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Bioaccumulation of metals in ryegrass (*Lolium perenne* L.) following the application of lime stabilised, thermally dried and anaerobically digested sewage sludge

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Abstract

The uptake and accumulation of metals in plants is a potential pathway for the transfer of environmental contaminants in the food chain, and poses potential health and environmental risks. In light of increased population growth and urbanisation, the safe disposal of sewage sludge, which can contain significant levels of toxic contaminants, remains an environmental challenge globally. The aims of this experiment were to apply municipal sludge, having undergone treatment by thermal drying, anaerobic digestion, and lime stabilisation, to permanent grassland in order to assess the bioaccumulation of metals (B, Al, Ti, V, Cr, Mn,

Co, Ni, Cu, Zn, As, Nb, Mo, Sb, Ba, W, Pb, Fe, Cd) by perennial ryegrass over a period of up to 18 weeks after application. The legislation currently prohibits use of grassland for fodder or grazing for at least three weeks after application of treated sewage sludge (biosolids). Five treatments were used: thermally dried (TD), anaerobically digested (AD) and lime stabilised (LS) sludge all from one wastewater treatment plant (WWTP), AD sludge from another WWTP, and a study control (grassland only, without application of biosolids). In general, there was no significant difference in metal content of the ryegrass between micro-plots that received treated municipal sludge and the control over the study duration. The metal content of the ryegrass was below the levels at which phytotoxicity occurs and below the maximum levels specified for animal feeds.

Keywords: Treated sludge; biosolids; ryegrass; metals; land application.

1. Introduction

In the European Union (EU), production of untreated municipal sewage sludge (MSS) was approximately 10 million tonnes in 2010 (Eurostat, 2014), and is estimated to rise to 13 million tonnes by 2020 (EC, 2010). Numerous directives, such as the Landfill Directive 1999/31/EC (EC, 1999) and the Waste Framework Directive 2008/98/EC (EC, 2008), amongst others, have meant that MSS is now increasingly recognised as a potential beneficial and valuable resource (Jiang et al., 2011). One of the more common ways to reuse MSS is as an agricultural organic fertiliser, but this may only be used subsequent to appropriate treatment such as composting, anaerobic digestion, lime stabilisation, or thermal drying (Koutroubas et al., 2014; Healy et al., 2015), after which it is commonly referred to as 'biosolids'. Nonetheless, there are still concerns surrounding the use of biosolids as a

fertiliser by food producers and consumers (Torri et al., 2014), and on account of this, its use as such is prohibited in many countries (Milieu et al., 2013a,b,c). These concerns are mainly based on public perception, but there is conflicting evidence with respect to the land application of biosolids and the levels of contamination of soil and water following rainfall (e.g., Cornu et al., 2001; Peyton et al., 2016). There has been particular concern over the bioaccumulation of metals in the food chain (Latare et al., 2014), which could create a conflict for their use in an industry that promotes sustainable products to an international market. While numerous studies have examined issues surrounding surface runoff water quality following biosolids application to land (Ojeda et al., 2006; Quilbé et al. 2005; Eldridge et al 2009; Lucid et al., 2014; Peyton et al., 2016) and the subsequent uptake of metals by plants (Dijkshoorn et al., 1981; Smith, 1994; Sukkariyah et al., 2005; Antoniadis et al., 2008; Gu et al., 2013; Bermanee et al., 2016), to our knowledge no study has examined the relative impact of the land application of common types of biosolids (anaerobically digested (AD), lime stabilised (LS), thermally dried (TD)), applied at the same rate and at the same time, on metal bioaccumulation by *Lolium perenne* L. (ryegrass), a common grass species used globally for pasture and forage, at field scale. As different types of MSS treatments are used prior to land application, such a study may inform policy on land spreading of MSS, and on appropriate crop harvesting and livestock grazing exclusion times.

