Provided by the author(s) and University of Galway in accordance with publisher policies. Please cite the published version when available.

Title	Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil
Author(s)	Peyton, Dara P.; Healy, Mark G.; Fleming, Ger; Grant, Jim; Wall, David P.; Morrison, Liam; Cormican, Martin; Fenton, Owen
Publication Date	2015
Publication Information	Peyton, D.P., Healy, M.G., Fleming, G.T.A., Grant, J., Wall, D., Morrison, L., Cormican, M., Fenton, O. (2015) 'Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil'. Science Of The Total Environment, 541 :218-229.
Publisher	Elsevier
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0048969715307130
Item record	http://hdl.handle.net/10379/5281
DOI	http://dx.doi.org/doi:10.1016/j.scitotenv.2015.09.053

Downloaded 2024-04-23T16:53:14Z

Some rights reserved. For more information, please see the item record link above.

Published as: Peyton, D.P., Healy, M.G., Fleming, G.T.A., Grant, J., Wall, D., Morrison, L., Cormican, M., Fenton, O. 2015. Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil. Science of the Total Environment 541: 218-229. http://dx.doi.org/10.1016/j.scitotenv.2015.09.053

Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil

D.P. Peyton[1,2], M.G. Healy[2], G.T.A. Fleming[4], J. Grant[3], D. Wall[1], L. Morrison[5], M. Cormican[6], O. Fenton*[1]

[1]*Teagasc, Environment Research Centre, Johnstown Castle, Co. Wexford, Ireland*

[2] *Civil Engineering, National University of Ireland, Galway, Co. Galway, Ireland*

[3]*Teagasc, Ashtown, Co. Dublin, Ireland*

[4]*Microbiology, National University of Ireland, Galway, Co. Galway, Ireland.*

[5]*Earth and Ocean Sciences and Ryan Institute, National University of Ireland, Galway, Co. Galway, Ireland.*

[6] *School of Medicine, National University of Ireland, Galway, Co. Galway, Ireland.*

*Corresponding author: Tel: +353 53 9171271; Fax: +353 53 9171271; Email address: owen.fenton@teagasc.ie

ABSTRACT

Treated municipal sewage sludge ("biosolids") and dairy cattle slurry (DCS) may be applied to agricultural land as an organic fertiliser. This study investigates losses of nutrients in runoff water (nitrogen (N) and phosphorus (P)), metals (copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr)), and microbial indicators of pollution (total and faecal coliforms) arising from the land application of four types of treated biosolids and DCS to field micro-plots at three time intervals (24, 48, 360 hr) after application. Losses from biosolids-amended plots or DCS-amended plots followed a general trend of highest losses occurring during the first rainfall event and reduced losses in the subsequent events. However, with the exception of total and faecal coliforms and some metals (Ni, Cu), the greatest losses were from the DCS-amended plots. For example, average losses over the three rainfall events for dissolved reactive phosphorus and ammonium-nitrogen from DCS-amended plots were 5 and 11.2 mg L^{-1}, respectively, which were in excess of the losses from the biosolids plots. When compared with slurry treatments, biosolids generally do not pose a greater risk in terms of losses along the runoff pathway. This finding has important policy implications, as it shows that concern related to the reuse of biosolids as a soil fertiliser, mainly related to contaminant losses upon land application, may be unfounded.

Keywords: biosolids; dairy cattle slurry, rainfall simulator; surface runoff; nutrients; metals, faecal coliforms, total coliforms

1. Introduction

In the European Union (EU), implementation of directives and other legislative measures in recent decades concerning the collection, treatment and discharge of wastewater, as well as technological advances in the upgrading and development of wastewater treatment plants (WWTPs) (Robinson et al., 2012), has resulted in a rise in the number of households connected to sewers, increasing the loadings on WWTPs (European Community (EC), 2014). Production of untreated sewage sludge across the EU has increased from 5.5 million tonnes of dry matter (DM) in 1992 to an estimated 10 million tonnes in 2010 (Eurostat, 2014), with production further expected to increase to 13 million tonnes in all EU member states by 2020 (EC, 2010).

The treatment and disposal of sewage sludge presents a major challenge in wastewater management and, consequently, there is a need to find a cost-effective and innovative solution for its disposal (Hall, 2000). In the EU, the drive to reuse sewage sludge has been accelerated by legislation such as the Landfill Directive 1999/31/EC (EC, 1999), the Urban Wastewater Treatment Directive 91/271/EEC (EEC, 1991), the Waste Framework Directive 2008/98/EC (EC, 2008), and the Renewable Energy Directive 2009/28/EC (EC, 2009). This has prompted those involved in sewage sludge management to find alternative uses for it, such as in the production of energy, bio-plastics, polymers and other potentially useful materials (Healy et al., 2015). Recycling to land is currently considered the most economical and beneficial way for sewage sludge management (Haynes et al., 2009; Peters et al., 2009; Healy et al., 2015). However, before this can occur, it must be treated to prevent harmful effects on soil, vegetation, animals and humans (EC, 2014). Chemical, thermal or biological treatments, which may include composting (USEPA, 2002), aerobic and anaerobic digestion (USEPA, 2006a), thermal drying (USEPA, 2006b), or lime stabilisation (USEPA, 2000), produces a stabilised organic material frequently referred to as 'biosolids'. The term biosolids was formally adopted in 1991 by the Name Change Task Force of the Water Environment Federation (WEF, 2005) to differentiate raw, untreated sewage sludge from treated and tested sewage sludge that can legally be utilized as a soil amendment and fertiliser.

The are many benefits of recycling biosolids to grassland: (1) their use completes the urban-rural cycle (Fehily, Timoney and Company, 1999) (2) they may be used as a soil conditioner, improving its physical, chemical and biological properties, and reducing the possibility of soil erosion (Lucid et al., 2014) and (3) they are a cheap organic alternative to commercial fertilizer (Lu et al., 2012).

There are many potential problems associated with the land application of biosolids, and these have been reviewed by Lu et al. (2012) and Singh et al. (2008), amongst others. Nutrient losses in runoff are affected not only by biosolid type, but also application rate. In the EU, land application of biosolids is based on the pH, metal and nutrient content of the soil and the nutrient and metal content of the biosolids (Fehily, Timoney and Company, 1999). Frequently, the phosphorus (P) content of the biosolids becomes the limiting factor in determining the land application rate (Lucid et al., 2013). In the USA, the application of biosolids to land is governed by the standard for the use or disposal of sewage sludge (USEPA, 1993) and as a result, the rate of application of biosolids to land are applied based on an estimate of crop nitrogen (N) need and biosolids N availability (Lu et al., 2012), and is not based on a soil test (McDonald et al., 2011). However, due to concerns about the effects of repeated manure or biosolids applications on the soil and the risk of P loss to surface water, some states (e.g. Maryland) have introduced regulations based on the P content of the biosolids (Lu et al., 2012).

Losses of nutrients to surface or subsurface waters bodies originates in two ways: as chronic (long-term, due to the build-up of nutrients in soil), or as incidental (short-term losses within 48 hr of application) losses following episodic rainfall events soon after land application of a fertiliser or amendment (Brennan et al., 2012). Such losses to a surface waterbody occur via direct discharges, surface and near surface pathways, and/or groundwater discharge, where there is a hydrological transfer continuum between a nutrient source (chronic or incidental) and surface water receptor (Wall et al., 2011). Losses of P have been reported by Lucid et al. (2013, 2014) following the application of thermally dried (TD), lime stabilised (LS) and anaerobically digested (AD) biosolids. Increased N losses have also been reported following biosolids application to land. For example, Ojeda et al. (2006) reported elevated concentrations of ammonium (NH_4-N) and nitrate (NO_3-N) in surface waters following the application of TD and composted biosolids at rates of 10 t DM ha^{-1}. Quilbé et al. (2005) measured elevated runoff NH_4-N concentrations following the spreading of AD biosolids applied at 7.5 DM ha^{-1}, whereas LS biosolids had no significant effect on such concentrations in runoff when applied at the same rate.

Although many studies have not reported elevated metal concentrations in runoff following the application of various types of biosolids (Joshua et al., 1998; Dowdy et al., 2001; Eldridge et al 2009; Lucid et al., 2013), there is a dearth of data comparing the impact of several types of biosolids, applied during the same application, on surface runoff of metals. In addition, concerns have been raised about the accumulation of heavy metals in both soil and crops after repeated applications of biosolids (McBride, 2003; Bai et al., 2010) and the migration of metals from the soil profile to surface and subsurface waters (Lu et al., 2012).

Other concerns associated with the land spreading of biosolids have focused around human enteric pathogens found in biosolids, as inactivation of pathogens is difficult to achieve (Sidhu et al., 2009). Typically, the densities of pathogens are reduced by two to three orders of magnitude by the wastewater treatment and biosolids processing (Apedaile, 2001). Whilst these reductions are significant, appreciable numbers of pathogens survive, which may subsequently re-grow to hazardous levels when exposed to favourable environmental conditions (Zaleski et al., 2005), especially during storage (Iranpour et al., 2006). Pathogen survival is evidenced by the survival of faecal coliforms (FC) as indicators for the possible presence of microbial pathogens. The use of indicator organisms allows for the limitation of potential contaminating effects (Sidhu et al., 2009).

Studies have shown that elements of pathogen population may exhibit enhanced survival due to advantageous physiological properties or colonisation of more favourable sites (Brennan et al., 2012). However, as the soil environment is very hostile to the survival of pathogens, their survival time, following the land application, is 2 to 4 months (Brennan et al., 2012). Consequently, pathogens are more likely to be transported to watercourses in incidental rainfall events soon after land application. Studies examining the transport of pathogens in runoff following the application of biosolids have generally shown increased runoff of FC compared to control plots (Dunigan et al., 1980; Nelson et al., 2005; Wallace et al., 2014).

Understanding the environmental persistence and fate of enteric pathogens introduction following land application of biosolids and organic amendments is necessary, as it provides a sound scientific basis for management practices designed to mitigate the potential microbiological health risks associated with spreading on agricultural land (Lang et al., 2007). The risk associated with biosolids-derived and other organic amendment pathogens is largely determined by their ability to survive and maintain viability in the soil environment after land spreading. In general, enteric pathogens are poorly adapted to survival in the soil environment, and pathogens that are land applied from biosolids and dairy cattle slurry (DCS) are influenced by climatic and agronomic variables (Lang et al., 2003).

