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Mobility and toxicity of heavy metal(loid)s arising from contaminated wood ash application to a pasture grassland soil☆

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ABSTRACT

Heavy metal(loid) rich ash (≤10,000 mg kg^{-1} total As, Cr, Cu and Zn) originating from the combustion of contaminated wood was subjected to several experimental procedures involving its incorporation into an upland pasture soil. Ash was added to soil that had been prior amended with local cattle manure, replicating practices employed at the farm scale. Metal(loid) concentrations were measured in soil pore water and ryegrass grown on soil/manure plus ash mixtures (0.1–3.0% vol. ash) in a pot experiment; toxicity evaluation was performed on the same pore water samples by means of a bacterial luminescence biosensor assay. Thereafter a sequential extraction procedure was carried out on selected soil, manure and ash mixtures to elucidate the geochemical association of ash derived metal(loid)s with soil constituents. Predictive modelling was applied to selected data from the pot experiment to determine the risk of transfer of As to meat and milk products in cattle grazing pasture amended with ash.

The inclusion of manure to soils receiving ash reduced phyto-toxicity and increased ryegrass biomass yields, compared to soil with ash, but without manure. Elevated As and Cu concentrations in pore water and ryegrass tissue resulting from ash additions were reduced furthest by the inclusion of manure due to an increase in their geochemical association with organic matter. Zinc was the only measured metal(loid) to remain uniformly soluble and bioavailable regardless of the addition of ash and manure. Risk modelling on pot experimental data highlighted that an ash addition of >1% (vol.) to this pasture soil could result in As concentrations in milk and meat products exceeding acceptable limits.

The results of this study therefore suggest that even singular low doses of ash applied to soil increase the risk of leaching of metal(loid)s and intensify the risk of As transfer in the food chain.

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1. Introduction

The majority of studies of wood ash application to land have been carried out on agricultural or forest soils, normally with the feedstock used to generate the ash coming from virgin timber (reviewed in Pitman, 2006). Agricultural soils display well-documented benefits to their properties after wood ash addition, including an increase in soil pH and nutrient status (Bougnom et al., 2009; Klemedtsson et al., 2010; Pérez-Cruzado et al., 2010; Arshad et al., 2012; Bougnom et al., 2012; Saarsalmi et al., 2012; Podmirseg et al., 2013) and resultant improvements in crop biomass and yields (Pérez-Cruzado et al., 2010; Bougnom et al., 2012; Materechera, 2012; Moilanen et al., 2012; Saarsalmi et al., 2012). Combustion of wood that has been previously treated with weatherproof protectants, paints and preservatives, or impregnated/coated with heavy metal(loid)s and organic compounds can result in an ash that is highly concentrated in both trace nutrients and heavy metal(loid)s, arising from the additive treatments (Balasoiu et al., 2001). For example, Solo-Gabriele et al. (2002) measured concentrations ranging from 730 to 99,300 mg kg^{-1} total As, ≤165,000 mg kg^{-1} total Cr and 98,450 mg kg^{-1} total Cu in samples of ash produced from wood previously treated with a commonly used anti-fungal/

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bacterial treatment 'chromated copper arsenate (CCA)'. Studies that have applied wood ash generated from reclaimed [contaminated] feedstocks have reported that heavy metals from the ash are bioavailable and potentially phytotoxic. Lucchini et al. (2013) studied the addition of a Cu rich ash (~200 g kg^{-1}) to two agricultural soils (pH 5–6) finding that increased soil Cu concentrations and uptake to sunflower (*Helianthus annuus* L.) resulted, compared to the control soil without ash. This study also noted adverse effects to plant biomass and yield resulting from this ash addition. In these experiments the authors noted that the pH increases to soils immediately after ash addition were not maintained throughout the experiment due to the leaching of alkaline metals. Jones and Quilliam (2014) experimented with applications of ash produced from various ratios of contaminated and non-contaminated wood combusted together, to a podzol soil (pH 5). In common with the findings of Lucchini et al. (2013), the addition of Cu contaminated ash to soil resulted in an increased bioavailability of Cu in soil and concomitant uptake to wheat (*Triticum aestivum* L.). Whilst ash addition to agricultural soils can potentially be beneficial, the application of wood ash from waste wood sources is likely to increase metal(loid) loadings in soils but there is little experimental evidence available related especially to As and Cr fate in soils amended with contaminated wood ashes.

In the following study we examined the fate of the metal(loid)s As, Cr, Cu and Zn which were present in excessive concentration in wood ash generated from the combustion of mixed source waste wood. The aim was to 1) determine the bioavailability and toxicity of metal(loid)s from the ash when added to an agricultural soil, 2) ascertain whether co-application of ash with manure could change the geochemical fractionation of ash-derived metal(loid)s and reduce their bioavailability and phyto-toxic effects, and 3) use modelling to indicate the longer-term risk of As from the ash entering the food chain.

2. Methods

2.1. Soil, amendments and their characterisation

Soil, manure and ash were obtained from a 1,000Ha upland farming estate in north-east Scotland (UK) stocked with 450 Scottish Blackface and 500 crossbred ewes, 50 suckler cows and 100 farmed red deer hinds. Soils on the farm are freely drained humus iron podzols developed on old red sandstone and part of the Strichen Association (Glentworth and Muir, 1963). The soil collected for this experiment was from part of the farm designated for experimental work having received no prior amendment of manure or ash and being lightly grazed seasonally (sandy-loam texture, pH (H_2O) 5.8; organic carbon (OC) contents of ~7% and nitrogen (N) contents of ~0.6%). After sward removal a horizontal soil pit was excavated and the top 30 cm of soil bagged and returned to the laboratory (A and B horizons). The soil was then air dried at 25 °C for 1 week before it was homogenised by hand and sieved to <10 mm. Ash was sampled from a 70 kW wood fired biomass burner that provides heat for the farm buildings. The feedstock for the biomass boiler had consisted of mixed virgin timber and waste wood such as fencing, pallets and doors (chromated copper arsenate-CCA-treated wood). Aside from heavy metal(loid)s, detailed in Table 1, the sampled ash was additionally composed of Ca (20%), K, (7%), Mg (3%), C (2.5%), P (1%), Fe and Al (<1%). The N content was <0.1%. Ash was obtained directly from the boiler's combustion chamber, bagged and returned to the laboratory. The manure, consisting of cattle excreta, was obtained from the farm's manure pit where it had partly decomposed. It was dried at 25 °C for ease of handling and then homogenised by hand. Relevant chemical characterisation of the soil, manure and ash was carried out as follows and is presented in Table 1.

