters were chosen because they filtered out all of the sediment in the sample, could be used quickly without application of heat to reduce as much DCM and metaldehyde volatility as possible, and were consistent between samples.

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