As demands for food and energy are expected to increase from a growing population (FAO, 2009), the demands for N, P, and potassium (K) are also expected to increase at an average rate of 2.5% per year to 2020 (Heffer et al., 2013) and as a result, the price of chemical fertiliser is also expected to rise (Heffer et al., 2013). As biosolids are often considered a waste product, they may be used as a cheap source of fertiliser and may provide an excellent opportunity to improve crop profit margins by means of reducing the input costs of chemical fertilisers. However, any nutrient recovery from biosolids must be considered against possible adverse impacts associated with their use. Therefore, there is a need for continued research into land spreading practices to ensure that environmental losses and associated concerns are minimised.

The objectives of this study were to (1) quantify runoff losses of nutrients (N, P), metals (copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr)), and microbes (total coliforms (TC) and FC), from experimental micro-plots at time intervals of 24, 48 and 360 hr, following application of four types of biosolids at the legal application rate based on current EU legislation (2) compare the losses arising from the application of the biosolids to land to losses on similar micro-plots following application of another commonly spread organic fertiliser in Ireland, DCS. At the scale of the present study, any losses represent worst case scenario losses, as further attenuation is expected along the transfer continuum before discharge to a waterbody.

2. Materials and Methods

2.1 Field Site characterisation

The study site was a 0.6-ha plot located at Teagasc, Johnstown Castle Environment Research Centre, Co. Wexford, Ireland (latitude 52.293415, longitude -6.518497) in the southeast of Ireland. The area has a cool maritime climate, with an average temperature of 10^{o}C and mean annual precipitation of 1002 mm. The site has been used as a grassland sward for over twenty years with nutrient inputs (organic and inorganic) applied based on routine soil testing. The site has undulating topography with average slopes of 6.7% along the length of the site and 3.6% across the width. Overall, the site is moderately drained with a soil texture gradient of clay loam to sand silt loam, as classified by Brennan et al. (2012). Soil nutrient analysis for the field site was characterised by dividing the site into an upper, middle and lower section, and by taking three composite soil samples (n=20) to characterise each section separately. The soil nutrient status at these locations (Morgan's P (P_m), K, and magnesium (Mg)) was determined using Morgan's extractant (Morgan, 1941), and **is** presented in Table 1. Mehlich-3 P extractant was also used to determine P levels (Mehlich, 1984). Soil pH (n=3) was determined using a pH probe (Mettler-Toledo Inlab Routine) and a 2:1 ratio of deionised water to soil as determined previously in Brennan et al. (2012).

2.2 Micro-plot installation and characterisation

Thirty grassland micro-plots, each 0.9 m in length and 0.4 m in width (0.36 m^2), were isolated using continuous 2.2 m-long, 100 mm-wide rigid polythene plastic strips, which were pushed to a depth of 50 mm into the soil to isolate three sides of the plot. All the edges were sealed with clay to prevent infiltration along the strips into the ground. A 0.6-m polypropylene plastic runoff collection channel was fitted at the end of each plot (Fig. 1). Micro-plots were orientated with the longest dimension in the direction of the slope. Once installed, plots were left uncovered to allow natural rainfall to wash away any soil that had been disturbed during their construction.

For textural analysis, each micro-plot was tested at before start of experiment (t_0) for particle size distribution (% sand/silt/clay) using the hydrometer method (ASTM D422, 2002). Results of analyses are presented in Table 2. Soil nutrient status of each micro-plot was taken at t_0 and analysed for soil pH, Mehlich 3-P, P_m, K, Mg, water extractable P (WEP), organic matter (OM) and lime requirement (LR) (Table 2). In addition, composited soil samples were oven dried and grinded to 2mm before being sent to ALS Environmental Global, Co. Dublin, Ireland at t_0 for metal content (Cu, Ni, Pb, Zn, Cd, Cr) by Inductively Couples Plasma Optical Emission Spectrometry (ICP-OES) (MEWAM, 1992), following aqua-regia digestion (MEWAM, 1986) (Table 3). Soil nutrient and metal status analysis was also repeated immediately at the end of the experiment (t_{360}) (Tables 2 and 3). Background checks were performed on the soil microbial status (TC and FC) (Table 4) at t_0 and t_{360} by taking composite soil samples from the four corners outside the micro-plots (top left, top right, bottom left, bottom right). Total coliforms were tested in accordance with ISO 4832 (ISO, 2006) at both t_0 and FC were tested in accordance with ISO 16649-2 (ISO, 2001) at t_0 and ISO 4831 (ISO, 2006) at t_{360}.

2.3 Biosolids characterisation

Four types of biosolids were examined in this study: two types of AD sludge, one sourced from a WWTP in Ireland (ADIRE) and another used in an EU-funded FP7 project (END-O-SLUDG, 2014) (ADUK); TD and LS biosolids (Fig. 1). With the exception of ADUK, all biosolids were sourced from the same WWTP in Ireland. As the Irish WWTP only employed two methods to treat sludge (anaerobic digestion and thermal drying), an untreated, dewatered sewage sludge cake was also collected from the same WWTP, so that it could be manually lime treated. The treated sludge and the dewatered sludge cake were collected in sealed, 50 L-capacity plastic storage boxes and transported to Teagasc, Environment Research Centre, Johnstown Castle, Co Wexford, South East Ireland, where they were labelled and stored at 4^{o}C. In accordance with standard methods in Ireland (Fehily, Timoney and Co., 1999), the AD treatment process must have a retention period of at least 1 hr at 70°C or 2 hr at 55°C, the TD treatment process must result in a product of approximately 90% solids, and lime (calcium oxide (CaO) of 98% purity sourced from Clogrennan Lime Ltd) must be added, if necessary, to the raw dewatered sewage sludge to raise the pH to greater than 12 and to generate heat. The treated sludge samples (each at n=3) were tested by Brookside Laboratories Inc, Ohio, USA for: DM, total Kjeldahl nitrogen (TKN), nitrite (NO_2-N), NH_4-N, organic-N, total P (TP), P as phosphorus pentoxide (P_2O_5), K, K as potassium oxide (K_2O), pH, and metal content (Cu, Ni, Pb, Zn, Cd, Cr, Hg) (Blinc, 2000) (Table 5). Water extractable P was tested after Kleinman et al. (2007) (Table 5). In addition, the biosolids samples (each at n=3) were also tested for TC and FC immediately after collecting using the same methods as for soil (Table 4).

2.4. Slurry Characterisation

Dairy cattle slurry was collected from the dairy farm unit at the Teagasc, Environmental research centre, Johnstown Castle. Cattle slurry was collected from a large underground slurry tank (25 long × 4.8 wide × 2.9 m deep), which had been filled with slurry in the previous 4 months. Prior to sampling, the tank was fully agitated (using a mechanical tractor-mounted agitator) to mix and homogenise the slurry. Following this, the slurry sample was collected by dropping a bucket, attached to a rope, into the tank and retrieving the sample. This slurry was placed into a sealed 25 L container, which was kept refrigerated (4°C). Prior to application, the slurry in the container was thoroughly mixed to suspend any solids that may have settled during the short-term storage.

Slurry pH was determined using a pH probe and a 2:1 ratio of deionised water to soil (Table 5). The DCS (each at n=3) were tested for (Southern Scientific Ireland, Co. Kerry, Ireland): DM, N (Kjeldahl, 1883), P and K and metal content (Cu, Ni, Pb, Zn, Cd and Cr) (Table 5). In addition, the DCS samples (each at n=3) were also tested for TC and FC immediately after collection using the same methods as for soil (Table 4).

2.4 Rainfall event simulation and application

One Amsterdam drip-type rainfall simulator, as described by Bowyer-Bower et al. (1989), was used to provide rainfall in this study. It was designed to form droplets with a median diameter of 2.3 mm, spaced 30 mm apart in a 1000 mm × 500 mm × 8 mm Perspex plate over a 0.5 m^2 simulator area. The simulator was calibrated to deliver a rainfall intensity of 11 mm hr^{-1}. Water samples, used in the rainfall simulations, were collected over the duration of the three rainfall events, and had average concentrations of: 0.07±0.0 mg NH_4-N L^{-1}, 3.81 ±0.02 mg NO_3-N L^{-1}, 3.80±0.02 mg total oxidised nitrogen (TON) L^{-1}, 0.01±0.00 mg dissolved reactive phosphorus (DRP) L^{-1}, 0.02±0.0 mg TP L^{-1}, 0.30±0.09 µg Cd L^{-1}, 0.38±0.07 µg Cr L^{-1}, 10.10±0.75 µg Cu L^{-1}, 0.65±0.46 µg Ni L^{-1}, 0.93±1.25 µg Pb L^{-1}, 78.91±6.67 µg Zn L^{-1}, 11.04±1.05 µg aluminium (Al) L^{-1}, 0.00±0.00 µg iron (Fe) $L^{-1}$and 9.95±0.05 µg manganese (Mn) L^{-1}.

The six treatments (four biosolids, DCS and one soil-only study control) used in this study were assigned to 30 micro-plots by dividing the plots in five blocks (five 'blocks' each containing six micro-plots). As metal content was not limiting in soil, DCS or biosolids application to the micro-plots was governed by the P content of the biosolids, and DCS and the P index of the soil. For comparable results, all micro-plots were classified into Index 2 P soil, which meant that all biosolids and DCS treatments were applied to all plots at a rate of 40 kg P ha^{-1} (Coulter and Lalor, 2008). As a result of the P content and the DM of each individual biosolid, application rates per individual plot was of 96.6 g of TD, 242.2 g of ADIRE, 1063.3 g of LS, 243.9 g of ADUK biosolids were applied to each designated plot. The DCS was spread at 2880 g per individual plot.