Table 1
Metal(loid) concentrations (pseudo-total) of soil, manure and ash. All values are the mean of replicates (n = 5) ± s.e.m.

mg kg^{-1}	As	Cr	Cu	Zn
Soil	4.5 ± 0.2	23.9 ± 2.1	8.8 ± 0.6	23.2 ± 1.4
Manure	5.4 ± 0.4	20 ± 2	23 ± 2	169 ± 12
Ash	9259 ± 649	9914 ± 715	8793 ± 632	4667 ± 374

Sub samples of soil, manure and ash were block digested using nitric acid (Meharg et al., 2013) to determine heavy metal(loid) concentrations. In brief, sub samples were oven dried, ball-milled and then 0.1 g was weighed into a glass digestion tube; 2.5 ml of concentrated nitric acid was applied and the sample left overnight. A total of 2.5 ml of hydrogen peroxide was added to the samples prior to being heated on a heating block which was gradually increased to 140 °C and then maintained at 140 °C for 4 h. After digestion the samples were made up to a total volume of 100 ml. In addition to the sample, 3 replicates of reference material (NCS ZC73007) and 3 analytical blanks were also digested and included for quality control. Samples were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Agilent Technologies 7500).

A further subsample of ash was subjected to a column leaching procedure to determine the solubility of metal(loid)s in the ash during a pre-determined time. Glass columns (dimensions 8 cm length and 1.5 cm internal diameter) were filled with 10 g of ash (Fig. 1A.). To avoid movement of ash material out of the columns during leaching, glass-wool was packed into the column at both ends after 0.2 μm filter papers had been prior inserted. The filter papers ensured that no particulate matter remained in the sample to be analysed. The columns were leached from their base via a peristaltic pump using de-ionised water as the eluent at a flow rate of 1 ml min^{-1}. A second pump was attached to the top of the column, at the same flow rate, in order to maintain neutral pressure in the columns. The purpose of leaching from the base of the column was to eliminate gravitation effects and the preferential passage of eluent through least resistant pathways in the ash inside the column, to ensure as far as possible that the whole mass of ash was leached uniformly. The columns were constantly leached for 2000 min^{-1} during which time an auto-sampler distributed 2 ml of leachate into tubes at pre-determined intervals of 10, 100 and 1000 $mins^{-1}$ until 2000 $mins^{-1}$. At the end of the experiment samples were analysed using the ICP-MS, as described above. Leachate pH was also determined and reported in Table 2.

The obtained raw data were converted from mg l^{-1} into cumulative mass of each metal(loid) (mg kg^{-1}), based on the volume of eluent passed through the column in each time step and the mass of ash in the column. Therefore a total mass of metal(loid)s extracted from the ash at pre-determined times could be calculated as a percentage of the total extracted throughout the duration of the experiment; as presented in Table 2.

2.2. Experimental pot setup

A total of 4 different treatments involving mixtures of soil, manure and ash were tested in this experiment in addition to 3 controls, with 5 replicates in all cases (see Fig. 1B). To create those treatments, soil and manure (M; 10% v:v) was first mechanically mixed using an end-over-end method to create a homogenous soil and manure mixture. Manure is mixed with ash at the farm scale to prevent ash dispersal by wind-blowing, so this process was replicated here in a controlled fashion. Ash (A) was applied to the soil/manure mixture at the following 4 vol and mixed in the same way;

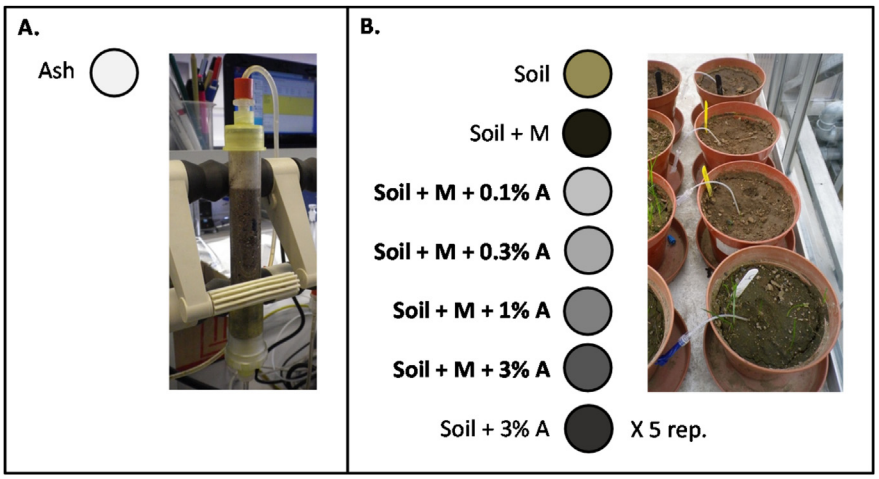

Fig. 1. Diagramatic representation of A.) column leaching test and B.) pot experimental treatments and set-up.

Table 2
Leachate pH and solubility of metal(loid)s in the ash as determined by the column leaching test. Values represent the percentage of total mass of metal(loid) leached from the ash at 10, 100 and 1000 $mins^{-1}$ out of a total duration of 2000 $mins^{-1}$.

