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Water treatment residuals as soil amendments: examining element extractability, soil porewater concentrations and effects on earthworm behaviour and survival

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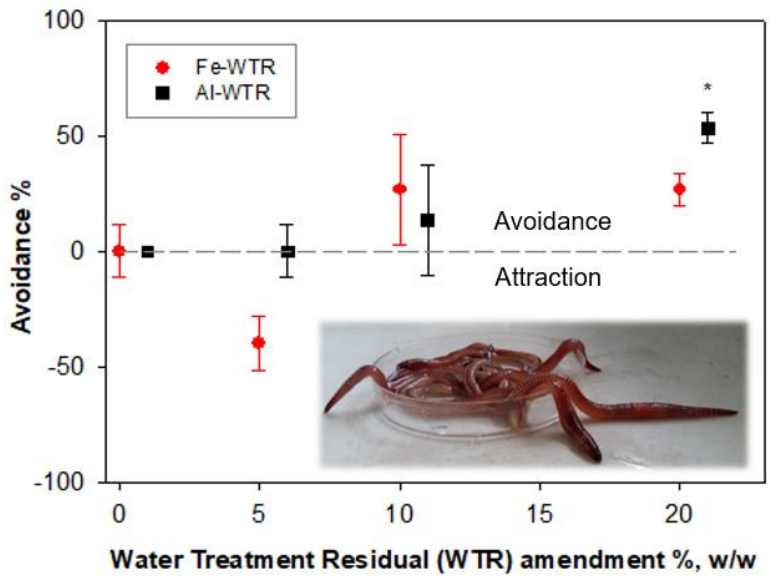
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Land application of Water treatment residuals (WTRs) offers environmental benefits
- Leachability of elements from WTRs was very low
- Al was only released from WTRs when the pH was lowered to 4.4
- Earthworms did not avoid soil amended with WTRs up to 10% w/w
- Earthworms accumulated marginally higher tissue concentrations of some elements

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Abstract

Drinking water treatment residuals (WTRs), the by-product of water clarification processes, are routinely disposed of via landfill however there is a growing body of research that demonstrates the material has great potential for beneficial use in environmental applications. Application to agricultural land is one option showing great promise (i.e. a low cost disposal route that provides organic matter input to soils and other potential benefits), however questions remain as to the impact such applications may have on earthworm survival and behaviour and also on the potential effects it may have on soil porewater chemistry. This study examined the leachability of elements within two types of WTRs (one Al- and one Fe- based) from England via 0.001 M $CaCl_2$ solution, at varying pH, and via the Community Bureau of Reference (BCR) sequential extraction scheme. Earthworm avoidance, survival, growth, reproduction and element concentrations were examined in WTR-amended sandy soils (0%, 5%, 10%, 20% w/w), while soil porewaters were also recovered from experimental units and examined for element concentrations. The results revealed leachable element concentrations to be very low in both types of WTRs tested and so element leaching from these WTRs would be unlikely to pose any threat to ecosystems under typical agricultural soil conditions. However, when the pH was lowered to 4.4 there was a substantial release of Al from the Al-WTRs (382 mg/kg). Soil porewater element concentrations were influenced to some degree by WTR addition, warranting further examination in terms of any potential implications for nutrient supply or limitation. Earthworm avoidance of WTR-amended soil was only observed for Al-WTRs and only at the maximum applied rate (20% w/w), while survival of earthworms was not affected by either WTR type at any application rate. Earthworm growth and reproduction (cocoon production) were not affected at a statistically significant level but this needs further examination over a longer period of exposure. Increased assimilation of Al and Fe into earthworm tissues was observed at some WTR application rates (maximum fresh weight concentrations of 42 mg/kg for Al and 167 mg/kg for Fe), but these were not at levels likely to pose environmental concerns.

Key words: Earthworms; *Eisenia fetida;* water treatment residuals; soil amendment; BCR