Prior to application, grass on all plots was cut to 50 mm, 48 hr before the first rainfall simulation (RS1). For better control of rainfall simulations and to prevent runoff losses caused by natural rainfall events, individual micro-plots were covered from the time of grass cutting to the end of the last rainfall event by 'rainout' shelters (Fig. 1f) (Hoekstra et al., 2014). Biosolids were hand surface applied to each micro-plot. To ensure even distribution, each micro-plot was divided into four quadrants (each 0.09 m^2 in area) and a proportionate amount of biosolids was applied in each quadrant (Fig. 1e). The DCS was applied in rows using a watering can to replicate normal trailing shoe application. The biosolids and DCS were then left 24 hr with the soil before RS1. The RS1 event occurred 24 hr after biosolids and DCS application, so as to demonstrate losses representative of a worst-case scenario. The second rainfall event (RS2) was two days (48 hr) after initial biosolids/DCS application, which was representative of current legislation, and the third (RS3) 15 days (360 hr) after initial application.

Volumetric water content of the soil in each plot (n=3) was measured immediately prior to each rainfall event using a time domain reflectrometry device (Delta-T Devices Ltd., Cambridge, UK), which was calibrated to measure resistivity in the upper 50 mm of the soil in each plot. Prior to each rainfall event, collection channels from the micro-plots were also rinsed with boiling hot water to sterilise them.

2.5 Runoff sample collection

Surface runoff was judged to occur once 50 mL of water was collected from the runoff collection channel from the start of simulated rainfall to runoff. The collection of the first 50 mL (t=0) was used to indicate time to runoff (TR), and was used for part of the microbial analysis. Samples for nutrient and metal analysis were collected every 10 min (t=10, T=20, T=30) from TR to allow for the flow weighted mean concentration (FWMC) to be calculated (Brennan et al., 2012). After this time, another 50 ml of surface runoff water was collected for microbial analysis, so that it could be bulked with the first 50 ml of runoff to create a 100 ml sample for microbial analysis. The rainfall simulator was then switched off and a final sample was collected to determine the final runoff ratio. This sample was also analysed for nutrient and metal content. Immediately after collection, all samples were stored in cool boxes with ice until they were returned to the laboratory for analysis.

2.6 Nutrient and metal runoff analysis

Runoff water samples were filtered through 0.45 µm filters (Sarstedt - Filtropur S 0.45) and a sub-sample was analysed calorimetrically for DRP, NO_3-N, NO_2-N and NH_4-N using a nutrient analyser (Aquachem Labmedics Analytics, Thermo Clinical Labsystems, Finland). A second filtered sub-sample was analysed for total dissolved phosphorus (TDP) using acid persulphate. Unfiltered runoff water samples were analysed for TP with an acid persulphate digestion and total reactive phosphorus (TRP) using the Aquachem Analyser. Metal analysis was tested on the filtered samples using inductively coupled plasma optical emission spectroscopy (ICP-OES). Particulate phosphorus (PP) was calculated by subtracting TDP from TP. The DRP was subtracted from the TDP to give the dissolved un-reactive phosphorus (DUP). All samples were tested in accordance with the Standard Methods (APHA, 2005).

2.7 Total and faecal coliform analysis

Samples (2 × 50 ml aliquots) of runoff water were collected at the start and towards the end of rainfall simulation experiments, and were stored in cool boxes filled with ice until they were returned to the laboratory for analysis. The time interval between the first collection and analysis was always less than 9 hr, with samples maintained at 4°C. Samples were appropriately diluted using sterile water from a Millipore automatic sanitization module, and 100-ml aliquots were apportioned for analysis in accordance with standard methods (APHA, 2005). Total and faecal coliforms were enumerated using the IDEXX Coilisure Quanti Tray/2000 method (IDEXX Laboratories, Westbrook, ME) after incubation at 37±0.5°C for 24 hr. Results were expressed as the Most Probable Number (MPN) of TC and FC per 100 ml.

2.8 Data analysis

The structure of the data set was a blocked one-way classification (treatments) with repeated measures over time (rainfall events (RS1– RS3)). The analysis was conducted using Proc Mixed in SAS software (SAS, 2013) with the inclusion of a covariance model to estimate the correlation between rainfall events. A large number of covariates were recorded, including measurements on the simulators and for each analysis; this set of covariates was screened for any effects that should be included in an analysis of covariance. The interpretation was conducted as a treatment by time factorial. Comparisons between means were made with compensation for multiple testing effects using the Tukey adjustment to p-values. Significant interactions were interpreted using simple effects before making mean comparisons. For comparison of soil characteristics before and after the experiment, the relationship between the paired measurements, adjusted for treatment, was tested and, given a significant relationship, the difference between each pair of results was analysed by treatment. In some cases an intercept-only model was fitted to determine if there had been an overall change across all treatments. Residual checks were made in all cases to ensure that the assumptions of the analyses were met.

3. Results

3.1 Nutrient losses in runoff

The average FWMC of TP, comprising DUP, PP and DRP, for all treatments and rainfall events is shown in Fig. 2. The application of TD and ADIRE biosolids and DCS increased the average FWMC of DRP in RS1 and RS2 compared to the study control, but this highly mobile P fraction was low for the other biosolids treatments. The highest median FWMC of DRP in the biosolids treatments (0.86 mg L^{-1}) was measured during RS1 for TD-amended plots, and this decreased significantly (p=0.02) over subsequent rainfall events to 0.14 mg L^{-1} for RS3. In comparison, the median FWMC of DRP from the ADIRE treatment was highest for RS2 (0.78 mg L^{-1}), although results for the three events were similar. However, losses for DRP from biosolids treatments were low compared to the DCS. Dissolved reactive phosphorus loss for DCS during RS1 was 7.0 mg L^{-1} and remained higher than any of the biosolids treatment losses during all simulation events.

Losses of PP were detected across all treatments, including the study control. Particulate P comprised >45% of TP losses for ADUK, ADIRE and LS biosolids, and the study control. Particulate P losses comprised only 14% and 32 % of TD biosolids and DCS, respectively, due to the high proportion of DRP losses. However, when only considering the PP losses, DCS plots for RS1 and RS2 had higher PP losses ($p < 0.05$) than all other measurements, which were statistically indistinguishable.

The average FWMC of TN across all treatments is shown in Fig. 2. There was a significant interaction between treatment and the rainfall simulation for NH_4-N. The application of all biosolids treatments and DCS increased the average FWMC of NH_4-N for RS1 compared to the study control, and while there was a downward trend between RS1 and RS3 for all treatments except the control, the decrease was not significant for LS. The ADUK-amended plots had the highest FWMC of surface runoff of NH_4-N for all biosolids treatments in RS1 (15.3 mg L^{-1}). Thermally dried and ADIRE treatments had the next highest FWMCs of NH_4-N, but these were not significantly different from each other or from the LS runoff during RS1. While total losses from DCS were greatest, they were significantly different only from LS (p=.005) and the control (p<0.001). The median FWMC of NH_4-N in RS1 for DCS was 17.4 mg L^{-1}. The addition of biosolids and DCS had no effect on FWMCs of NO_3-N in runoff, except for LS biosolids, which significantly reduced, relative to the control, the incidental losses of NO_3-N during RS1 and RS2 ($p<0.001$), before it increased during RS3. Nitrite losses were negligible in all treatments, with only exception being the DCS.

3.2 Metal losses in runoff

The average FWMC of metals (Cu, Ni, Pb, Zn, Cd, Cr) in runoff **is** shown in Fig. 3. All runoff samples were below their respective drinking water standards intended for human consumption (S.I. No. 122 of 2014). There was no difference in the FWMCs in surface runoff of Cd and Cr of any treatment compared to the study control, except for DCS. Cadmium losses for DCS during RS1 were significantly lower than other treatments, but were significantly higher during RS3. For Cu, the LS-amended plots had significantly higher FWMCs than all other treatments ($p<0.001$), with the highest median concentration of 202 µg L^{-1} measured during RS1. There was a decreasing trend in Ni concentrations across all treatments from RS1 to RS3, except for the study control, but there were no significant differences within treatments. All Ni concentrations were elevated compared to control. The highest median FWMC for Pb (1.5 µg L^{-1}) was measured during RS3 for the DCS and the second highest was 0.82 µg L^{-1} during RS1 for TD-amended plots. However, there was no significant difference between the treatments and the study control. The highest median FWMC of Zn (30.8 µg L^{-1}) was during RS1 for DCS-amended plots, but there were no significant differences across treatments or events.

3.4 Microbial losses in runoff (Total and faecal coliform)

The average losses of TC and FC are shown in Fig. 4. The ADUK-amended plots produced runoff with the lowest number of TC (averaged over the three rainfall simulations), but produced the highest average number of FC: 7.1×10^3 MPN per 100 ml during RS1 and RS2. For TC losses there was an interaction between treatment and event ($p=0.01$), but only the highest and lowest event outcomes were significantly different. While median losses from the

TD-amended plots increased with successive rainfall events from 1.9×10^5 MPN per 100 ml during RS1 to 1.0×10^6 MPN per 100 ml during RS3, there were no significant differences within treatments. There was no evidence of interaction between treatment and event for TC, so it is impossible make inference about the factors separately. There was no change from RS1 to RS2, but there was a decrease from RS2 to RS3 ($p<0.0001$) from a median of 7.6×10^1 MPN per 100 ml during RS1 to 5.4×10^1 MPN per 100 ml during RS3. Overall losses from DCS (3.1×10^2 MPN) were greatest and significantly greater than LS, ADIRE and the control. ADUK losses (1.7×10^2 MPN) were not statistically different from DCS, but were significantly greater than the control ($p=0.009$). The highest median count of TC and FC measured in LS biosolids-amended plots was 5.6×10^5 and 1.5×10^1 MPN per 100 ml, respectively. The highest median loss of TC for DCS-amended plots was 1.5×10^5 MPN per 100 ml.