$Mins^{-1}$	pH	As %	Cr %	Cu %	Zn %
10	11.7	33	51	7	<1
100	9.3	65	82	36	3
1000	7.9	74	94	70	30

0.1%, 0.3%, 1.0% and 3.0% ash (Soil + M + A). In preliminary experiments a volumetric addition of 10% ash was also tested, but this was completely phyto-toxic, so treatments including a maximum ash addition of 3% were used in order to ensure ryegrass germination and growth. By way of controls, pots containing only soil (Soil), soil plus manure (Soil + M) and soil plus 3% ash without manure (Soil + A) were included. Treatments and controls were potted into 2 L plastic pots and left to equilibrate for 3 weeks before any further experimental work was carried out. Then, one 10 cm Rhizon sampler (Eijkelkamp Agrisearch equipment, Netherlands) was inserted into each pot at a 45° angle (Fig. 1B). Once the sampler was inserted into the pots 15 rye grass (*Lolium perenne* L.) seeds were sown in each pot and soil moisture brought to 75% field capacity, which was maintained throughout the experiment. Perennial ryegrass was used because it is the primary sward species used in the pasture from where the soil was collected. Throughout the experiment pots were kept in a greenhouse with controlled temperature between 16 and 20 °C and natural light. After 9 weeks pore water was extracted from each of the pots using removable plastic syringes and immediately split into three sub-samples, one of which was used to determine pH, one used for the bacterial biosensor assay (Section 2.3) and the last sub-sample was acidified to a final concentration of 1% nitric acid for elemental analysis by ICP-MS.

Following pore water extraction (at approx. 10 weeks after the start of experimental work) rye grass was harvested, washed using deionised water and dried at 100 °C for 24 h; the dry biomass weight of the grass was recorded. The plant material was then ball milled and microwave acid digested (Meharg et al., 2013). In brief 0.2 g of dried ball milled material was accurately weighed into a 50 ml tube; 2.5 ml of concentrated nitric acid was added to the sample and left overnight. Then 2.5 ml of hydrogen peroxide was added to the sample prior to microwave digestion. Microwave digestion was performed at 95 °C for 30 min. Once the digestion was complete the samples where made up to a final volume of 50 ml. In addition to the rye grass samples analytical blanks and reference material (CTA-OTL-1) was also digested. Digested material was used for total elemental analysis. For ryegrass and pore water total elemental analysis was performed by ICP-MS as described above, and 10 μg l^{-1} rhodium was run as an internal standard. Analysis was performed as detailed in Sun et al. (2009).

2.3. Bacterial biosensor assay

Pore water samples were subject to a genetically modified luminescent bacteria *Escherichia coli* HB101 pUCD607 for toxicity (Tiensing et al., 2001). In brief, 280 μl of sample which had been pH adjusted to between 5.5 and 6.0 pH, was added to 96 well plates. A total of 20 μl of bacterial biosensor was added into the plates and the luminescence (relative light units; RLU) recorded after 15 min saturation using a Microplate Luminometer (LB 962, CentroPRO). The luminescence was calculated into the percentage luminescence compared to the soil control pore water.

2.4. Sequential extraction procedure

After the pot experiment was complete, soils from two treatments were used for the determination of geochemical associations of metal(loid)s from the ash with organic matter from the manure. Soil +3% ash and soil + manure +3% ash were used for this procedure representing the treatments with maximum loading of ash derived metal(loid)s (≤200 mg kg-1 total As, Cr, Cu and Zn; data not shown). After removal from the pots, soils were air dried at 25 °C for 1 week prior to being sieved to <2 mm to remove ryegrass root material remaining. Samples were then subject to a sequential extraction procedure according to McGrath and Cegarra (1992) with a slight modification; fraction F1) 0.1 M $CaCl_2$ (1:10, w/v) for 16 h; *metals in soil solution and in exchangeable forms*; F2) 0.5 M NaOH (1:10, w/v) for 16 h followed by HNO_3:H_2O_2 digestion of the extract; *metals associated with organic matter*; F3) 0.05 M Na_2H_2EDTA (1:10, w/v) 1 h; *metals mainly in the carbonate fraction*; F4) Digestion with HNO_3:H_2O_2; *residual metals*. This method was used because there is generally no single method optimised for both metals and arsenic.

2.5. Predicting transfer of metal(loids) to meat and milk products

Predictive modelling was used to estimate theoretical

accumulation of As in the tissues and milk of dairy cattle grazing pasture amended with the study ash. The transfer of As to the tissues and milk of cattle shows a clear time-dependency (Crout et al., 2004) and thus here the transfers of As to the milk [As_{milk}], liver [As_{liver}] and kidney [As_{kidney}] of grazing dairy cattle were estimated using time-dependent transfer coefficients. These are defined as the ratio of the concentration of As in animal tissue (mg kg^{-1}) to the daily intake of As in the diet (mg d^{-1}; Table 4) as determined by Crout et al. (2004). Arsenic accumulates within animal tissues with time so the concentrations of As in milk and tissues were estimated for the life span of a typical 7000 l milk y^{-1} head of dairy cattle (4.5 years; Chadwick et al., 1997). Cattle were assumed to feed entirely on grass silage produced on the farm for 185 days per year whilst, for the other 180 days, cattle were assumed to be grazing pasture fields in rotation (Chadwick et al., 1997). Any supplements fed to the cattle were assumed not to be contaminated with As.