Various elements, such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), sulphur (S), boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), cobalt (Co), silicon (Si) and sodium (Na), are essential micro- and macro-nutrients required by plants for growth. Metal bioavailability and uptake by plants is affected by contamination levels and several soil properties and factors: pH, organic matter and clay content (Hettiarachchi et al., 2003),

element speciation in the soil, absorption of the element onto the root, and translocation into the plant (McGrath and Fleming, 2006), and uptake may also be species specific (Basta et al., 2005; Deram et al., 2006). Alkaline soil conditions reduce metal bio-availability, but metal cations are more active under acid conditions, with increases of Mn, Zn, Cu, nickel (Ni) and cadmium (Cd) content in ryegrass being reported when soil pH is reduced (to around 4) following biosolids application (Dijkshoorn et al., 1981; Smith, 1994). In addition to modifying soil pH (Singh and Agrawal, 2010a,b), the rate at which biosolids is applied also potentially impacts metal uptake by ryegrass (Antoniadis et al., 2008) to a point at which plant phytotoxicity (effects on photosynthesis, mineral nutrition, respiration and gene expression, resulting in impaired growth and biomass yield) may be likely. Some elemental ranges for phytotoxicity of plants are listed in Table 1.

Land application of biosolids is governed by various regulations or instructions, such as EU Directive 86/278/EEC (EEC, 1986) in Europe and 40 CFR Part 503 (US EPA, 1993) in the US. Land spreading of biosolids is highly controlled in Europe, but in the US under the 503 rules, Class A 'EQ' sludges and sludge products (exceptional quality sludges that have undergone treatment to eliminate pathogens) can be applied without restriction in amount. In Europe, often these rules are enacted at a local level, with detailed guidelines governing the specifics of land application (e.g. 'Codes of Good Practice for the Use of Biosolids in Agriculture'; Fehily Timoney and Company (1999) in Ireland) published in separate documents. These guidelines frequently place constraints on crop harvesting and livestock grazing subsequent to land application of biosolids. For example, in Ireland no animal fodder, including kale, fodder beet or silage, may be harvested until at least three weeks after application of biosolids, and livestock should not be turned out onto pasture which has been fertilised with biosolids until three to six weeks after the date of application (Fehily Timoney

and Company, 1999). In contrast, the 503 regulations do not restrict grazing on lands to which Class A sludges have been applied, but allow grazing approximately four weeks after application of Class B sludges (sludges in which pathogens have been reduced but are still present).

The aims of this study were to examine (1) if biosolids (Class B sludges) application at the legal rate in Ireland (which is currently based on the metal content of biosolids and the available P content of the soil) to ryegrass will increase the metal content of the plant biomass (2) if the method used to generate the biosolids (anaerobic digestion, lime stabilisation, thermal drying) resulted in different metal bioavailability and subsequent uptake and bioaccumulation rates in ryegrass, and (3) if the metal content of the ryegrass, following the application of biosolids, will reduce as the ryegrass plants grow. To address these aims, biosolids were applied to ryegrass at the legal permissible rate in Ireland, and ryegrass samples were collected and tested for metal content at time intervals of up to 18 weeks from the time of application.

2. Materials and Methods

2.1 Study Site and instrumentation of micro-plots

The location of the study was a 0.6-ha plot located at Teagasc, Johnstown Castle Environment Research Centre, Co. Wexford, Ireland in the southeast of Ireland. The area has a cool maritime climate, with an average temperature of 10°C and mean annual precipitation of 1002 mm. The site has been used as a grassland sward planted with ryegrass for over twenty years with nutrient inputs (organic and inorganic) applied based on routine soil testing.

Twenty-five grassland micro-plots, each 0.9 m in length and 0.4 m in width (0.36 m²), 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 each micro-plot. A 0.6-m polypropylene plastic runoff collection channel was fitted at the end of each micro-plot to facilitate surface runoff. Micro-plots were orientated with the longest dimension in the direction of the slope. Once installed, micro-plots were left uncovered to allow natural rainfall to wash away any soil that had been disturbed during their construction.