3.5 Soil test P, Mehlich-3 P, K, LR, pH and metal

Morgan's P, Mehlich-3 P, WEP, Mg, K, pH, LR and metals results from analysis of plots before (t_0) and at the end of the experiment (t_{360}) are presented in Tables 2 and 3. Average P_m (3.6 to 4.8 mg L^{-1}), Mehlich-3 P (38.0 to 47.4 mg L^{-1}), K (58.2 to 94.94 mg L^{-1}), LR (2.3 to 2.6 t ha^{-1}) and pH (5.90 to 5.99) across all plots before application of treatments were similar. At the end of the experiment, P_m increased across all treatments ($p<0.0001$), with no significant differences between treatments. The Pm of the control plots also increased by 18%. Mehlich-3 P decreased across all treatments ($p=0.0001$), with no significant differences between treatments. Potassium concentrations showed no significant decrease for LS and TD treatments, while the greatest reduction was in the ADUK plots (35%) and the lowest in the lime-amended plots (10%). Magnesium showed no significant changes over the duration of the experiment. Lime requirement increased in the ADUK, TD, control plots and ADIRE by 11%, 10% 8% and 3.8%, respectively, but reduced by 56% in the lime-amended plots.

Average metal results across all treatments before the start of the experiment were similar (Table 3). At the end of the experiment, Cd and Cr ($p<0.0001$) increased across all treatments, while Cu showed a significant decrease only for TD. Lead ($p=<0.0001$) and Ni ($p<0.0001$) increased across all treatments, but there were no significant differences between treatments. The average increase for Pb was 50.8% and was 27.6% for Ni. Zinc decreased ($p<0.0001$) across all treatments, but there was no difference between treatments.

4. Discussion

4.1 Incidental nutrient losses for all rainfall events

With the exception of LS biosolids, FWMCs of TP and DRP across all treatments were significantly higher than the study control and, in some cases, were in breach of maximum admissible concentrations (MACs) for surface water. The volumetric water content of all study micro-plots was approximately 40% and the runoff ratio (the volume of runoff as a percentage of the volume of water applied to each micro-plot) was broadly similar across treatments (data not shown). Therefore, the nutrient load from each micro-plot was proportional to the FWMCs.

The FWMCs of TP and TN generally decreased across successive rainfall events. This trend is similar to several studies that have examined runoff of nutrients resulting from the land application of different types of biosolids and DCS (Rostagno and Sosebee, 2001; Penn and Sims, 2002; Ojeda et al., 2006; Eldridge et al., 2009; Lucid et al., 2014). The DRP losses measured in the current study were proportional to the WEP of the biosolids. Several studies have shown that WEP is an effective quantitative indicator of dissolved P losses from surface applied biosolids (Kleinman et al., 2002; Elliot et al., 2005; Kleinman et al., 2007). Thermally dried and ADIRE biosolids, which also had high WEPs (Table 5), had the highest losses of dissolved P from their respective plots.

All biosolids treatments had elevated FWMCs of NH_4-N in runoff compared to the study control across all rainfall simulations, whereas the study control and biosolids-amended plots had the same NO_3-N concentrations. Ammonium can be volatilised (or rapidly mobilised by runoff and leaching) after organic matter spreading (Quilbé et al., 2005). ADUK biosolids, which had the highest initial NH_4-N concentration in the biosolids at the time of application (3846 mg kg^{-1} DM; Table 5), also had the highest FWMC of NH_4-N in runoff compared to biosolids treatments during RS1. Similar trends were noted for the ADIRE and LS biosolids. However, the initial concentration of NH_4-N in TD biosolids before application (573 mg kg^{-1}; Table 5) was lower than the ADIRE biosolids (3428 mg kg^{-1}; Table 5), but had similar losses of NH_4-N in surface runoff during RS1. These types of anomalies may be due to the consistency of the biosolids, which means that different types of biosolids will have varying surface area exposure to rainfall. Therefore, TD biosolids could possibly be easier diluted and transported in the runoff compared to the ADIRE, ADUK and LS biosolids, due to their finer particle granulated consistency. This is also the reason for the high proportion of runoff measured for the DCS. Dairy cattle slurry had the highest FWMC of NH_4-N and DRP. A possible reason for this is that DCS had a DM of 8%, and was highly mobile following an episodic rainfall event. This study shows that biosolids, although having a higher DM than DCS, are not as easily mobilised.

4.2 Incidental metal losses for all rainfall events

The concentrations of metals in runoff were below drinking water standards intended for human consumption (S.I. No. 122 of 2014). Similar results have been reported for several runoff studies using different types of biosolids at higher application rates than the current study (Joshua et al., 1998; Dowdy et al., 1991; Eldridge et al., 2009; Lucid et al., 2013). This shows that the codes of good practice for the use of biosolids in agriculture (Fehily Timoney and Company, 1999) are appropriate in limiting metal application and, therefore, losses to waterbodies. The metal content in the biosolids was not the limiting factor in terms of runoff for the spreading rate, and the soil metal content was also below maximum permissible guidelines (Fehily Timoney and Company, 1999). The soil pH and clay content were within the recommended guidelines set out in code of good practices (Fehily Timoney and Company, 1999).

While there was generally low FWMC of metals over all rainfall simulations, the LS biosolids-amended plots released the highest quantity of Cu, Ni and Zn compared to other plots. One possible explanation for this is that Cu, Ni and Zn are more soluble metals (Joshua et al., 1998), and as LS biosolids consists of larger sized particles of a more compact consistency, time to runoff increased (results not shown), giving these metals more contact time to dissolve and subsequently be released compared to the other biosolids treatments. The pH adjustment and temperature increase, resulting from the LS treatment, reduced the biological activity within the biosolid material, affecting both N mineralisation and nitrification. This reduced the NO_3-N concentration initially after application (RS1 and RS2). Copper is more likely to be complexed as pH increases; however, under these circumstances Cu is likely to be complexed with soluble organic matter. Following rainfall and transport of dissolved organic matter-Cu complexes, high concentrations of Cu were transported in surface runoff from the LS treatment compared to others.
Metal concentration was low in DCS in comparison to the biosolids (Table 5) before application. However, the FWMC of Cd and Cr in DCS-amended plots were higher than any of the biosolids plots, with peak concentrations of 1.68 μg L^{-1} during RS3 for Cd and 3.89 μg L^{-1} during RS1 for Cr, respectively. However, even at these concentrations, they were still well below drinking water standards.

4.3 Incidental pathogen losses for all rainfall events

When biosolids and DCS are incorporated into the soil, pathogen survival is affected by factors such as pH, OM, soil texture, temperature, moisture content, and competition with other microorganisms (Lang et al., 2007). These factors have been reviewed by Erickson et al. (2014). However, when biosolids and DCS are surface applied, as in the current study, desiccation and ultraviolet light are the key factors in the decay of pathogens (Lu et al., 2012). Desiccation of pathogens is influenced by the soil, biosolids and DCS moisture content. In the current study, soil moisture remained consent at approximately 40%, which was unlikely to affect pathogen survival or regrowth. However, as the rainfall simulator provided moisture to the biosolids, there may have been regrowth of the FC in the ADIRE and LS biosolids between RS1 and RS2. Similar FC regrowth in AD biosolids was also reported by Zaleski et al. (2005). All TC and FC in biosolids decayed by RS3, which was most likely due to desiccation of pathogens rather than the influence of UV, as all plots were covered by the rainout shelter, which prevented natural rainfall between RS2 and RS3.

ADUK biosolids had significantly higher concentrations of FC in runoff during RS1 and RS2 compared to other treatments. At the start of the experiment, the ADUK biosolids were above the recommended standards of $>1 \times 10^3$ MPN g^{-1} (Fehily Timoney and Company., 1999), and, as a result, were equivalent to Class B microbial matter under the US EPA Part 503 regulations (USEPA, 1993), which allows detectible levels of FC up to 2×10^6 MPN g^{-1} DS. All the Irish biosolids were some 10-fold below the Class A Irish standard (Table 4). Dairy cattle slurry had high FC losses compared to the Irish biosolids, suggesting that pathogen losses to surface water bodies following land application of untreated organic fertiliser may be a concern in Ireland. This may be particularly important, given that incidence of shiga-toxigenic *E. coli* infection (STEC) in Ireland is amongst the highest in Europe and that waterborne transmission from cattle (zoonotic source) to humans is considered to play an important role in human infection in rural areas.

It is important to evaluate the risks arising from the application of biosolids to land relative to other common agricultural practices such as the land application of animal waste (Vinten et al., 2010), which is commonly spread as an organic fertiliser. Hubbs (2002) reported that land application of DCS as a fertiliser had FC concentrations in surface runoff of up to 1.2×10^5 CFU per 100 ml, two days after application, and after five rainfall events over 30 days, the mean FC concentrations in runoff, although decreasing, remained at high levels compared to the biosolids in the same study (4.0×10^3 CFU per 100 ml). This was also observed in the current study, as the DCS had the second highest FC during RS1 and RS2, but was the highest by RS3, showing that FC survive for a longer period in DCS compared to biosolids, and may result in losses of pathogen to waterbodies for a longer period following application. Moreover, Payment et al. (2001) found that the pathogen concentration was lower in untreated sludge (3×10^2 to 6×10^2 cfu g^{-1}) compared to fresh and stored cattle slurries (2.6×10^8 to 7.5×10^4 cfu g^{-1}) (Hutchison et al., 2004). When considered within this context, the risk of infectious diseases arising from the land application of biosolids appears to be low in magnitude. This study also provided no buffering capacity to the runoff samples, and overland flow was not sampled at delivery end of the transfer continuum, so the bacterial results represent a worst case scenario.

While this study and many others focus on the TC group as an indicator of the presence of pathogens, the drawback of relying on them is that it they are a poor indicator for the presence of viruses and parasitic protozoa, which may survive for much longer periods (NHMRC, 2003). However, due to the lack of well-developed methods for the detection and enumeration of these pathogens (Sidhu et al., 2009), the use of indicator organisms allows for the limitation of potential contaminating effects.

4.4 Soil characteristics before and after experiment.

In the current study, differences in soil nutrient concentration following amendments were observed. The application of all biosolids increased the P_m in all amended plots from an Index 2 soil to an Index 3. Whilst the P_m of the control plots also increased from an Index 2 soil to an Index 3 soil, the increase was less than half the increase of the nearest biosolids amendment (ADIRE). Lime stabilised biosolids had the greatest increase in P_m, and this may have been a result of the evaluated pH in the soil, as liming improves the availability of soil P. This result also shows that although LS biosolids are low in nutrient content, they can be applied for their pH adjusting characteristics and, as a result, may enhance nutrient availability to soil and plants.