1. Introduction

Clarification of drinking water supplies is commonly achieved by treatment with aluminium (Al) or iron (Fe) salts, which remove impurities through coagulation and co-precipitation into a sludge like material referred to as drinking water residuals (WTRs). Thus WTRs are primarily composed of $Al(OH)_3$ or $Fe(OH)_3$ plus organic matter, clay particles and other components (e.g. nutrients, contaminant metals and other impurities) removed from the raw water (Bugbee and Frink, 1985; Graveland et al., 1993). Vast quantities (i.e. millions of tons) of WTRs are produced globally (Babatunde and Zhao, 2007), with the majority disposed of via landfill. However, landfill disposal is increasingly expensive and may be wasting a potentially useable material; an increasing array of potential beneficial uses of WTRs have been researched and demonstrated over the last two decades, including use in constructed reedbeds or as a soil amendment to manage phosphorus (P) mobility within catchments (Babatunde et al., 2011; Ippolito, 2015; Oliver et al., 2011), land application to increase organic matter and water holding capacity and related soil parameters (e.g. Ahmed et al., 1998; Bugbee and Frink, 1985), and most recently as a way of remediating polluted soils through immobilization of contaminants by WTRs (Garau et al., 2014; Garau et al., 2017; Wang et al., 2012). Beneficial use of WTRs is therefore an attractive option that offers financial advantages and facilitates development of a more circular economy with greater levels of materials recycling. However, while land applications of WTRs can be beneficial there are uncertainties that remain, including the mobility of elements within WTRs (particularly Al) and any ecotoxicological impacts on soil ecosystems linked to that or other changes brought about by WTR addition. For this reason there are still tight controls on where WTRs can be applied (e.g. in the UK it is only permitted on soils with pH>6.0). Some studies have found no negative impacts on plants or plant yield increases following WTR application to 'clean' agricultural soils (Ahmed et al., 1998; Geertsema et al., 1994), while others have noted plant yield reductions that were attributed to restrictions in bioavailable P (Lombi et al., 2010; Oladeji et al., 2007). While a number of studies have investigated the effects on microbes following soil amendment with WTRs (e.g. Garau et al., 2017), very few, if any, have examined the influence of WTR application on earthworms. This is a major gap in current understanding of the risks and benefits of using these materials in agricultural soils, especially considering that earthworms are widely recognized as essential ecosystem engineers that provide a host of advantages for soil health and development (e.g. creation of pore channels, improved aeration and hydraulic conductivity, nutrient cycling, etc). The aims of the present study were to fill this gap, and to further scientific understanding of the behavior and ecological effects of WTR components when the materials are applied to soils, by examining two WTR types from central England, UK, and determining i) the leachability of elements via single solution extraction at varying pH, ii) the fractionation of key elements within WTRs, iii) the influence of WTRs on the survival, growth and reproduction of the earthworm *Eisenia fetida*, and iv) the influence of WTR application on soil porewater element concentrations (because the majority of soil biota assimilate nutrients and contaminants via the soil porewater).

2. Methods

2.1 Water treatment residuals and soil

Partially dewatered WTRs from two water treatment plants in Staffordshire, England, one of which primarily uses Al salts (producing Al-WTRs; once dry, pH 7.34±0.06, OM 28.0±0.1%, Al 11.64±1.08%, Fe 0.91±0.08%, w/w) and the other primarily Fe salts (producing Fe-WTR; pH 7.37±0.01, OM 25.9±0.2%, Al 0.71±0.12%, Fe 17.69±0.19%) in their respective water treatment processes, were supplied by Severn Trent Water. The original 'as received' water content was high (~80% of total mass, determined on subsamples oven dried at 105°C) so the WTRs were air-dried with the assistance of an oven set at 30°C. During the ~2 week drying period required to reach stable mass, the WTRs were broken down to small pieces by hand on a daily basis to avoid large clods forming that, once dried, would present difficulties for hand crushing using a pestle and mortar. Once dried, the WTRs were crushed to pass a 2 mm sieve. Organic matter content was determined by loss on ignition at 450°C, pH was determined in 0.001 M $CaCl_2$ extracts using a Jenway 3510 pH meter and probe, and pseudo total element concentrations were determined via microwave (CEM Mars 6) assisted mineral acid digestion (0.3-0.5 g solid; 9 ml HCl + 3 ml HNO_3, i.e. *aqua-regia*, n=3) and analysis via ICP-OES (Optima 5300 DV instrument, Perkin Elmer, UK) as per USEPA method 3052 (see Supplementary Information Table 1). Due to the high organic matter content, samples were combusted for 4h at 450°C prior to digestion. All acids used in the digestions were trace analysis grade (e.g. Aristar and Primar plus) and a certified reference material (CRM033 Loamy Sand; Trace Metals - Loamy Sand 10, Sigma-Aldrich) was digested and analysed alongside samples for quality assurance purposes. Measured values for relevant elements in the CRM closely matched certified values (e.g. 97-117% for Fe, Pb and Zn).

A sandy soil from Sevenoaks, Kent, UK, provided by a commercial supplier (Bourne Amenity) and known to be free from contaminants, was used in the experiments. A sandy soil was selected because this would maximize the likelihood of identifying elements that leach from the WTRs into the soil and therefore into soil porewater. Organic matter content (1.1%) and pH (6.78±0.1) were determined while particle size distribution (1% clay, 2% silt and 97% sand) was determined by first combusting at 450°C, soaking in calgon solution and then analysing on a Coulter LS230 optical laser particle size analyser. Water holding capacity (WHC) was determined as 0.37±0.02 mL/g by fully saturating 100 g, allowing to drain and then measuring retained water.