Bovine intake of As, [As_{intake}], mg kg^{-1} d^{-1}, was calculated multiplying the concentration of As in the feed (in this case perennial ryegrass) by the mass of feed consumed, as shown in Equation (1) below:

$$[As_{intake}] = \left(\left[As_{feed}\right] I_{feed}\right) + \left([As_{soil}] W_{soil}\right) \quad (1)$$

In this case As_{feed} is the concentration of As in the feed, herbage or silaged ryegrass (mg kg^{-1}); I_{feed} is the intake of feed (kg dw d^{-1}); As_{soil} is the concentration of As in the soil (mg kg^{-1}) and W_{soil} is the amount of soil that is ingested. This calculation was based on several assumptions; that I_{feed} = 13 kg pasture/silage (dw) d^{-1} in the case of the average head of cattle (after Fayers, 1996) and that, during periods of outdoor grazing, W_{soil} = 0.52 kg soil (dw) d^{-1} (after Fayers, 1996). At those times of year when the cattle were kept indoors and fed only on silaged ryegrass, a value of 0.02 was given to W_{soil} to represent typical levels of soil deposition on crop plant surfaces (after Pinder and McLeod, 1988).

2.6. Statistical analysis

Where appropriate the data were analysed using one-way ANOVA with a Tukey post-hoc analysis. Correlation analyses were performed using Spearman's Rank Correlation. Prior to statistical analysis the data were tested for normality and transformed (square root or log) where necessary. For the purposes of these experiments, statistical significance was defined as the probability of the null hypothesis being 5% or lower (i.e. $p \leq 0.05$).

3. Results

3.1. Characteristics of soil pore water

The concentration of the measured metal(loid)s in the pore water from the control pots without ash (Soil; Soil + M) were low in comparison to the treatment pots containing ash, with the exception of Zn where there was no significant difference between controls or any treatments (Fig. 2). These results reflected the elevated metal(loid) concentration of the ash (Table 1) and the strongly soluble nature of As and Cr, compared to Cu and the weakly soluble Zn, as determine by the column leaching test (Table 2).

In the case of As, the addition of all volumes of ash to the soil with manure significantly increased concentrations of this metal(loid) in pore water, compared to the control soil, but significant differences between treatments including manure and ash were not seen (Fig. 2). The addition of the uppermost ash volume (3%) to the soil with and without manure significantly increased Cr in the pore water above the control; variation between replicates resulted in non-significant increases for treatments with less than 3% ash addition, against the control (Fig. 2). There was a significant increase in the concentration of Cu in the pore water when ash was added without manure, compared to any other treatment but the volume of ash added to soil with manure had no significant influence on Cu in pore water, except compared to the control (Fig. 2). No significant differences between any treatment was found in the case of Zn.

The pH of the pore water extracted from the control soil was ~5.0 increasing significantly to >6.0 with the addition of the manure. The addition of ash increased pH further to neutral, whereas the addition of ash to soil in the absence of manure resulted in a pH > 7.5; data not shown.

Table 3
Geochemical association (fractionation) of metal(loid)s in soil with 3% ash, with and without manure addition. Values represent the percentage of the total concentration of metal(loid) (determined by aqua-regia) extracted by each step. Values in parentheses are from soils including manure.

Fraction	As %	Cr %	Cu %	Zn %
'F1' $CaCl_2$	<1 (<1)	<1 (<1)	<1 (<1)	<1 (2.7)
'F2' NaOH	34 (67)	4.7 (6.5)	25 (33)	7.4 (5.5)
'F3' EDTA	12 (<1)	7 (3.2)	13 (8)	25 (16)
'F4' Residual	53 (32)	87 (90)	62 (58)	67 (75)

Table 4
Estimates of transfer coefficients (d kg^{-1}) for As to the muscle, kidney, liver and milk of dairy cattle after 10 days, 100 days and 1000 days of feeding on a contaminated diet at a constant intake rate (adapted from Crout et al., 2004).

$Days^{-1}$	Muscle	Kidney	Liver	Milk
10	1.92×10^{-04}	1.74×10^{-03}	4.02×10^{-03}	1.17×10^{-04}
100	4.60×10^{-04}	3.50×10^{-03}	4.73×10^{-03}	1.19×10^{-04}
1000	1.11×10^{-03}	4.21×10^{-03}	4.93×10^{-03}	1.19×10^{-04}

3.2. Plant elemental composition and biomass

The addition of ash to soil plus manure at 1 and 3% significantly increased As in ryegrass tissue compared to the lower ash additions; in all cases the addition of ash resulted in significantly greater concentrations of As in rye grass compared to the control (Fig. 2). The addition of ash to the soil also significantly increased the concentration of Cr in the ryegrass compared to the control soil, though there were no significant differences within treatments containing ash (Fig. 2). The highest measured concentration of Cr in ryegrass was from the soil and manure treatment, without ash, though this was not significantly greater than the treatments including ash, due to the large range between replicates (see error bars; Fig. 2). The addition of ash to soil without manure increased Cu in ryegrass significantly compared to all other treatments whilst, in common with pore water results, no treatments significantly altered ryegrass Zn concentration compared to the soil with no amendment (Fig. 2).

In terms of ryegrass biomass, the addition of ash alone to the soil did not significantly alter this compared to the control but the inclusion of ash with manure resulted in significantly greater biomass than the control and ash without manure, though this was dependant on the volume of ash included (Fig. 3).

3.3. Biosensor toxicity assessment

The toxicity of the pore water was determined using the universal bacterial biosensor (*E.coli* HB101 pUCD607). The luminescence of this bacteria exposed to pore water collected from