2.2 Biosolids application to plots

Three types of biosolids were examined in this study: two types of AD sludge, one sourced from a wastewater treatment plant (WWTP) in Ireland (AD) and another used in an EU-funded FP7 project (END-O-SLUDG, 2014) (ADUK); TD and LS biosolids. With the exception of ADUK, all biosolids were sourced from the same WWTP in Ireland.

2.3 Rainfall event simulation and application

Legislation governing the application of any type of organic or inorganic waste in Ireland states that a period of 48 hr should elapse between the land application and the first rainfall event (SI 610 of 2010). As most organic, nutrient, metal and microbial matter is released from the soil surface in rainfall events close to the time of waste application, an Amsterdam drip-type rainfall simulator, similar to that described by Bowyer-Bower et al. (1989), was used in this experiment, so as the rainfall events, intensity and durations could be controlled for the first 360 hr after biosolids application (this time interval was to facilitate rainfall-surface runoff experiments that were being conducted at the same time as the study described

in this paper). The rainfall simulator was calibrated to deliver a rainfall intensity of 11 mm hr⁻¹.

The five treatments (four biosolids and one soil-only study control) used in this study were assigned to 25 micro-plots by dividing the plots into five blocks (five 'blocks' each containing five micro-plots on which each of the five treatments were examined). Biosolids application to the micro-plots commenced in January 2014 (winter time in Ireland). As surface runoff water samples, arising from controlled rainfall simulations using the rainfall simulator, were collected from each micro-plot as part of another study (Peyton et al., 2016), 51 days elapsed between the application of biosolids to the first micro-plot of the first experimental block and the final micro-plot of the final block (Figure S1). The biosolids were applied to each micro-plot at a rate of 40 kg P ha⁻¹ (0.04 tonne P ha⁻¹), and the rate of application was governed by the P content of the biosolids and the P concentration (agronomic soil P index) of the soil (soils tested using Morgan's extractable P, Morgan (1941)). As a result of the P content and the dry matter (DM) of each type of biosolid, application rates per individual plot were 96.6 g (2.6 tonnes DM ha⁻¹) for TD biosolids, 242.2 g (6.7 tonnes DM ha⁻¹) for AD biosolids, 1063.3 g (29.5 tonnes DM ha⁻¹) for LS biosolids and 243.9 g (6.8 tonnes DM ha⁻¹) for ADUK biosolids.

Prior to application, grass on all micro-plots was cut uniformly to 50 mm using a plastic hand shears, 48 hr before the first rainfall simulation. Biosolids were hand surface applied to each micro-plot and in order to ensure even distribution, each micro-plot was divided into four quadrants (each 0.09 m² in area) and a proportionate amount of biosolids was applied in each quadrant. The biosolids were then left 24 hr on the soil before the first rainfall simulation. The first rainfall simulation event occurred 24 hr after biosolids application, which was less

than the 48 hr legal limit, but allowed a worst-case surface runoff scenario to be evaluated. The second rainfall event was two days (48 hr) after initial biosolids application, which was representative of current legislation, and the third 15 days (360 hr) after initial application. Between these three rainfall applications, a 'rainout' shelter was used (a large, plastic shelter on a steel frame that protects the soil from direct rainfall, while allowing air to circulate over the soil surface; Hoekstra et al., 2014), but after 360 hr (the time of the third rainfall simulation), the micro-plots were exposed to normal weather conditions. The daily rainfall over the study duration is shown in Figure S1.