2.2 pH buffering capacity and element leachability

The pH buffering capacity of WTRs and their extractable element contents were determined in 0.001 M $CaCl_2$ (3 g solid; 30 mL solution; n=3) extracts (Degryse et al., 2007; Hamels et al., 2014) that had been adjusted to varying acid levels. For Fe-WTR samples, the solutions were adjusted to four acid levels using high purity HCl (0, 0.013, 0.032 and 0.064 M HCl), while for Al-WTRs three acid levels were imposed (0, 0.013 and 0.064 M HCl). Once solutions were added samples were sealed, shaken by hand for 30 s, then shaken for 48 h on an end-over-end shaker, centrifuged and then a portion used for pH measurement and the remainder filtered (0.45 µm cellulose acetate syringe filter) before analysis by ICP-OES and ICP-MS (Agilent 7500ce).

2.3 Element fractionation (BCR sequential extraction)

Many sequential extraction schemes have been devised that attempt to identify fractions within soils and sediments with which elements of interest are associated. All have limitations and all generate operationally defined fractions (see review by (Bacon and Davidson, 2008), but they are nonetheless useful for identifying easily extractable vs recalcitrant element contents and for comparative purposes. The scheme devised by the Community Bureau of Reference (BCR) (Ure et al., 1993) has been employed extensively to examine metal fractionation in river sediments (Martinez-Santos et al., 2015; Pulford et al., 2009), aquaculture sludges (Nemati et al., 2011), sewage sludge (Scancar et al., 2000), urban soils (Gál et al., 2008; Madrid et al., 2007), agricultural soils (Kosolsaksakul et al., 2014), upland peat soils (Bacon et al., 2006), battlefield soils (Oliver et al., 2008) and in soils were pollution remediation trials (e.g. immobilisation with biochar or by zeolite formation) have been conducted (Belviso et al., 2010; Ippolito et al., 2017), hence it was chosen for this study. In the BCR procedure, 1.0 g oven dry equivalent samples are subjected to the following extraction regime. Step 1 (targeting the 'exchangeable' fraction): 40 ml 0.11 M acetic acid, shaken over-night, centrifuged, supernatant removed and filtered (0.45 µm cellulose acetate syringe filter) before analysis by ICP-OES. Step 2 (targeting the 'reducible fraction', indicative of Fe/Mn oxide-bound): 40 ml 0.1 M hydroxyl ammonium chloride adjusted to pH 2.0 with concentrated (15.8 M) HNO_3 is added to the residue from step 1, shaken over-night, then centrifuged, with the solution removed, filtered and analysed as in step 1. Step 3 (oxidisable fraction, indicative of organic matter bound): residues from step 2 were treated with 10 ml hydrogen peroxide (>30% w/v, added as supplied), left to stand at room temperature for 1 h, heated in a water bath at 85°C for 1 h then reduced to near dryness (<1 ml volume). Each sample then received 40 ml 1.0 M ammonium acetate (adjusted to pH 2.0 with 15.8 M HNO_3) and was shaken over-night followed by extraction, filtration and analysis performed as above. Step 4 (residual fraction): this additional recommended (Rauret et al., 1999), and widely adopted, step to the original BCR procedure enables assessment of element mass balances (i.e. sample recoveries). Here, residues from the above 3-step sequence were digested in *aqua-regia* as described in section 2.1 and analysed by ICP-OES. Analyses of BCR fractions were conducted using matrix-matched standards (range 0.1 – 100 mg/L).

2.4 Earthworm avoidance tests

Earthworms (*Eisenia fetida*) originally obtained from Wormery UK (Hertfordshire, England) were maintained in a bonsai compost and coir substrate and fed with oatmeal for several weeks to allow acclimitisation to the laboratory prior to avoidance and survival/ reproduction tests. Only adult earthworms with well-developed clitellum were employed in the ecotoxicology assays. The avoidance tests were conducted according to ISO guideline 17512-1:2008 (avoidance test for determining the quality of soils) using the two-chamber method, where plastic vessels of dimensions 15x10x15 cm (length x width x depth) are divided into two chambers using a removable plastic partition. One side of the vessel was filled with 500 g of unamended (or control) soil and the other with 500 g of soil amended with either Al- or Fe-WTRs at rates of 0%, 5%, 10% or 20% (w/w). A 5% WTR (w/w) amendment rate was selected to represent the upper range of what is likely to be practical in a typical field application scenario, with the 10% and 20% rates selected as extreme worse case scenarios that have been tested and discussed in the literature (Nagar et al., 2014; Sarkar et al., 2007). The soil-WTR mixtures were thoroughly homogenized via hand mixing. Prior to placement in vessels, soils were moistened with de-ionised water to 50-60% WHC. To commence the avoidance test the plastic partition was removed and 10 earthworms were placed in the centre of the vessel. The vessels were covered with cling film into which holes were pierced to allow air movement. The vessels were then left for 48 h under conditions of 20°C ±2°C and the natural photoperiod for March/April in England, after which covers were removed, partitions replaced and the locations of earthworms determined by hand-sorting the soil from each chamber. Any earthworm divided by the partition was counted as being in both chambers. Three replicates were conducted for each treatment.