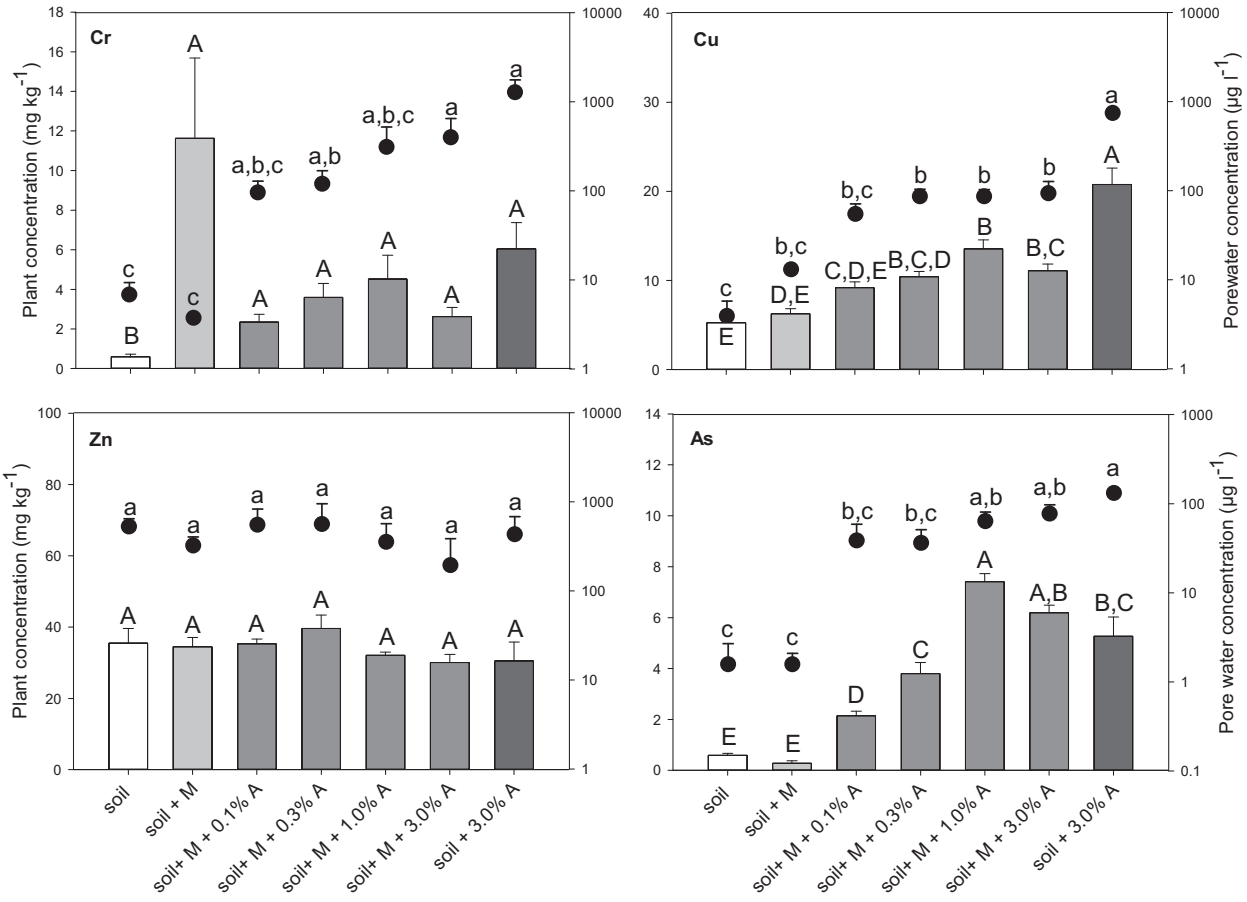

Fig. 2. Concentrations of metal[loid]s in pore water (circles) and in ryegrass tissue (bars) from each of the different soil treatments (+M = with manure; +A = with ash). Values are means of the replicates and the error bar is the s.e.m (n = 5). Means that share the same letter are not significantly different. Note that pore water concentrations are displayed on a log-scale.

treatments containing ash did not differ significantly from the luminescence of this biosensor grown in pore water collected from the soil and manure treatment. However, the pore water collected from the pots that contained soil and ash without manure resulted in a significantly lower percentage luminescence from the *E.coli* HB101 pUCD607 compared to the *E.coli* HB101 pUCD607 cultured in pore water sampled from the soil and manure treatment, indicating gross toxicity (Fig. 4).

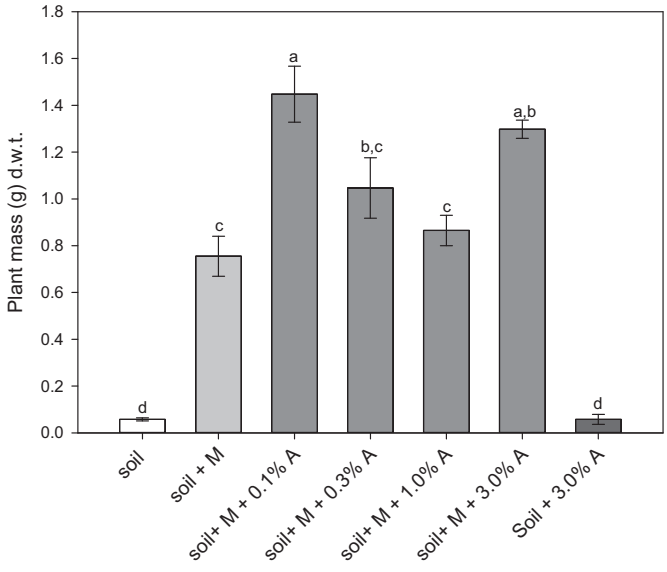

Fig. 3. Plant biomass of the rye grass for each of the different treatments (+M = with manure; +A = with ash). The bars represent the average of the replicates and the error bar the s.e.m (n = 5). Means that share the same letter are not significantly different.

3.4. Geochemical fractionation of metal(loids)

A greater proportion of the total concentration of metal extracted by the four-stage sequential extraction was present in the organic matter fraction of the soil + ash with manure than without manure for As, Cr and Cu (Table 3). In the case of Zn the addition of manure resulted in an increase in the exchangeable fraction (>2.5%, compared to < 0.5% in the soil without manure; Table 3). In all cases the residual fraction accounted for >50% of total concentration of extracted metal, except in the case of As in the presence of manure, where it was reduced to ~30% (Table 3).