2.4 Collection of ryegrass samples

Based on the findings of a preliminary study designed to determine the minimum amount of grass blades required for metal testing (approximately 0.1 – 0.2 g dry weight), three ryegrass samples, each comprising a composite of six to eight blades or shoots of ryegrass, were cut at the soil surface in each of the 25 micro-plots immediately prior to the second (at 48 hr after application) and third (at 360 hr after application) rainfall simulations, and finally at a time varying between 55 and 130 days from the time of the application of biosolids. This variation was due to the staggered nature of the initial application of biosolids to the micro-plots and the fact that the final collection of grass blades occurred on the same day in June 2014. Nitrile gloves were used to do collect the grass samples, and were changed between plots to avoid cross-contamination. Each grass sample was thoroughly washed with ultra-pure water (18.3 mΩ, Milli-Q Element SystemTM, Merck Millipore, Cork, Ireland) to remove any particulate or adhered material, before being placed into separate, clean, sealed bags, frozen at -20°C and transported to the laboratory.

Plant roots absorb metals from the soils which can be transported to the shoots or foliar parts (Clemens, 2001). This study focused on ryegrass above-ground shoots to assess the impact of biosolids application, as these represent the plant biomass portion that is consumed by livestock and harvested for fodder crops. In addition, there are many analytical difficulties associated with the sampling and analysis of root tissue without avoiding contamination (Lynch, 1995).

2.5 Preparation and analysis of ryegrass samples

Ryegrass were freeze-dried at -52 °C (Freezone 12, Labconco, Kansas City, MO, USA) and approximately 0.1 g of sample was digested with an optimised microwave digestion procedure (Anton Paar Multiwave 3000, Graz, Austria) using 3 mL of trace metal grade 67-69% HNO₃ (*ROMIL-SpA*[™], USA) and 3 mL of 30% H₂O₂ (*TraceSELECT*[®] Ultra ≥30%, SIGMA-ALDRICH, USA) (Morrison et al, 2008). The digested samples were transferred into trace metal-free centrifuge tubes (Labcon, Petaluma, CA, USA). The determination of Cr, Cu, Pb, Ni, Zn, Cd, B, Co, Fe, Mn, Mo, Al, V, As, Nb, Sb, Ba, and W in the ryegrass digests were performed on a PerkinElmer ELAN DRCe (Perkin Elmer, Waltham, USA) inductively coupled plasma mass spectrometer (ICP-MS) using both standard and dynamic reaction cell (DRC) mode (Staunton, et al., 2014; Ratcliff, et al., 2016) in a class1000 (ISO class 6) clean room. A certified reference material (CRM) of ryegrass (ERM[®] – CD281 RYE GRASS, European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium) was used with method blanks and duplicate samples for method validation and assurance purposes.

2.6 Statistical analysis

The data were assessed using ANOVA. Minitab statistical software (Pennsylvania, USA) was used for the analysis, with a two-sided confidence interval level set at 95%. Variances were not assumed to be equal across treatments and control, and consequently the Welch's ANOVA test was utilised in the Minitab software. The same approach was used to test the statistical significance of the observed reduction in metal content with test program duration across all treatments and control.

3. Results and Discussion

Soil textural, pH, nutrient and metal analyses, immediately prior to the application of biosolids, are presented in Table 2. Although the changes in the soil characteristics were not measured over the study duration, soil attributes may have an impact on the metal content of the above-ground tissue. However, there are inconsistencies in the literature, with some studies finding good correlation between metal fractions in the soil and plant metal content (Torri and Lavado, 2009), and other studies finding no correlation (Maiz et al., 2000).

All biosolids used in this study are characterised in Table 3. The metal concentrations of the biosolids were all within EU regulatory limits (EEC, 1986), but Healy et al. (2016) noted that non-regulated metals, such as antimony (Sb) or tin (Sn) (results not reported in the current study), for which no intentional standards exist, may build up in a soil over time after repeated applications of biosolids.

The recovery of metals from the analysis of the certified reference material is shown on Table S1. The rainfall simulator water had average concentrations of $0.30 \pm 0.09 \mu\text{g Cd L}^{-1}$, $0.38 \pm 0.07 \mu\text{g Cr L}^{-1}$, $10.10 \pm 0.75 \mu\text{g Cu L}^{-1}$, $0.65 \pm 0.46 \mu\text{g Ni L}^{-1}$, $0.93 \pm 1.25 \mu\text{g lead (Pb)}$

L^{-1} , $78.91 \pm 6.67 \mu\text{g Zn } L^{-1}$, $11.04 \pm 1.05 \mu\text{g aluminium (Al) } L^{-1}$, $0.00 \pm 0.00 \mu\text{g Fe } L^{-1}$ and $9.95 \pm 0.05 \mu\text{g Mn } L^{-1}$.