This study also investigated the accumulation of metals before and after the experiment. Results showed that while there was an increase for some metals, none exceed the recommended guideline limits for soil set out in code of good practices (Fehily Timoney and Company, 1999). It should be noted, however, that the current study encompassed a single application of biosolids, and that concerns have been raised about the accumulation of metals in both soil and crops after repeated applications of biosolids (McBride, 2003; Bai et al., 2010). However, in Ireland, the application rate of biosolids to land is governed by legislation and whilst best practice is followed, problems in terms of metal or nutrient build-up will be avoided.

5. Conclusions

The results of this plot-scale study showed that there were elevated losses of nutrients (nitrogen and phosphorus), faecal coliforms and some metals (Cu, Ni, Pb, Zn) from biosolids-amended plots compared to unamended plots. However, surface runoff concentrations of nutrients, metals (with the exception of Cu and Ni), total coliforms (from both types of anaerobically digested biosolids used in this study) and faecal coliforms (from thermally dried, lime stabilised and biosolids originating from a WWTP in Ireland) were lower than the concentrations in surface runoff from plots treated with dairy cattle slurry. This means that in these respects, biosolids do not pose a greater risk than dairy cattle slurry in terms of surface runoff losses following land application. This study did not examine the surface runoff for the presence of emerging contaminants, such as pharmaceuticals, personal care products, micro-plastics, or nanomaterials. While the findings of this study suggest that surface runoff of nutrients, metals and microbial matter for biosolids and dairy cattle slurry are comparable, the surface runoff water from the biosolids-amended micro-plots of the current study must be tested for these, and other, emerging contaminants.

Acknowledgements

The authors acknowledge funding from the EPA (Project reference number 2012-EH-MS-13). They are also grateful to the End-o-sludg project (http://www.end-o-sludg.eu/) for providing samples.

References

Apedaile, E. "A perspective on biosolids management." The Canadian journal of infectious diseases= Journal canadien des maladies infectieuses 12.4 (2001): 202-204.

APHA. Standard methods for the examination of water and wastewater. American Public Health Association (APHA). Washington: APHA; 2005.

ASTM D422-63, Standard Test Method for Particle-Size Analysis of Soils, ASTM International, West Conshohocken, PA, 2002

Bai, Y., Chen, W., Chang, A. C., & Page, A. L. "Uptake of metals by food plants grown on soils 10 years after biosolids application." Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes 45, no. 6 (2010): 531-539.

Blinc.,. Biosolid (Sludge) Analysis, Brookside Laboratories INC; 2000. Available online at: http://www.blinc.com/worksheet_pdf/biosolidanalysis.pdf.

Bowyer-Bower, T. A. S., & Burt, T. P.. "Rainfall simulators for investigating soil response to rainfall." Soil Technology 2, no. 1 (1989): 1-16.

Brennan, R. B., Healy, M. G., Grant, J., Ibrahim, T. G., & Fenton, O. "Incidental phosphorus and nitrogen loss from grassland plots receiving chemically amended dairy cattle slurry." Science of the Total Environment 441 (2012): 132-140.

Coulter BS, Lalor S. “Major and micro nutrient advice for productive agricultural crops”. Third edition. Castle Wexford: Teagasc Johnstown; 2008):116 pp.

Dowdy, R. H., Page, A. L., & Chang, A. C. Management of agricultural land receiving wastewater sludges: Soil Management for Sustainability. Soil and Water Conservation Society, Ankeny, Iowa. (1991). p 85-101. 5 fig, 10 tab, 54 ref.

Dunigan, E. P., Dick, R. P. "Nutrient and coliform losses in runoff from fertilized and sewage sludge-treated soil." Journal of Environmental quality 9, no. 2 (1980): 243-250.

EC., European Commission, Environment, Waste, Studies, Sewage Sludge. (2014). Available at: http://ec.europa.eu/environment/waste/sludge/.

EC., European Commission. Environmental, economic and social impacts of the use of sewage sludge on land. Final Report Part I: Overview Report: (2010). Available at:: http://ec.europa.eu/environment/archives/waste/sludge/pdf/part_i_report.pdf.

EC., European Commission. Council Directive 2009/28/EC of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. (2009) Available at:: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32009L0028.

EC., European Commission. Council Directive 2008/98/EC of 19 November 2008 on waste (Waste Framework Directive). (2008). Available online at: http://eur-lex.europa.eu/legal content/EN/TXT/?uri=CELEX:32008L0098.

EC., European Commission. Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste. (1999). Available at: http://www.central2013.eu/fileadmin/user_upload/Downloads/Document_Centre/OP_Resources/Landfill_Directive_1999_31_EC.pdf.

EEC., European Economic Community. Council Directive 91/271/EEC of 21 May 1991 concerning urban waste water treatment. (1991). Available at: http://www.igemportal.org/Resim/Council%20Directive%20Concerning%20Urban%20Wastewater%20Treatment.pdf.

Eldridge, S. M., Chan, K. Y., Barchia, I., Pengelly, P. K., Katupitiya, S., & Davis, J. M."A comparison of surface applied granulated biosolids and poultry litter in terms of risk to runoff water quality on turf farms in Western Sydney, Australia." Agriculture, ecosystems & environment 134, no. 3 (2009): 243-250.

Elliott, H. A., Brandt, R. C., & O'Connor, G. A. "Runoff phosphorus losses from surface-applied biosolids." Journal of environmental quality 34, no. 5 (2005): 1632-1639.

Erickson, M. C., Habteselassie, M. Y., Liao, J., Webb, C. C., Mantripragada, V., Davey, L. E., & Doyle, M. P. "Examination of factors for use as potential predictors of human enteric pathogen survival in soil." Journal of applied microbiology 116, no. 2 (2014): 335-349.

END-O-SLUDG, (2014). Available at: http://www.end-o-sludg.eu/.

EUROSTAT. EC, European Commission. Sewage sludge production and disposal. (2014). Available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=env_ww_spd&lang=en.

FAO., Food and Agriculture Organization. Global agriculture towards 2050., from Food and Agriculture Organization of the United Nations. (2009). Available at: http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf.

Fehily Timoney and Company.. Codes of good practice for the use of biosolids in agriculture – guidelines for farmers. (1999). Available at: http://www.environ.ie/en/Publications/Environment/Water/FileDownLoad,17228,en.pdf

Hall, J. Ecological and economical balance for sludge management options. Technology and innovative options related to sludge management. (2000). Available at: http://ec.europa.eu/environment/archives/waste/sludge/pdf/workshoppart4.pdf.

Haynes, R. J., Murtaza, G., & Naidu, R. "Inorganic and organic constituents and contaminants of biosolids: implications for land application." Advances in agronomy 104 (2009): 165-267.

Healy, M.G., Clarke, R., Peyton, D., Cummins, E., Moynihan, E.L., Martins, A., Beraud, P., Fenton, O., 2015. Resource Recovery from sludge. In (K. Konstantinos, Ed.): Sewage treatment plants: economic evaluation of innovative technologies for energy efficiency. IWA, London.

Heffer, P., Prud'homme, M., "Fertilizer outlook 2013–2017," in Proceedings of the 81st International Fertilizer Industry Association Conference, p. 8, Chicago, Ill, USA, May 2013, Paper No.: A/13/78. (2013). Available at: http://www.fertilizer.org/.

Hoekstra, N. J., Finn, J. A., Hofer, D., & Lüscher, A., "The effect of drought and interspecific interactions on depth of water uptake in deep-and shallow-rooting grassland species as determined by δ 18 O natural abundance."Biogeosciences 11, no. 16 (2014): 4493-4506.

Hubbs, A. K. B. Fecal coliform concentration in surface runoff from pastures with applied dairy manure (Doctoral dissertation, Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in Biological and Agricultural Engineering in The Department of Biological and Agricultural Engineering by Alyson Kristine Bertges Hubbs BS, Louisiana State University). (2002).

Hutchison, M. L., Walters, L. D., Avery, S. M., Synge, B. A., & Moore, A. "Levels of zoonotic agents in British livestock manures." Letters in Applied Microbiology 39, no. 2 (2004): 207-214.

IDEXX Laboratories. Colilert brochure. IDEXX Laboratories, Carlsbad, CA. (2010).

ISO., International Standards Organisation. ISO 4831. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - MPN Technique. (2006)

ISO., International Standards Organisation ISO 4832. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms – Colony-count technique (2006)

ISO., International Standards Organisation. ISO 16649-2. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli. Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. (2001)

Iranpour, R., & Cox, H. H. "Recurrence of fecal coliforms and Salmonella species in biosolids following thermophilic anaerobic digestion." Water environment research (2006): 1005-1012.

Joshua, W. D., Michalk, D. L., Curtis, I. H., Salt, M., & Osborne, G. J. "The potential for contamination of soil and surface waters from sewage sludge (biosolids) in a sheep grazing study, Australia." Geoderma 84, no. 1 (1998): 135-156.

Kleinman PJA, Sullivan D, Wolf A, Brandt R, Dou Z, Elliott H, et al.. "Selection of a water-extractable phosphorus test for manures and biosolids as an indicator of runoff loss potential." Journal of environmental quality 36, no. 5 (2007): 1357-1367.

Kleinman, P. J., Sharpley, A. N., Wolf, A. M., Beegle, D. B., & Moore, P. A. "Measuring water-extractable phosphorus in manure as an indicator of phosphorus in runoff." Soil Science Society of America Journal 66, no. 6 (2002): 2009-2015.

Kjeldahl, J. "A new method for the estimation of nitrogen in organic compounds." Z. Anal. Chem 22, no. 1 (1883): 366.

Lang, N. L., Smith, S. R., Bellett-Travers, D. M., Pike, E. B., & Rowlands, C. L. "Decay of Escherichia coli in soil following the application of biosolids to agricultural land." Water and Environment Journal 17, no. 1 (2003): 23-28.

Lang, N. L., Bellett-Travers, M. D., & Smith, S. R. "Field investigations on the survival of Escherichia coli and presence of other enteric micro-organisms in biosolids-amended agricultural soil." Journal of applied microbiology 103, no. 5 (2007): 1868-1882.