Percent avoidance was calculated according to Equation 1.

$$(C-T)/n * 100 \qquad \text{(Eq. 1)}$$

where C = number of earthworms in the un-amended control chamber, T = number of earthworms in the treatment chamber and n = number of earthworms in the test (Amorim et al., 2005). A positive percentage indicates avoidance of the treated soil, zero indicates no avoidance, while a negative percentage indicates an attraction to the treated soil (Amorim et al., 2005).

For quality control purposes a preliminary avoidance test experiment was first conducted, in which un-amended control soil was placed in both halves of the test vessels (n=4). Results confirmed no avoidance or attraction bias was apparent within the experimental setup (see Supplementary Information Fig. 1), and that the avoidance tests met the validity criteria of the protocol.

2.5 Earthworm survival, growth and reproduction tests

Tests were conducted in large (1 L) plastic beakers following OECD protocol 222 and included six control replicates (500 g un-amended soil) and three replicates of each WTR treatment (5%, 10% and 20% (w/w) for each of Al- and Fe-WTRs; each treatment was thoroughly homogenized via hand mixing). The soils were moistened to 50-60% WHC with de-ionised water and then 10 adult earthworms were weighed and added to each vessel. Oatmeal (~2 g) was added as a food source and then each vessel was covered with cling film that was pierced to facilitate air flow. The mass of each vessel was monitored and de-ionised water added to compensate for any moisture loss. Additional oatmeal was provided on day 7 and after 14 days the earthworms were recovered by hand sorting. Survival/mortality was determined and living earthworms were weighed and allowed to depurate for 24h in petri dishes lined with moistened filter paper, after which they were rinsed with deionized water, patted dry with paper towel, re-weighed and frozen to euthanize and preserve prior to digestion in concentrated HNO_3 (Primar plus) and analysis for element content via ICP-MS. The soil was returned to the test vessels, any moisture loss replaced with deionized water, and the vessels were then maintained for a further 7 days after which each vessel was emptied into a plastic tray and the number of cocoons present determined by careful hand sorting. A portion of the recovered soil from each treatment was then used to determine the pH that had become established after 21 days of equilibration, with the outcome being that the soil pH of 6.78 was elevated to above 7.15 in all WTR treatments and that a maximum pH of 7.48±0.03 was observed in the 20% Al-WTR treatment.

2.6 Soil porewater extraction

Following recovery of cocoons (section 2.5) soil solution (soil porewater) was obtained from each treatment by centrifugation, following the double chamber method described by Smolders et al.

(Smolders et al., 1999). This involved removing the plunger from 20 mL disposable plastic syringes, placing a small plug of cotton wool into the bottom and then filling with ~50 g moist soil. Four syringes were filled for each treatment, which were then centifuged for 20 minutes at 3500 rpm and the resulting extracted solutions pooled, filtered at 0.45 µm and acidified with 0.1 mL concentrated HNO_3 (16 M, Primar Plus, Trace Metal Analysis grade).

2.7 Statistical assessment

Statistical assessment of differences amongst treatments and controls were conducted via t-tests and ANOVA, when underlying assumptions of the tests were met (i.e. normality of distribution), or via Mann-Whitney tests. All statistical assessments were conducted using Minitab-17 and Sigmaplot-10 software.

3. Results and discussion

3.1 pH buffering capacity and element leachability

Although the initial pH of the two WTRs were similar (~7.3), their response to acid addition and resulting pH buffering capacities varied (Fig. 1). The Fe-WTR had a consistent buffering capacity across the range of acid concentrations applied, such that a linear model described the data suitably (R^2 0.9741, Fig. 1) and a buffering capacity of 0.34 moles H^+/kg Fe-WTR/ pH unit was determined. The Al-WTR showed a varying buffering capacity across the pH range imposed, with a much lower initial buffering capacity of just 0.065 moles H^+/kg Al-WTR/ pH unit calculated between the initial pH of 7.3 and the pH of 5.5 observed after equilibration with the 0.013 mol H^+/ L solution. However, below pH 5.5 the Al-WTR had a buffering capacity of 0.45 moles H^+/kg Al-WTR/ pH unit, similar to that of Fe-WTR (Fig. 1).