The average legislated and unlegislated (in the Republic of Ireland) metal concentrations in the ryegrass for each treatment over the duration of the study are displayed in Figures 1, S2 and S3. For most metals examined, there was no statistically significant difference between the biosolid treatments and the study control. In this respect, the results obtained in this study were quite different to those of Antoniadis et al. (2008), who measured appreciable differences in Cd, Ni, Pb and Zn concentrations in ryegrass between AD biosolids-amended and unamended plots. However, in the current study, statistically significantly different concentrations between the treatment and the study control were detected for some metals: Co (ADUK biosolids; from 15 d onwards) and Ni (LS biosolids; at 15 d).

As the application rate of the biosolids to each micro-plot was determined by their P content, and not their metal content, application of metals was different between treatments.

Therefore, for example, the application rate of Cu was much higher for LS and AD biosolids-amended micro-plots (119 and 183 mg per micro-plot, respectively) than the other micro-plots (70 mg for ADUK-amended micro-plots and 49 mg for TD-amended micro-plots). Over the 18-week study duration of the current study and with the exception of Ni, Cu and Al, there was no statistically significant difference in metal uptake by the ryegrass between treatments.

In general, the element concentration of the ryegrass followed the temporal fluctuation of the study control. On the basis of these findings, biosolids application at the legal rate in Ireland (which is currently based on the metal content of biosolids and the available P content of the

soil) did not increase the metal content of the plant biomass and the method used to generate the biosolids (anaerobic digestion, lime stabilisation, thermal drying) did not result in different metal bioavailability and subsequent uptake and bioaccumulation rates in ryegrass. While the maximum average concentrations of As, Cr, Fe and V were at, or above, the ranges of trace elements in Irish pastures, no measured metals were within, or above, the concentration at which phytotoxicity of ryegrass occurs (Table 1). The metals were also below the maximum levels specified for animal feeds (EC, 2002; Table 1).

There was a statistically significant downward trend with time from around 15 d onwards in the concentration of all metals in all treatments, including the study control. Concentration of some metals such as As, Nb and Sb, reduced from low initial concentrations (average initial concentrations for each were 0.2, 0.07 and 0.01 mg kg⁻¹, respectively) to below the limits of the instrumental technique over the duration of the study, indicating the impact of metal dilution by shoot growth. There was a residual metal concentration in the ryegrass (prior to biosolid application) at the start of a growing season, but this became more dilute as the growing season progressed. When the metal uptake in the biosolids-amended micro-plots were taken away from the metal uptake in the control micro-plots at each of the time intervals sampled, the metal content of the shoots reduced from the time of first sampling (at 2 d) through all subsequent sampling times.

On the basis of this study, with respect to the parameters tested, it would seem that the existing legislation constraining crop harvesting and livestock grazing subsequent to land application of biosolids is overly conservative. In fact, in a larger study examining surface runoff of which the current study formed a part, Peyton et al. (2016) found that the greatest losses of nutrients and metals (with the exception of Ni and Cu) occurred from micro-plots

amended with dairy cattle slurry applied at the same P application rate to the biosolids. As the land application of biosolids may offer a sustainable, relatively cost-effective source of nutrients and micro-nutrients required for plant and crop growth (Jeng et al., 2006), improve soil physical and chemical characteristics (Mondini et al., 2008), and address EU legislation regarding the sustainable use of resources (COM, 2014), it is somewhat surprising that continued resistance to their reuse on land exists. However, the presence of other contaminants, such as micro-plastics (Magnusson and Norén, 2014), personal care products and pharmaceuticals (Clarke and Cummins, 2014), which may be present in biosolids, was not examined in this study, and this may prove decisive in their acceptance for use as a fertiliser. Further work examining the uptake of these parameters by ryegrass should take place before the existing legislation is altered.