Lu, Q., He, Z. L., & Stoffella, P. J.. "Land application of biosolids in the USA: A review." Applied and Environmental Soil Science 2012 (2012).

Lucid, J. D., Fenton, O., Grant, J., & Healy, M. G. "Effect of Rainfall Time Interval on Runoff Losses of Biosolids and Meat and Bone Meal when Applied to a Grassland Soil." Water, Air, & Soil Pollution 225, no. 8 (2014): 1-11.

Lucid, J. D., Fenton, O., & Healy, M. G., "Estimation of maximum biosolids and meat and bone meal application to a low P index soil and a method to test for nutrient and metal losses." Water, Air, & Soil Pollution 224, no. 4 (2013): 1-12.

Ojeda, G., Tarrasón, D., Ortiz, O., & Alcaniz, J. M. "Nitrogen losses in runoff waters from a loamy soil treated with sewage sludge." Agriculture, Ecosystems & Environment 117, no. 1 (2006): 49-56.

Payment P, Plante R, Cejka P. "Removal of indicator bacteria, human enteric viruses, Giardia cysts, and Cryptosporidium oocysts at a large wastewater primary treatment facility." Canadian Journal of Microbiology 47, no. 3 (2001): 188-193.

Peters, G. M., & Rowley, H. V. "Environmental comparison of biosolids management systems using life cycle assessment." Environmental science & technology 43, no. 8 (2009): 2674-2679..

Penn, C. J., & Sims, J. T. "Phosphorus forms in biosolids-amended soils and losses in runoff." Journal of Environmental Quality 31, no. 4 (2002): 1349-1361.

SAS., Statistical Analysis System. SAS for windows.Version 9.4.SAS/STAT® User's Guide. Cary, NC: SAS Institute Inc.;

Statutory instrument. (2014). S.I. No. 122 of 2014, European Union (drinking water) Regulations 2014 Arrangement of Regulations. The Stationary Office. Available online at: http://www.irishstatutebook.ie/pdf/2014/en.si.2014.0122.pdf.

Sidhu, J. P., & Toze, S. G. "Human pathogens and their indicators in biosolids: a literature review." Environment International 35, no. 1 (2009): 187-201.

Singh, R. P., & Agrawal, M. "Potential benefits and risks of land application of sewage sludge." Waste management 28, no. 2 (2008): 347-358

McBride, M. B. "Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks?." Advances in Environmental Research 8, no. 1 (2003): 5-19.

McDonald, N., & Wall, D. Soil specific N advice – utilising our soil nitrogen resources. National Agri-environment Conference 2011 10 November 2011, Athlone. Available at: http://www.teagasc.ie/publications/2011/1050/Agrienvironment_Proceedings.pdf. (2011).

Mehlich, A. "Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant." Communications in Soil Science & Plant Analysis 15, no. 12 (1984): 1409-1416.

MEWAM,. Methods for the Examination of Waters and Associated Materials. “Methods for the determination of metals in soils, sediments and sewage sludge and plants by hydrochloric - nitric acid digestion”. HMSO. London. ISBN 0 11 751908 1 (1986)

MEWAM., Methods for the Examination of Waters and Associated Materials. “Information on concentration and determination procedures in atomic spectrophotometry”. HMSO . London. ISBN 0 11 752375 5. (1992).

Morgan MF., (1941). “Chemical soil diagnosis by the Universal Soil Testing System”. Connecticut agricultural Experimental Station Bulletin 450 Connecticut. New Haven. Connecticut. (1941).

Nelson, N.B., & Choi, C. Microbial Quality Analysis of Water Runoff for Biosolid Applied Fields in Southern Arizona. Available at: http://wsp.arizona.edu/sites/wsp.arizona.edu/files/Nelson.pdf (2005).

NHMRC. (2003). National Health and Medical Research Council, Australian Government. Available at: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/eh32.pdf. (2003).

Quilbe, R., Serreau, C., Wicherek, S., Bernard, C., Thomas, Y., & Oudinet, J. P. "Nutrient transfer by runoff from sewage sludge amended soil under simulated rainfall." Environmental monitoring and assessment 100, no. 1-3 (2005): 177-190.

Robinson, K. G., Robinson, C. H., Raup, L. A., & Markum, T. R. "Public attitudes and risk perception toward land application of biosolids within the south-eastern United States." Journal of environmental management 98 (2012): 29.

Rostagno, C. M., & Sosebee, R. E., "Biosolids application in the Chihuahuan Desert." Journal of environmental quality 30, no. 1 (2001): 160-170.

USEPA., US Environmental Protection Agency. Biosolids Technology Fact Sheet. Multi-Stage Anaerobic Digestion. Available at: http://water.epa.gov/scitech/wastetech/upload/2006_10_16_mtb_multi-stage.pdf. (2006a).

USEPA., US Environmental Protection Agency. Biosolids Technology Fact Sheet. Heat Drying. Available at: http://water.epa.gov/scitech/wastetech/upload/2006_10_16_mtb_heat-drying.pdf. (2006b).

USEPA., US Environmental Protection Agency Biosolids Technology Fact Sheet. Use of Composting for Biosolid Management. Available at: http://water.epa.gov/scitech/wastetech/upload/2002_10_15_mtb_combioman.pdf. (2002)

USEPA., US Environmental Protection Agency Biosolids Technology Fact Sheet. Alkaline Stabilization of Biosolids. Available at: http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_alkaline_stabilization.pdf. (2000).

USEPA., US Environmental Protection Agency 40 CFR Part 503. Standards for use or disposal of sewage sludge; final rules. Federal Reg 1993; 58(32): 9248. (1993).

Vinten A.J.A., Douglas J.T., Lewis D.R., Aitken M.N & Fenlon D.R. "Relative risk of surface water pollution by E. coli derived from faeces of grazing animals compared to slurry application." Soil Use and Management 20, no. 1 (2004): 13-22.

Wall, D., Jordan, P., Melland, A. R., Mellander, P. E., Buckley, C., Reaney, S. M., & Shortle, G. "Using the nutrient transfer continuum concept to evaluate the European Union Nitrates Directive National Action Programme." Environmental Science & Policy 14, no. 6 (2011): 664-674.

Wallace, C. B., Burton, M. G., Hefner, S. G., & DeWitt, T. A."Sediment, Nutrient, and Bacterial Runoff from Biosolids and Mineral Fertilizer Applied to a Mixed Cool-and Native Warm-Season Grassland in the Ozark Mountains." Applied and Environmental Soil Science 2014 (2014).

WEF., Water Environment Federation (2005). National Manual of Good Practice for Biosolids. Available at: http://www.wef.org/Biosolids/page.aspx?id=7767.

Zaleski, K. J., Josephson, K. L., Gerba, C. P., & Pepper, I. L. "Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil." Applied and environmental microbiology 71, no. 7 (2005): 3701-3708.

Captions for Figures

Fig. 1. A) ADUK, B) TD, C)LS, D)ADIRE, E) Plot dimensions with application quadrant F) Rainout shelter

Fig. 2. Flow weighted mean concentrations of phosphorus (top) and nitrogen (bottom) in the runoff over three successive rainfall events at 24 hr (RS1), 48 hr (RS2) and 360 hr (RS3) after application to grassland.

Fig. 3. Flow weighted mean concentrations of cadmium (A), chromium (B), copper (C), nickel (D), lead (E), zinc (F), aluminium (G) and iron (H) in the runoff over three successive rainfall events at 24 hr (RS1), 48 hr (RS2) and 360 hr (RS3) after application to grassland.

Fig. 4. Total coliforms (top) and faecal coliforms (bottom) in the runoff per 100ml over three successive rainfall events at 24 hrs (RS1), 48 hrs (RS2) and 360 hrs (RS3) after application to grassland.

Captions for Tables

Table 1. Soil characteristics from the upper, middle and lower section of the 0.6 ha field site.

Table 2. Average topographical and soil characteristics for the 25 individual micro-plots pooled together as per treatment applied, on the day before experiment (t_0) and immediately after the experiment ended (t_{360})

Table 3. Average soil metals concentration of copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr) before start of experiment (t_0) and after the experiment (t_{360})

Table 4. Average nutrient and metal characteristics of the biosolids (±standard deviation) before start of experiment (t_0)

Table 5. The average total and faecal coliforms (±std. dev.) for soil and biosolids on the day before experiment (t_0) and after the experiment (t_{360})

Table 1. Soil characteristics from the upper, middle and lower section of the 0.6 ha field site.

Position	pH	Morgan P	Mehlich 3-P	WEP	P index	K[a]	Mg[a]	LR[a]	Sand[b]	Silt[b]	Clay[b]	Textural[c] class
		mg L^{-1}	mg L^{-1}	mg kg^{-1}		mg L^{-1}	mg L^{-1}	t ha^{-1}	%	%	%	
UPPER	5.6	2.3	36.1	6.8	1.0	128.9	133.0	4.0	44%	36%	21%	Clay Loam
MIDDLE	5.4	2.3	35.3	5.6	1.0	70.5	108.8	5.5	47%	36%	18%	Sandy Silt Loam
LOWER	5.5	2.6	25.9	9.0	1.0	121.6	137.0	5.0	52%	30%	18%	Sandy Loam
AVERAGE	5.5	2.4	32.6	7.1	1.0	107.0	126.3	4.8	47.7	34	19	
STD. DEV	0.1	0.2	4.6	1.4	0.0	26.0	12.5	0.6	4	3.5	1.7	

[a]Morgan's extractable potassium (K) and magnesium (Mg), lime requirement (LR)

[b]Brennan et al. (2012)

[c]USDA classification system

Table 2. Average topographical and soil characteristics for the 25 individual micro-plots pooled together as per treatment applied, on the day before experiment (t_0) and immediately after the experiment ended (t_{360}).