3.5. Estimation of potential risks to the food chain

At present there is no European-level legislation governing the maximum permissible concentration of As in meat or milk products. Maximum permissible limits are only available for rice and rice products (0.2 mg kg^{-1}). The USEPA has published a chronic reference dose (RfD, mg kg^{-1} d^{-1}) for inorganic arsenic of 0.0003 mg kg^{-1} d^{-1} based on a no-observed-adverse-effect-level (NOAEL) of 0.0008 mg kg^{-1} d^{-1} for dermal effects in a Taiwanese agricultural community exposed to As in well water (Tseng, 1977;

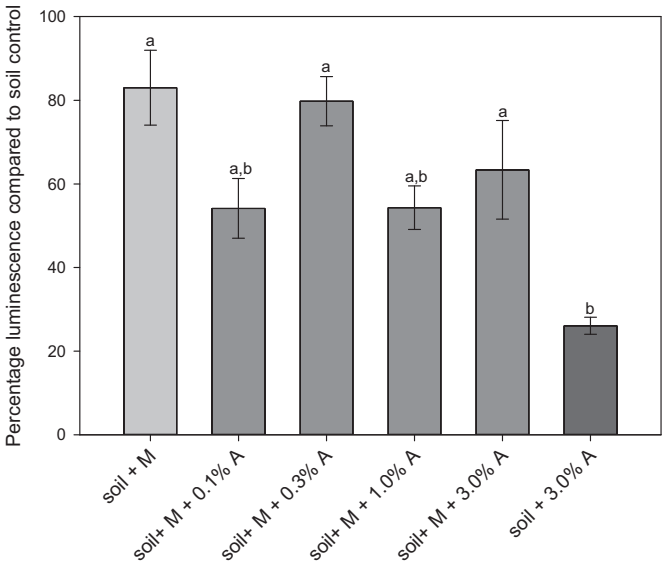

Fig. 4. Bacterial biosensor toxicity tests of pore water for the different treatments (+M = with manure; +A = with ash). Bar represent the average of the replicates and the bar is the s.e.m (n = 5). Means that share the same letter are not significantly different.

Tseng et al., 1968). This RfD will be used here as a comparator to estimate the levels of meat (muscle), offal and milk consumption required to exceed the RfD.

Fig. 5 shows the cumulative build-up of As in muscle, milk and offal (kidney and liver) after 10, 100, and 1000 days grazing of an average dairy cow producing 7000 l milk y^{-1}, based on a typical life span of a herd. For muscle, assuming a 1000 day production period, daily consumption would have to exceed 0.03 kg d^{-1} for a consumer to exceed the As RfD for beef produced on none ash-amended pasture (ie ryegrass grown on soil only). Given that the 95th %ile beef muscle consumer eats about 1.7×10^{-5} kg muscle d^{-1}, a dietary risk to consumers is not implied. At the upper ash treatments (≥1.0% ash), daily muscle consumption would have to exceed 3.82×10^{-3} kg d^{-1} (approx. the 20th %ile consumption rate) in order to exceed the RfD for As.

According to the criteria above, the daily consumption of milk would have to exceed 0.25 l d^{-1} for a consumer to surpass the As RfD for milk produced on un-amended pasture. The 95th %ile consumer drinks about 0.13 l d^{-1} of milk so a risk cannot be implied here. At the highest ash treatment consumption would need to exceed 0.036 l d^{-1} (about one fourth of the 95%ile milk consumption rate) to surpass the As RfD.

In the case of offal (kidney and liver, or other internal organs used for food), daily consumption of kidney and liver would have to exceed 0.007 kg d^{-1} and 6.05×10^{-3} kg d^{-1} respectively for a consumer to exceed their As RfD from offal produced on pasture not amended with ash. The 95th %ile offal consumer eats about 1.40×10^{-8} kg kidney d^{-1} and about 3.20×10^{-7} kg liver d^{-1}, so once again a dietry risk cannot be implied. At the highest ash treatments, kidney and liver consumption would need to exceed 1.01×10^{-3} kg d^{-1} and 8.60×10^{-4} kg d^{-1} (approx. the 50th %ile consumption rates) in order to exceed the RfD for As.

4. Discussion

4.1. Combined impacts of ash and manure on As and Cr toxicity

The concentration of metal(loid)s in pore water reflects the fraction to which plants and soil animals are exposed, and has been proposed as a simple indicator of environmental risk posed from contaminated soils (Moreno-Jiménez et al., 2011). As it is used in the present study, it may provide a measure of the potential toxicity of the addition of contaminated ash to soils, as demonstrated by its use in the bacterial biotoxicity test (Fig. 4). However, in the present study there is some disparity between the input of As [the volume of ash applied to the soil increased 30-fold between the lowest and highest ash treatments] and the concentration of As in the pore water and in the plant [2 and ~3-fold increases with ash respectively]. Arsenic speciation and therefore solubility, mobility, bioavailability and toxicity depend on a large number of factors including organic matter, redox potential, pH and phosphate concentration (Masscheleyn et al., 1991; Caetano and Vale, 2002; Tu and Ma, 2003; Warren et al., 2003; Cao and Ma, 2004). The unbalanced relationship between the total amount of As present in the soil and the concentration in the pore water/plant in the present study may be explained by the effect that the amendments have on the pH; stark pH effects have been observed in previous studies involving ash application to soils (Bougnom et al., 2009; Klemedtsson et al., 2010; Pérez-Cruzado et al., 2010; Arshad et al., 2012; Bougnom et al., 2012; Saarsalmi et al., 2012; Podmirseg et al., 2013). The pH of the leachate from the ash in the present study decreased by ~3 units during the column test (Table 3), which demonstrates that pH effects in ash amended soils will change over time, concomitantly influencing metal(loid) mobility.

Organic matter influenced As fractionation in the present study to the greatest extent of all measured metal(loid)s, with a profound shift from mostly residual towards a predominance of As bound to organic matter with the addition of manure, as shown by the sequential extraction data (Table 3). It can therefore be extrapolated that the release of As into more exchangeable forms (such as soluble in water) could occur upon the natural mineralisation of organic matter in soil. The column study indicates that both As and Cr are the more soluble of the measured metal(loid)s, with their leaching from ash being rapid (Table 2); it is therefore likely that, upon ash addition to soil plus manure, sorption of As to organic manure was rapid.