4. Conclusions

This study found that, in general, there was no statistically significant difference in the shoot metal concentration of ryegrass cultivated on biosolids-amended plots and unamended plots, when the biosolids were spread at the maximum permissible rate in Ireland. Over a period of 18 weeks after land application of biosolids, a downward trend in the concentration of metals in the shoots of ryegrass was observed, which was attributed to a dilution effect as the ryegrass grew. On the basis of the parameters measured in this study, it would appear that the legislation governing livestock exclusion rates from land after biosolids application are overly strict. However, a short period of withdrawal (e.g. 3 weeks) seems reasonable to reduce the risk of biosolid ingestion by the animals (as would be the case with cattle slurries). Any further restriction may be overly strict for a single application to land at compliant application rates.

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Table 1. Ranges of trace elements in Irish pastures (mg kg^{-1}), recommended herbage concentrations to meet animal requirements, maximum concentrations for products intended for animal feed (2002/32/EC; EC, 2002), phytotoxicity of trace elements in ryegrass (adapted from McGrath and Fleming, 2006) and maximum average concentrations of trace elements measured in this study (mg kg^{-1} dry matter). Type of sewage sludge treatment (LS – lime stabilised; TD – thermally dried; AD – anaerobic digested) applied to micro-plots in brackets after metal content. Values in bold indicate elements in the current study that are at, or above, herbage content.

Element	Herbage concentration ^a	Animal requirement ^a	Maximum concentrations for products intended for animal feed ^b	Phytotoxicity of elements in ryegrass ^a	Maximum average concentration s measured in this study ^a
----- mg kg^{-1} dry matter -----					
Arsenic	0.05 – 0.3		4		0.3 (TD)
Boron	1 – 20	nil		>190	5.6 (AD)
Cadmium	0.01 – 0.3	nil	1	>100	0
Chromium	0.1 – 0.3			>100	1.3 (TD)
Cobalt	0.03 – 0.2	0.1 – 0.2		>100	0.5 (ADUK)
Copper	2 – 15	10 – 20		>20	10 (LS)
Iron	20 – 300	25 – 40			393
Lead	0.5 – 20	nil	30		1.1 (TD)
Manganese	20 – 300	25 – 300		>500	145 (LS)
Molybdenum	0.05 – 2				1.3 (LS)
Nickel	0.5 – 3			>80	2.1 (LS)
Vanadium	0.05 – 0.5				1.33 (TD)
Zinc	20 - 60	25 - 100		>400	57 (TD)

^a as dry matter

^b maximum content relative to a feeding stuff with a moisture content of 12%.

1 **Table 2.** Average topographical and soil characteristics (\pm standard deviation) for the 25 individual micro-plots pooled together as per treatment
 2 applied, on the day before experiment commenced (adapted from Peyton et al., 2016).