The extractability of Fe, As, Cd, Cr and Pb in 0.001 M $CaCl_2$ solutions was extremely low or nil for both Al-WTRs and Fe-WTRs at all pH levels (Fig. 2). The extractability of Zn was very low in Fe-WTRs at all pH levels (<1 mg/kg) and slightly higher in Al-WTRs in which it rose from 1.4 mg/kg at natural (un-amended) pH to 3.8 mg/kg at pH 4.4 (Fig. 2). The extractability of Al from Fe-WTRs was modest, rising from ~3 mg/kg at un-amended pH to 4.5 mg/kg at pH 5.5. The extractability of Al from Al-WTRs was similar to that of Fe-WTRs across the pH range 5.5-7.5 (i.e. ~5 mg/kg), but at the lower pH of 4.4 realised in the Al-WTR samples extracted with 0.001 M $CaCl_2$ in 0.064 mol H^+/ L solution the extractable Al rose markedly to 382 mg/kg (Fig. 2). The results are in line with observations by Lombi and co-workers (Lombi et al., 2010) who found that $CaCl_2$ extractable Al in two Al based WTRs from South Australia rose to ~400 mg/kg or greater when the pH was lowered to <4.5. They also found that WTR application rates equivalent to between 5 and 500 t/ha produced $CaCl_2$ extractable Al concentrations that were always <0.5 mg/kg in the sandy soil (pH 6.3) tested. That study also tested an acidic clay soil (39% clay, pH 4.3) and found that with no WTRs applied the extractable Al was 39 mg/kg, rising to a maximum of 53 mg/kg at a WTR application rate equivalent to 5 t/ha and then falling below 25 mg/kg for applications equivalent to 50 t/ha and above (where the pH had risen to > 5.0) (Lombi et al., 2010). One of the main reasons for the current restrictions on where WTRs can be used as amendments in agricultural soils is the concern that Al may become mobilized. The results of the present study, when added to those from previous works, indicate that above pH 5.5 Al is not released from either Al- or Fe-WTRs at levels that would raise any ecological issues. The higher pH buffering capacity of Fe-WTRs assessed in the present study also indicates that they may offer greater protection than Al-WTRs in terms of preventing soil pH from dipping to undesirable levels in the event of acidic inputs, though this needs further examination to determine whether it is universally so. The protection offered by the pH raising or 'liming' capacity of both Al- and Fe-WTRs shown here (section 2.5) and in other studies also needs to be considered in this context.

3.2 Element fractionation (BCR sequential extraction)

Particularly considering the potential heterogeneity of the material, the mass balances observed for the BCR procedures (i.e. sum of recoveries in BCR fractions/ total digest) were good for the majority of elements examined (Fig. 4; Sup Inf Table 1). An exception was the recovery of Cr in Fe-WTR fractions and the consequent mass balance for that element. Other studies have similarly noted the difficulty in achieving a reliable mass balance for Cr in BCR fractionation procedures (Bacon and Davidson, 2008), thus this is not uncommon.

The BCR fractionation results (Fig. 3) support the $CaCl_2$ extract data in that very few elements were found to be readily extracted from either type of WTR. For Fe-WTRs, <0.1% of the total Fe and total Al were found to be in the acetic acid extractable fraction (65.0±0.7 mg/kg for Fe and 6.6±0.4 mg/kg for Al; Fig. 3 and SI Table 1) while only 6% (13.1±0.8 mg/kg) of the total Zn was in this fraction. For Fe-WTRs much of the Zn was in the 'organic' BCR step 3 fraction (52%, or 109±8 mg/kg) and in the 'reducible' BCR step 2 fraction (19%, or 40±0.3 mg/kg) while all of the Cd and Cr was in the residual phase, along with the great majority of the Pb (Fig. 3). For Al-WTRs 8% of the recovered Al was in the acetic acid extractable fraction, somewhat matching the results of the $CaCl_2$ solution extracts where acidification of the solution led to release of a portion of the Al in Al-WTRs. The fractionation of Zn in Al-WTRs was similar to that in Fe-WTRs, except that a larger proportion was in the residual (BCR 4) phase. All of the Cr and Pb in the Al-WTRs was in the residual phase which, together with the results for the Fe-WTR fractionation, indicates that any Cd, Cr and Pb in these WTRs are unlikely to have any ecological significance when applied to soils. In a study of six WTRs from China, Wang et al. (Wang et al., 2014) similarly found that the majority of metals and metalloids within WTRs were typically in the residual phase according to a BCR protocol (e.g. 63% of the Al and 81% of the Fe) and moreover that according to the toxicity characteristic leaching procedure (TCLP) employed by the USEPA those materials could be classified as non-hazardous. However, in that Chinese study the amount of Cd in the acetic acid-soluble fraction (BCR 1) ranged from 5% to ~45% of the total in some WTRs tested, which contrasts sharply with the results of the present study where Cd was entirely in the residual phase when present at all. This indicates that local and regional variation can occur in terms of element fractionation and extractability and emphasizes the need to examine WTRs before application in the field.