The application of ash to soil without manure caused an increase in the concentration of Cr in the pore water and plant accumulation compared to soil and ash with manure addition, which is due to two factors. Firstly it is observable that the addition of manure to soil plus ash increases Cr in the organic matter fraction, simultaneously reducing it in the exchangeable fraction, albeit not in the same magnitude as that of As (Fig. 4). The relationship between Cr in the pore water and plant is complex; in the terrestrial environment the most common forms of Cr are the trivalent (Cr (III)) and hexavalent (Cr (VI)) forms (Kimbrough et al., 1999). Cr (VI) is more toxic than Cr (III) due to its increased solubility and high oxidising potential (Pawlisz et al., 1997). It has been demonstrated that dissolved organic carbon can cause the reduction of the more toxic Cr (VI) to Cr (III) (Nakayasu et al., 1999) and that Cr (III) can then bind to the humic acid matrix (Janos et al., 2009). However, although the organic matter may decrease the mobility and toxicity of Cr in the environment, an increased pH (above neutral) may favour the oxidation of Cr (III) back to Cr (VI) (Seaman et al., 2003).

4.2. Influence of soil treatments on Cu and Zn mobility and uptake

In contrast to As and Cr, the presence of bioavailable Cu and Zn in ash could add value to its application to soils as a means of fertilisation and fortification of plant tissues with trace metals. Both Cu and, especially Zn were not as strongly soluble as As and Cr from the ash (Table 2); Cu and Zn solubility increases greatly at pH < 5, but the effective liming capability of ash in the present study,

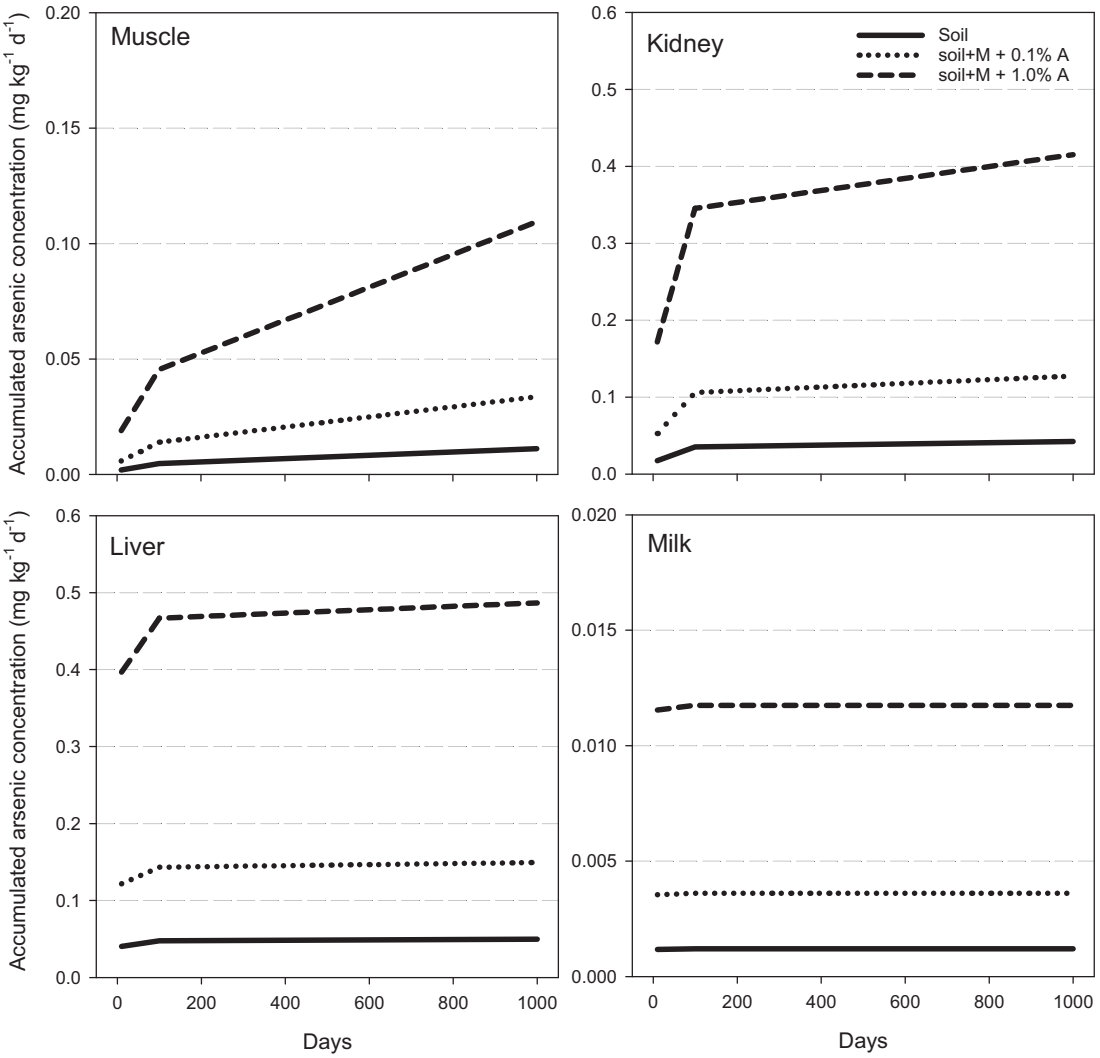

Fig. 5. Accumulated concentrations of As (mg kg^{-1} d^{-1}) in the muscle, milk, liver and kidney of dairy cattle consuming a diet of 49% grass grazed from pasture amended with the ash used in this study and 51% silage produced using the same grass (assuming all supplements to be uncontaminated).

increasing pore water pH by ~3 units, failed to prevent large quantities of free Cu and Zn in ash being mobilised in the pot experiment. Indeed without the organic matter binding capacity of manure addition, concentrations of Cu in pore water and subsequent plant uptake of Cu were significantly increased, compared to where manure was present. This suggests that both pH and organic matter content of the combined manure/ash additions to soil controlled Cu retention in semi-stable complexes; the sequential extraction demonstrated that Cu was increasingly fractionated into the organic matter phase in the presence of manure (Table 3). Several studies have reported the reduced mobility and availability of Cu in the presence of organic amendments (reviewed in Kumpiene et al., 2008) due to organo-metallic complexes formed between Cu and organic fractions in soil (Bernal et al., 2009).