Treatment	Slope	pH_0/pH_{360}	WEP_0/WEP_{360}	Morgan's P_0/P_{360}	Mehlich 3-P_0/P_{360}	K_0/K_{360}[a]	Mg_0/Mg_{360}[a]	LR_0/LR_{360}[a]	OM_0/OM_{360}[a]	Sand[b]	Silt[b]	Clay[b]	Textural class[C]
	%		**mg kg^{-1}**	**mg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**	**t/ha**	**%**	**%**	**%**	**%**	
ADUK	2.89	5.94/5.90	7.10/5.9	3.60/5.57	38.0/37.1	94.94/60.78	147.13/147.80	2.70/3.00	8.1/	45.70	39.49	14.82	Loam
TD	3.69	5.90/5.90	9.25/7.5	4.80/6.79	47.4/41.9	66.08/55.66	156.75/164.00	2.30/2.70	9.0/	47.41	37.63	14.97	Loam
LS	2.84	5.90/6.25	6.60/5.4	3.82/6.24	38.3/32.7	58.20/52.12	136.47/146.40	2.60/1.00	8.1/	48.74	36.58	14.69	Loam
ADIRE	2.87	5.96/5.93	7.7/6.1	4.32/6.11	41.4/35.7	78.39/55.74	152.68/147.40	2.40/2.70	8.1/	48.17	36.55	15.28	Loam
SOIL	**3.53**	**5.99/5.96**	**8.6/6.9**	**4.71/5.59**	**46.8/39.2**	**65.95/54.30**	**149.49/149.60**	**2.80/2.90**	**8.8/**	**45.52**	**39.43**	**15.05**	**Loam**
DCS	**2.73**	**5.81/6.10**	**2.86/1.63**	**5.00/9.13**	**31.93/-**	**62.40/208.42**	**84.20/167.17**	**3.30/1.60**	**8.3/**	**50.00**	**29.20**	**20.80**	**-**

[a] Morgan's extractable potassium (K) and magnesium (Mg), lime requirement (LR) and Organic Matter (OM)

[b]ASTM D422 (2002).

[c]USDA classification system

Table 3. Average soil metals concentration of copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr) before start of experiment (t_0) and after the experiment (t_{360}).

Treatment	Cd_0/Cd_{360}	Cr_0/Cr_{360}	Cu_0/Cu_{360}	Pb_0/Pb_{360}	$Ni_0/_{360}$	Zn_0/Zn_{360}
	----------mg kg^{-1}----------					
ADUK	<0.20/0.54	11.8/13.8	8.12/6.74	15.5/27.2	7.14/9	35.2/29.8
TD	<0.2/0.56	11.5/14.4	9.54/7.8	16.12/25	6.86/9.42	33.2/31.2
LS	<0.2/0.54	11.6/13.8	7.8/7.4	15/22	7.2/8.96	34.6/27.6
ADIRE	<0.2/0.54	12/14.4	8.42/7.34	16/21.8	7.66/9.4	36/30
SOIL	<0.2/0.56	11.8/14.4	8.62/7.16	17.22/24.4	7.28/9.34	35.2/31.2

Table 4. The average total and faecal coliforms (±std. dev.) for soil and biosolids on the day before experiment (t_0) and after the experiment (t_{360})

MICROBE	ADUK	TD	LS	ADIRE	SLURRY	SOIL
Presumptive Coliforms (cfu g^{-1}) (t_0)	$<1.0 \times 10^7$	$<1.0 \times 10^7$	$<1.0 \times 10^7$	$<1.0 \times 10^7$	5.43×10^4 (6.34×10^3)	<1.0 × 107
ß-Glucuronidase + E. coli (cfu g^{-1}) <100 (t_0)	6.5×10^3 (3.6×10^3)	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$	1.10×10^3	$<1.0 \times 10^2$
Total coliform (Product) (t_{360})	7.4×10^2 (4.5×10^2)	6.3×10^1 ($4.5 \times 10^{1)}$	1.3×10^1 (4.7×10^0)	5.0×10^1 ($5.0 \times 10^{0)}$	-	1.3×10^3 (6.9×10^2)
Faecal Coliforms (MPN) (t_{360})	1.7×10^1 (2.1×10^1)	1.9×10^0 (1.7×10^0)	$<3.0 \times 10^{-1}$ (0)	2.3×10^0 (0)	-	7.7×10^0 (4.9×10^0)

Table 5. Average nutrient and metal characteristics of the biosolids (±standard deviation) before start of experiment (t_0)

Treatment	DM	Total N	Total P	Total K	pH	WEP (dry)	OM	Cu	Ni	Pb	Zn	Cd	Cr	Hg	NO_3-N	NH_4-N	Organic - N	P_2O_5[a]	K_2O[b]
	%	--------------------mg kg^{-1}--------------------				g kg^{-1}	%	----------mg kg^{-1}----------											
ADUK	25	43216	23512	2146	8	16		287	140	115	683	2	31	0.0	3979	3847	39370	53876	2585
	(0.1)	(1671)	(274)	(40)	(0.0)	(8)		(4)	(2)	(1)	(3)	(0)	(1)	(0)	(14)	(294)	(1962)	(628)	(48)
LS	34	17621	3939	2230	13	9	28	111.7	12	11	219	0.4	8.1	0	2922	449	17171	9138	2686
	(0.2)	(396)	(396)	(44)	(0.0)	(0.3)	(1)	(11)	(0.3)	(1)	(20)	(0)	(0.3)	(0)	(13)	(29)	(395)	(790)	(52)
TD	87	51446	17114	2055	7	493	80	505	19.6	63	877	1.0	22	0.4	1148	573	50873	39216	2476
	(0.1)	(2897)	(187)	(51)	(0)	(26)	(2)	(19)	(2)	(1)	(6)	(0)	(0.1)	(1)	(1)	(32)	(2876)	(428)	(61)
ADIRE	24	54578	25186	2199	8	302	72	756	26.3	91.6	1110	2	32	0	4235	3428	51150	57711	2649
	(0.2)	(1530)	(609)	(78)	(0)	(1)	(1)	(21)	(1)	(3)	(22)	0	(2)	0	(38)	(240)	(1776)	(1395)	(95)
DCS	8	2	1	4	8	93		3.9	0.44	<0.25	14	<0.2	1						
	(0.2)	(0.2)	(0)	(0.4)	(0)	(3)		0	(0.3)	0	(0.2)	0	(1)						

[a] P_2O_5 - Phosphorus pentoxide

[b] K_2O - Potassium oxide

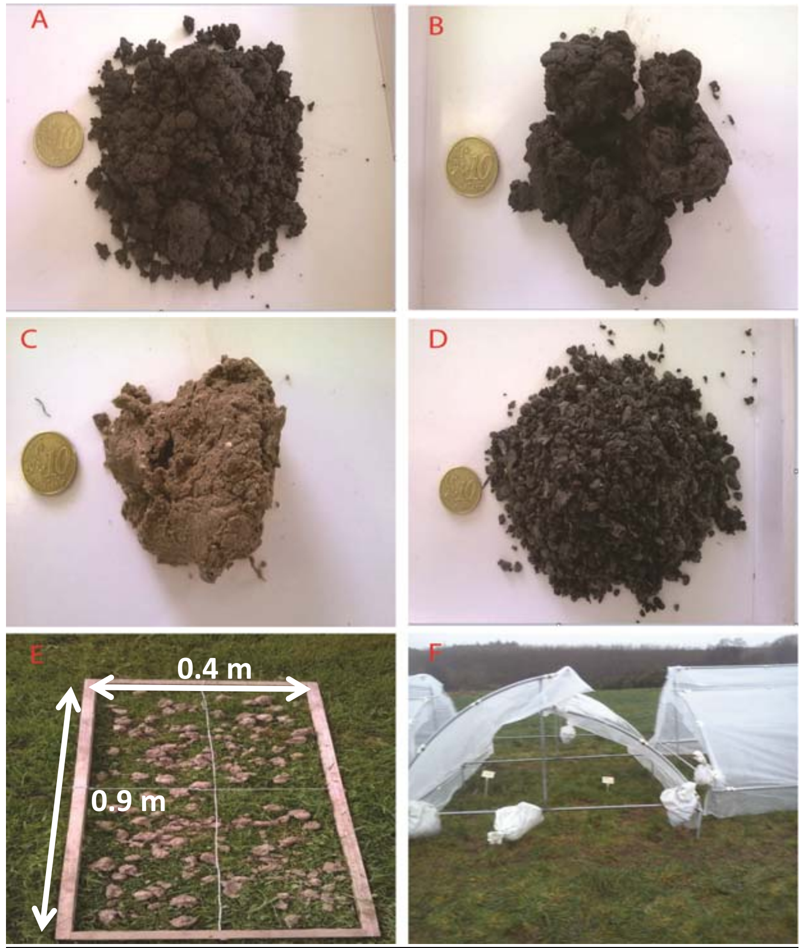

Fig. 1. A) ADUK biosolids; B); ADIRE biosolids, C) LS biosolids; D) TD biosolids; E) Plot dimensions with application quadrant; F) Rainout shelter.