Treatment	Slope	pH	WEP ^a	Morgan's P	K ^b	Mg ^b	Cd	Cr	Cu	Pb	Ni	Zn	OM ^b	Sand ^c	Silt ^c	Clay ^c	Textural class ^d
	%		mg kg ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	%	%	%	%	
ADUK	2.9	5.9	7.1	3.6 (1.1)	94.9	147.1			8.1	15.5	7.1	35.2	8.1	45.7	39.5	14.8	Loam
		(0.1)	(1.3)		(27.1)	(25.9)	<0.2	11.8 (1.5)	(0.87)	(6.45)	(1.55)	(10.1)	(0.8)	(3.6)	(3.9)	(1.8)	
TD	3.7	5.9	9.3	4.8 (1.9)	66.1 (5.4)	156.8			9.5		6.9		9.0	47.4	37.6	14.9	Loam
		(0.1)	(3.8)			(26.80)	<0.2	11.5 (1.2)	(3.5)	16.1 (7.7)	(1.01)	33.2 (5.8)	(1.1)	(4.7)	(4.9)	(1.2)	
LS	2.8	5.9	6.6	3.8 (0.8)	58.2 (9.1)	136.5			7.8		7.2		8.1	48.7	36.6	14.7	Loam
		(0.1)	(1.4)			(27.1)	<0.2	11.6 (1.0)	(0.9)	15 (3.4)	(1.2)	34.6 (6.5)	(1.1)	(4.5)	(4.1)	(0.6)	
AD	2.9	5.9	7.7	4.3 (0.9)	78.4	152.7			8.4		7.7		8.1	48.2	36.6	15.3	Loam
		(0.1)	(2.3)		(14.0)	(42.3)	<0.2	12 (1.4)	(1.7)	16 (2.2)	(1.2)	36 (6.6)	(1.0)	(3.4)	(2.3)	(1.0)	
SOIL	3.5	6.0	8.6	4.7 (1.7)	65.9 (9.9)	149.5			8.6		7.3		8.8	45.5	39.4	15.1	Loam
		(0.2)	(2.3)			(26.91)	<0.2	11.8 (0.9)	(1.6)	17.2 (5.7)	(0.7)	35.2 (5.4)	(1.1)	(3.6)	(4.6)	(1.2)	

^a WEP, water extractable phosphorus.

^b Morgan's extractable potassium (K) and magnesium (Mg), lime requirement (LR) and Organic Matter (OM)

^c ASTM D422 (2002).

^d USDA classification system

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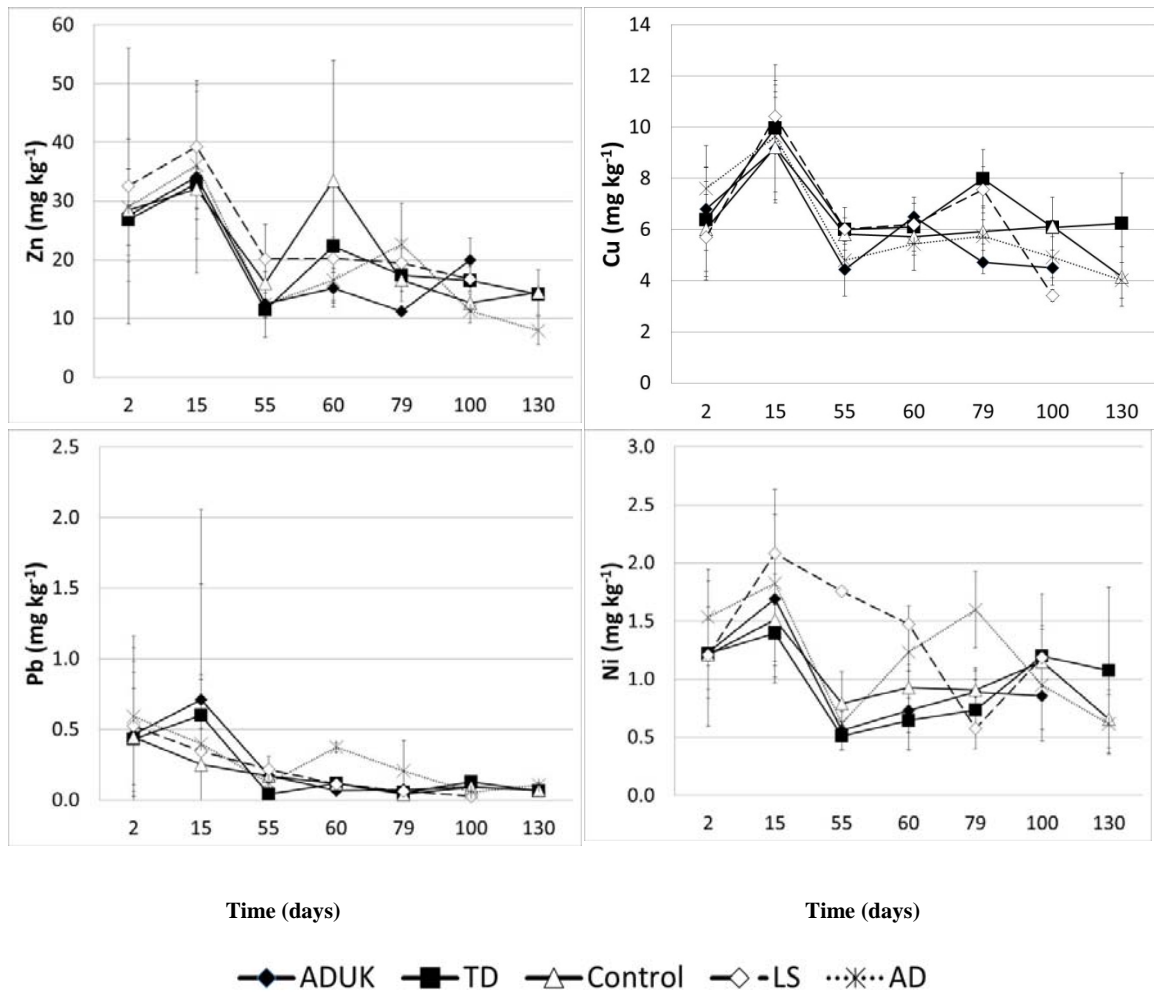
9 **Table 3.** Average nutrient and metal characteristics of the biosolids (\pm standard deviation) before start of experiment (adapted from Peyton et al.,
 10 2016).