3.3 Earthworm avoidance tests

There was no avoidance behavior in the dual control soil treatments (i.e. having un-amended soil on both sides of the partition), again confirming validation of the avoidance test (Fig. 4). An attraction to the 5% Fe-WTR treatment and a mild avoidance of the 10% and 20% Fe-WTR treatments seemed apparent (Fig. 4), but none of these constituted statistically significant variation from the controls (t-tests $p > 0.05$). A significant avoidance was observed for the Al-WTR at the 20% amendment rate (53.3±6.7% avoidance, Fig. 4). Li et al. (2011) found significant avoidance by *Eisenia fetida* of soils amended with 10% and 20% biochar produced from apple wood sawdust and concluded that increased desiccation, induced by the high water holding capacity of the biochar, may have been responsible. It is feasible that a similar issue, or possibly something linked to alteration in texture, caused the avoidance observed in the 20% Al-WTR treatment of the present study, however why this did not occur equally in the 20% Fe-WTR treatment requires further investigation.

3.4 Earthworm survival, growth and reproduction tests

Survival of earthworms was very high in all controls (98.3 ±1.7%) and all treatments (93.3±3.3% for 20% Fe-WTR treatment, and >96% for all other treatments), with no significant differences (ANOVA, $p > 0.05$) observed between survival rates in treatments and controls. However, while it cannot be quantified, the earthworms in treatments with 20% WTRs (both Al- and Fe-) did appear less active (i.e. moved more slowly) than those in other treatments at the time of recovery.

The mean mass gain per earthworm was very similar between the controls (0.23±0.08 g) and most of the treatments (Table 1). A lower mean mass gain was seemingly observed in the Al-WTR treatments (range 0.06±0.06 g to 0.17±0.14 g) and a mean mass loss was observed in the 20% Fe-WTR treatments (-0.17±0.14 g), however these mean mass gains/losses were not significantly different from the control for any of the treatments (ANOVA, $p > 0.05$), possibly reflecting the variability of this parameter in the control group (i.e. 34% relative standard error, RSE). The number of cocoons produced was also similar across all treatments and controls, with a single exception for the 10%Al-WTR treatment where the number was lower (Table 1). While an ANOVA test found no significant differences ($p > 0.05$) amongst treatments and controls in relation to cocoon production, a t-test of the control vs. Al-WTR 10% did identify a significant difference for that treatment if no correction for multiple comparisons is made ($p = 0.034$). This may indicate an effect at the borderline of significance that warrants further examination, although it must be acknowledged that higher rates of Al-WTR addition (20%) did not induce any reduction in cocoon development (Table 1). Future studies can examine this point and also probe for evidence of any more subtle effects of WTR amendment on earthworm fitness and function, such as any changes to earthworm protein content and enzyme function as has been investigated in relation to other soil amendments/contaminants (e.g. Li et al., 2011; Zhang et al., 2013a). It is also important that future studies examine any impacts on earthworms over a longer period of exposure. The results do however suggest that earthworms may be less sensitive to WTR addition than certain plant species, at least in the short term, as some studies have reported plant yield decreases that may be linked to restricted phosphorus availability; for example Lombi et al. (2010) found lettuce (*Lactuca Sativa*) yield in a 4-week study decreased at WTR application rates of <1% by dry mass (e.g. EC50s of 0.3 and 8.5 t/ha in two contrasting soils), while Oladeji et al. (2007) determined in glasshouse trials that WTR amendment had to be balanced with supplemental fertiliser to maintain optimal yields of Bahiagrass (*paspalum notatum*).

Table 1. Average mass gain per earthworm and number of cocoons produced (mean ± standard error)

Treatment	Mean mass gain, g	Mean number of cocoons
Control	0.23±0.08	2.5±0.7
5% Fe-WTR	0.25±0.03	2.7±0.7
10% Fe-WTR	0.25±0.06	3.7±1.8
20% Fe-WTR	-0.17±0.15	2.0±0.0
5% Al-WTR	0.17±0.14	3.7±0.7
10% Al-WTR	0.06±0.06	0.3±0.3*
20% Al-WTR	0.09±0.11	2.0±0.6