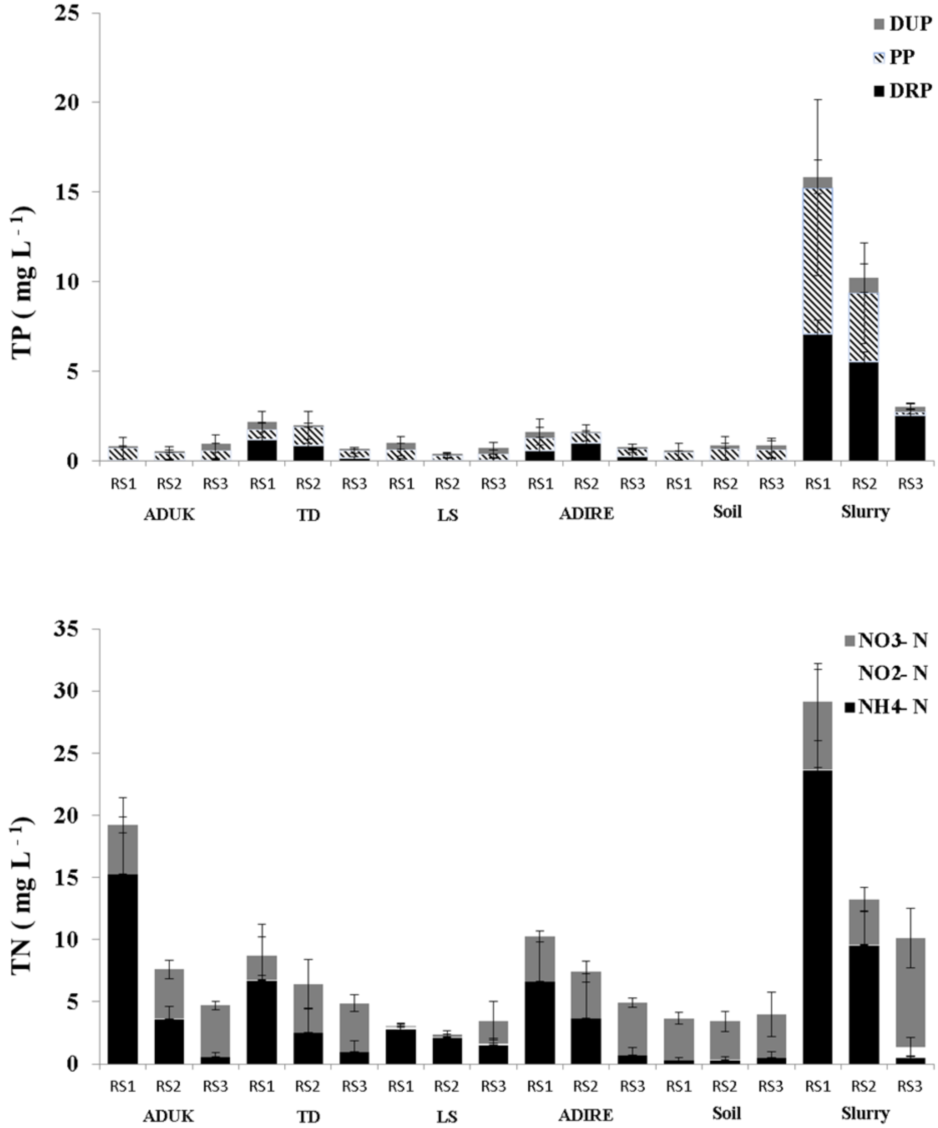

Fig. 2. Flow weighted mean concentrations of phosphorus (top) and nitrogen (bottom) in the runoff over three successive rainfall events at 24 hr (RS1), 48 hr (RS2) and 360 hr (RS3) after application to grassland. (std dev error bars)

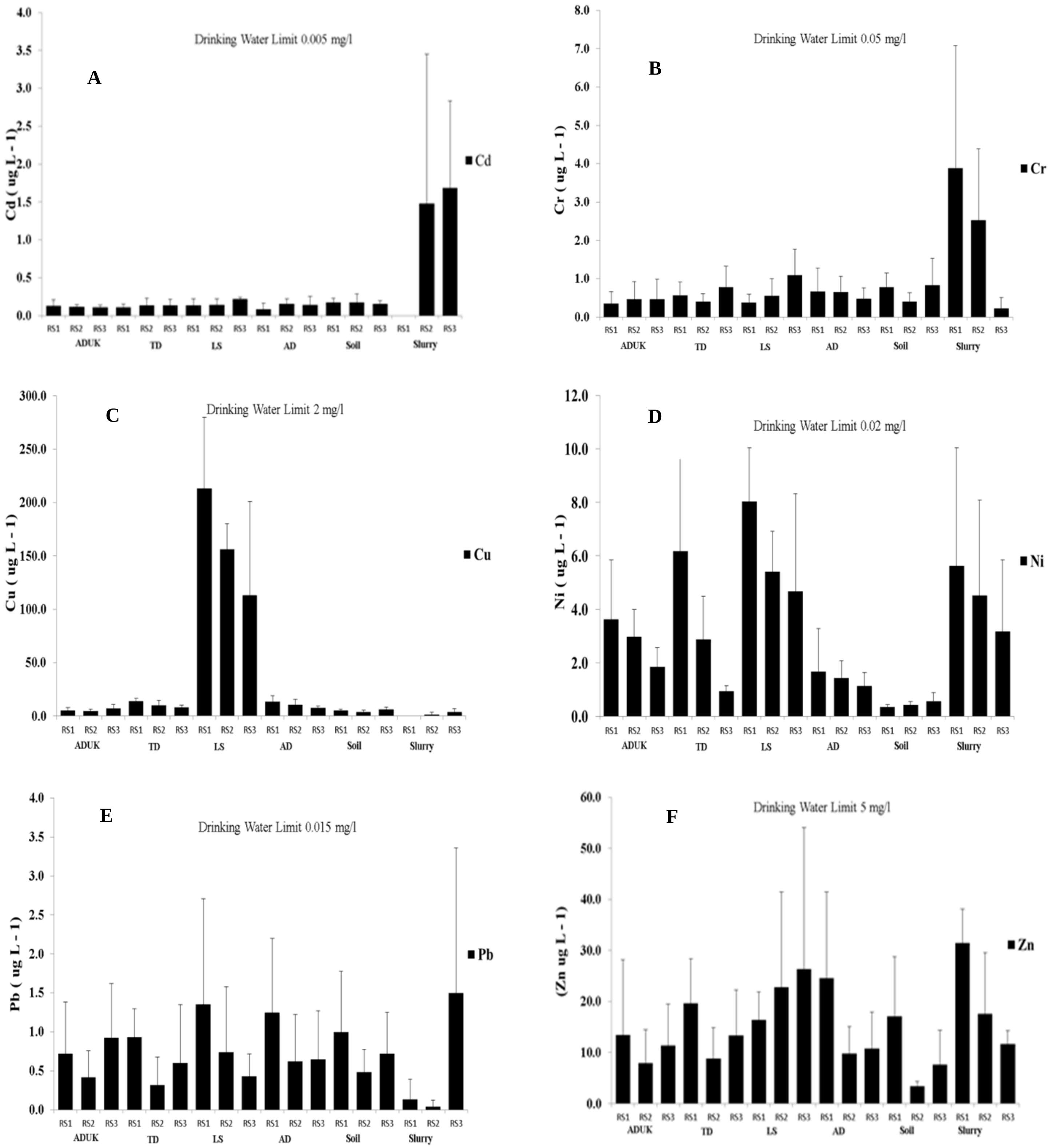

Fig. 3. Flow weighted mean concentrations of cadmium (A), chromium (B), copper (C), nickel (D), lead (E), zinc (F), in the runoff over three successive rainfall events at 24 hr (RS1), 48 hr (RS2) and 360 hr (RS3) after application to grassland. (std dev error bars)

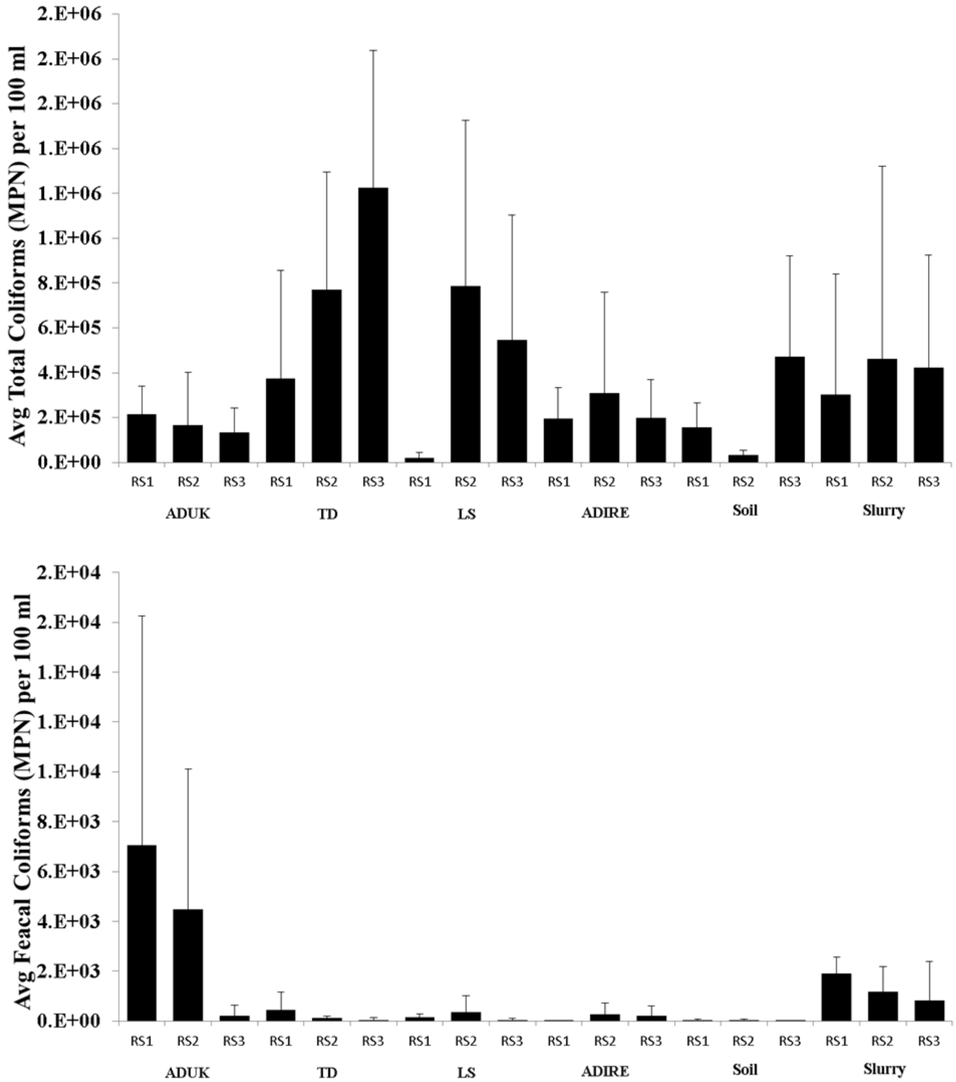

Fig. 4. Total coliforms (top) and faecal coliforms (bottom) in the runoff per 100ml over three successive rainfall events at 24 hrs (RS1), 48 hrs (RS2) and 360 hrs (RS3) after application to grassland (std dev error bars)