Treatment	DM	Total N	Total P	Total K	pH	WEP	OM	Cu	Ni	Pb	Zn	Cd	Cr	Hg
	%	-----mg kg ⁻¹ -----				(dry)	%	-----mg kg ⁻¹ -----						
						g kg ⁻¹								
ADUK	25.1 (0.1)	43216.3 (1670.8)	23512.1 (273.9)	2145.8 (39.8)	7.8 (0.0)	15.5 (7.6)	-	287.0 (4.0)	140.2 (1.5)	115.3 (0.8)	682.8 (3.0)	1.84 (0.0)	31.46 (0.5)	0.0 (0.0)
LS	34.2 (0.2)	17620.5 (395.5)	3938.7 (396.1)	2229.5 (43.8)	12.6 (0.0)	8.9 (0.3)	28.4 (0.5)	111.7 (11.4)	12.2 (0.3)	10.7 (1.0)	218.5 (20.1)	0.4 (0.0)	8.1 (0.3)	0.0 (0.0)
TD	87.10 (0.07)	51446.0 (2897.3)	17114.4 (186.9)	2055.1 (50.7)	6.9 (0.0)	492.7 (25.6)	79.5 (2.0)	504.8 (18.8)	19.6 (2.0)	62.2 (0.6)	876.9 (5.5)	1.0 (0.0)	22.1 (0.06)	0.4 (0.5)
AD	23.6 (0.2)	54577.8 (1530.3)	25185.7 (609.0)	2198.7 (78.4)	8.1 (0.0)	302.2 (1.0)	72 (1.0)	756.4 (20.7)	26.3 (1.3)	91.6 (3.1)	1109.6 (21.6)	1.5 (0.0)	31.7 (1.9)	0 (0.0)

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Figure 1. Measured metal content of legislated metals in the Republic of Ireland (zinc (Zn), copper (Cu), lead (Pb), nickel (Ni)) in ryegrass up to 130 d after a single application of either anaerobically digested (AD) biosolids originating from the UK (ADUK) or Ireland (AD), lime stabilised (LS) or thermally dried (TD) biosolids. Cadmium (Cd) and mercury (Hg) are not displayed as they were below the limits of detection. Control refers to ryegrass that did not receive any biosolids application.