Although the exchangeable fraction of Zn formed 2.5% of the total concentration for soil treated with manure against >0.5% in soil treated only with ash (Fig. 4), unlike As, Cr and Cu, the concentrations of Zn in pore water sampled from control soil, or any of the ash and manure treatments showed no significant variation (Fig. 2). Less soluble complexes of Zn can form with P, Ca or Fe-oxides (Kumpiene et al., 2008) and, as the carbonate fractioned Zn was rather greater in soil treated only with ash as against that also including manure in the present study, there could be multiple de-sorption/ad-sorption mechanisms at play to control Zn solubility and bioavailability in the presence and absence of ash and manure.

However, what is apparent is that the soil used in the present work, without amendments of manure and/or ash, had a significant soluble Zn pool, verified by the results of the pore water extractions (Fig. 2). For example, Moreno-Jimenez et al. (2011) extracted pore water from a range of UK mine, industrial and urban soils; under field conditions, recording pore water Zn concentrations of <500 μg l^{-1}. Those soils generally had total Zn concentrations of 25–1600 mg kg^{-1}, which greatly exceeds those of the present upland pasture soil (Table 1).

4.3. Implications of contaminated ash addition to environmental risk

Unlike pre-industrial times, modern waste wood streams can contain a variety of additives (for example; preservatives, veneers, glues, etc.) which, after combustion, produce a highly heterogeneous wood-ash waste. Such ash residues provide liming and nutrient supplement to soils, when applied, but also an unknown loading of heavy metal(loids). This was demonstrated in the current experiment by the beneficial effects to ryegrass biomass when

wood ash was added to pre-manured soil, contrasted with the increased metal(loid) uptake to ryegrass derived from the ash. Extrapolating the experimental results further using transfer modelling to meat and milk products suggested that, with lower applications of ash (<1.0% v/v) after 1000 days grazing, levels of As in milk and meat products would not pose a risk to consumers within the top 5% of average daily consumptions. However, at higher application rates (>1.0% v/v ash), offal accumulation could result in exposures above the RfD for As. It should be noted that the transfer coefficients used in this study were derived from an experimental design where equilibrium was not reached. Several factors may affect the model output therefore; 1) the pot experimental duration was very short and ryegrass uptake may have increased given a longer growth period and 2) the grazing period up to a maximum of 1000 days was assumed, during which time it was not known whether equilibration would have been reached. Therefore, under equilibrium conditions the model outputs could have been different. Equilibrium transfer coefficients for As into milk and meat products that have previously been recommended by Morgan (1991) and Stevens (1991, 1992) with those given by Morgan (1991) generally of a similar order of magnitude as those given in Table 2. Stevens (1991, 1992) recommended an equilibrium transfer coefficient of 3.0×10^{-2} d l^{-1} for milk which is about two orders of magnitude greater than the non-equilibrium coefficient detailed in Table 2. Transfer coefficients such as these are often considered 'conservative' so, in other words, one or two orders of magnitude greater transfer coefficients than the non-equilibrium coefficients used in this study can be found. The transfer coefficients detailed in Table 2 imply a time-dependency for the transfer of As from feed to the tissues and milk of dairy cattle which may render the use of equilibrium transfer coefficients to predict food product concentrations inappropriate. Additionally, non-equilibrium transfer coefficients are more representative of an agricultural system because of the relatively short life-spans of livestock. In this study, the life-span used to represent a 'typical' head of dairy cattle is 4.5 years (Chadwick et al., 1997), whilst beef production requires ~390 days (Dodsworth, 1972). It is also worth noting that the transfer calculations used in the present study include ingestion of soil directly which, in the context of the present study, may also include particles of ash, especially if it has not been incorporated thoroughly into soil, or just surface ripped.

The experimental design employed in the present study provides single doses of ash to soil but in the event of multiple applications of ash to farm land the accumulation of metals in soils may render an increased risk than that detailed above. The repeated application of fresh organic matter may not be able to provide effective binding sites for metals in solution, nor those that are bioavailable, especially during decomposition or humification. Also of concern would be the direct ingestion of ash that has been re-applied to soil, which would render increased food-chain transfer risk, regardless of organic matter binding capacity.

5. Conclusions

There are potential environmental risks associated with the application of metal(loid) rich wood ash to land related with leaching and food-chain transfer of contaminants. The present study amended an acidic agricultural soil with wood ash that had high total concentrations of As, Cu, Cr and Zn, and a strongly soluble As and Cr pool, so the immediate concern is that soil metal(loid) loadings will dramatically whelm, and leaching of As and Cr could occur to proximal waters. Soil alone has some capacity to mitigate against metal(loid) leaching but pre-manuring soil before adding ash buffers pH and reduces the solubility of some metal(loid)s further, whilst organic matter also provides binding sites for Cu and As. These effects serve to reduce the toxic impacts of the wood ash and increase ryegrass biomass in manure amended soils compared to soils with the addition of ash alone. However, from food-chain transfer modelling it is clear that, regardless of manure application, higher doses of ash application to soil are likely to induce increased probability of transfer of As to grazing animals and resulting foodstuffs. Therefore it can be concluded that the application of heavy metal(loid) rich ashes to soils should be avoided unless there is evidential proof that soils and added amendments have the capacity to render metal(loid)s immobile, bio-unavailable and non phyto-toxic.

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