* Significantly different from control

Acid digestion of earthworms revealed that the 10% and 20% Al-WTR treatments produced significantly higher (ANOVA, $p < 0.05$) tissue Al concentrations, viz. 3-5 fold greater than controls, but none of the other treatments generated significant increases in earthworm Al (Table 2 and sup. Inf. Table 2). The highest Al concentration was recorded in the 10%Al-WTR treatment, being 42 mg/kg fresh weight (fw) or 212 mg/kg dry weight (dw). The body burden at which Al becomes toxic for earthworms is unknown, however the concentrations observed here were all below the concentrations reported by Hartenstein (1980) for *E. fetida* in control soils (i.e. 437 mg/kg dw for unexposed earthworms, which rose to 940 mg/kg for earthworms that had been living for 2 weeks in sewage-sludge dressed soil), and were similar to concentrations reported by Bilalis et al (2013) for earthworms (*Octodrilus complanatus;* ~140 mg/kg dw) kept in untreated agricultural soils of similar pH. In terms of wider food-web considerations, a review by Scheuhammer (1987) reported that dietary Al at rates up to 1500 mg/kg had no negative impacts on ring doves (*Streptopelia risoria*), a passerine bird species, suggesting that Al concentrations observed in earthworms in the present study are of little environmental concern. The high pH buffering capacity of the WTRs and the resulting pH of the amended soils (>7), together with the widely understood low toxicity of Al at neutral pH, also support the notion that Al levels observed here are unlikely to be of concern.

Table 2. Element concentrations in *Eisenia fetida* earthworm tissues (mg/kg fresh weight)

	Al	Cd	Cr	Fe	Mg	Mn	Ni	Pb	Zn	P
Control	8.59±1.09	0.15±0.01	0.52±0.05	54.6±5.4	9.32±0.24	9.33±0.66	0.47±0.03	0.24±0.02	16.08±0.16	103.2±1.4
5% Fe	8.93±0.80	0.13±0.01	0.50±0.06	92.2±5.0*	9.21±0.13	12.81±1.14	0.51±0.05	0.25±0.03	15.26±0.78	93.7±2.6
10% Fe	5.20±1.39	0.11±0.01	0.37±0.08	81.3±25.7	6.49±1.40	11.57±2.52	0.42±0.08	0.19±0.04	14.33±0.65	73.1±19.5
20% Fe	7.23±0.88	0.13±0.01	0.47±0.02	167.3±13.3*	7.91±0.29	17.25±0.90*	0.56±0.02	0.26±0.01	14.53±0.36	97.0±1.6
5% Al	14.38±4.02	0.14±0.00	0.38±0.09	36.2±9.3	7.84±1.12	9.58±1.18	0.39±0.04	0.19±0.02	14.28±0.64	83.2±11.7
10% Al	42.44±11.61*	0.13±0.01	0.40±0.08	34.5±7.9	8.58±0.82	10.53±1.61	0.41±0.06	0.27±0.05	14.54±1.08	94.4±7.5
20% Al	28.41±8.35*	0.12±0.02	0.32±0.01	26.6±2.0	6.70±1.19	8.57±0.36	0.33±0.00	0.21±0.01	13.82±1.26	81.0±17.6

* Significantly different from control (α 0.05)

Addition of Fe-WTR significantly increased earthworm Fe concentrations at the 5% and 20% addition rate (Table 2), with concentrations of 167 mg/kg fw (equating to 837 mg/kg dw) recorded at the higher rate. The Al-WTR treatments all had lower mean Fe concentrations than the controls, and for the 20% Al-WTR treatment the difference was statistically significant (ANOVA, $p < 0.05$). The Fe concentrations observed in the 5% and 20% Fe-WTR treatments approximate those reported for *E. fetida* by Hartenstein (1980), who reported 684 mg/kg dw in control specimens rising to 1069 mg/kg in earthworms after 2 weeks of exposure to sewage sludge dressed soil. Similarly, Rida (1996) observed Fe concentrations in *Lumbricus terrestris* controls of 418 mg/kg dw, rising to 1066 mg/kg dw after 1 week in a metal contaminated soil. Importantly, in that study Rida (1996) found that even at the higher concentrations observed there were no correlations between earthworm Fe levels and either mass or relative growth rate. This suggests that the Fe concentrations observed in the present study would not be problematic for earthworms. The only treatment with Mn concentrations significantly different from controls was the 20% Fe-WTR treatment, but the difference was less than a factor of two (Table 2). With regards to Pb, none of the treatments resulted in concentrations that differed from controls, having all been <0.3 mg/kg fw (or <1.4 mg/kg dw equivalent). Langdon et al (2005) found earthworms living in un-amended control soil (i.e. no added metals) to have dw Pb concentrations ranging 0.43 mg/kg for *Aporrectodea caliginosa* to 16.43 mg/kg for *Eseinia andrei* (a closely related species to *E. fetida*), indicating that the Pb concentrations observed in all treatments of the present study with *E. fetida* can be considered normal for uncontaminated soils. It is worth noting that no significant differences were observed in terms of earthworm P concentration in any of the treatments, which contrasts with the studies mentioned above (e.g. Lombi et al 2010) that reported reduced P availability in WTR treated soils (albeit that the cited studies concerned plants rather than earthworms and so any differences in assimilation pathways also need to be considered).

3.5 Soil porewater extraction

Porewater Cd and Cr were at very low levels in all controls and treatments (Table 3). Amendment with Al-WTRs at 10% and 20% application rates decreased the soil porewater concentrations of Mg, Ni, Zn and, notably, P (by a factor of ~2), which accords with the P-sorbing capacity of WTRs noted previously (Lombi et al., 2010; Oliver et al., 2011). At all amendment rates Al-WTR addition increased the porewater Al concentration (Table 3), however it always remained below 40 µg/L which is still low by comparison with values reported for soils elsewhere (e.g. Graham et al., 2008). The pH values of the soil and the buffering capacity of the WTRs makes it very unlikely that this marginal increase in porewater Al will have any ecological significance.

While the porewater Zn concentrations were decreased by ~half in the 10% and 20% Al-WTR treatments, they were increased 5-8 fold in the Fe-WTR treatments (Table 3). This may be worth further consideration in terms of Zn nutrient supply capacity of Fe-WTRs, while noting that the higher concentrations observed in the Fe-WTR treatments were below negative impact thresholds reported elsewhere for Zn porewater concentrations (i.e. EC10 values for soil microbial processes (Smolders et al., 2004). Interestingly, the highest Fe-WTR application rate increased the porewater P concentration above that of the control, showing a clear difference to the Al-WTR treatments. The Fe-WTR treatments also increased the porewater Ni concentrations and, at the highest application rate, the Mg concentration, however the Ni concentrations were all below porewater toxicity thresholds previously reported (i.e. EC10 for root elongation >> 200 µg/L, Zhang et al., 2013b) while the Mg concentrations were at or below typical background soil porewater concentrations (e.g. Zhang et al., 2013b).

Table 3. Mean soil porewater concentrations (n=3, ± standard error; mg/L for Mg and µg/L for other elements)

	Mg	Al	P	Cr	Mn	Fe	Ni	Zn	Cd
Control	3.60±0.20	5.4±0.9	323.9±45.7	<0.65	54.97±6.6	16.3±1.2	22.3±0.4	6.8±0.6	<0.2
Al-5%	2.72±0.50	13.1±0.9*	301.2±127.7	<0.65	33.43±4.5	22.8±9.2	18.2±0.9*	7.7±4.6	<0.2
Al-10%	1.56±0.26*	28.8±1.8*	174.8±21.1*	<0.65	37.16±11.8	15.2±1.5	15.8±0.2*	3.2±0.4*	<0.2
Al-20%	1.11±0.04*	39.3±3.7*	141.6±45.6*	<0.65	19.04±2.6*	10.4±1.2*	13.2±0.1*	2.9±0.4*	<0.2
Fe-5%	5.74±0.61	11.31±3.0	445.8±114.7	<0.65	44.00±3.8	29.3±5.7	34.6±5.9*	45.3±37.3	<0.2
Fe-10%	6.12±3.1	17.5±2.8^	469.9±95.9	<0.65	59.71±8.9	34.4±1.8^	39.8±2.4*	59.0±33.5*	<0.2
Fe-20%	16.18±1.3*	15.3±3.1*	835.8±172.1*	<0.65	70.97±8.0	36.8±5.6*	52.1±1.7*	31.2±7.7*	<0.2

^N = 2 only; * significantly different from control at α 0.05.

4. Conclusions

The principal conclusions from the present study were that element leachability was low in the WTRs examined and would likely pose no threat to the soil ecosystem under most conditions observed in typical agricultural soils, however when the pH was lowered to 4.4 there was a substantial release of Al from the Al-WTRs. When applied to a sandy soil, WTR addition influenced soil porewaters to some degree and this warrants further examination in terms of any potential implications for nutrient supply or limitation. Earthworm avoidance of the WTR-amended soil was only observed for Al-WTRs and only at the maximum rate of 20% (w/w) applied, while survival of earthworms was not affected by either WTR type at any application rate. Earthworm growth and reproduction (cocoon production) were not affected at a statistically significant level but this should be examined over a longer period of exposure and, as with all the assessments conducted here, in a wider set of soil types. Increased assimilation of some elements (Al and Fe) into earthworm tissues was observed but not at levels likely to pose environmental concerns.

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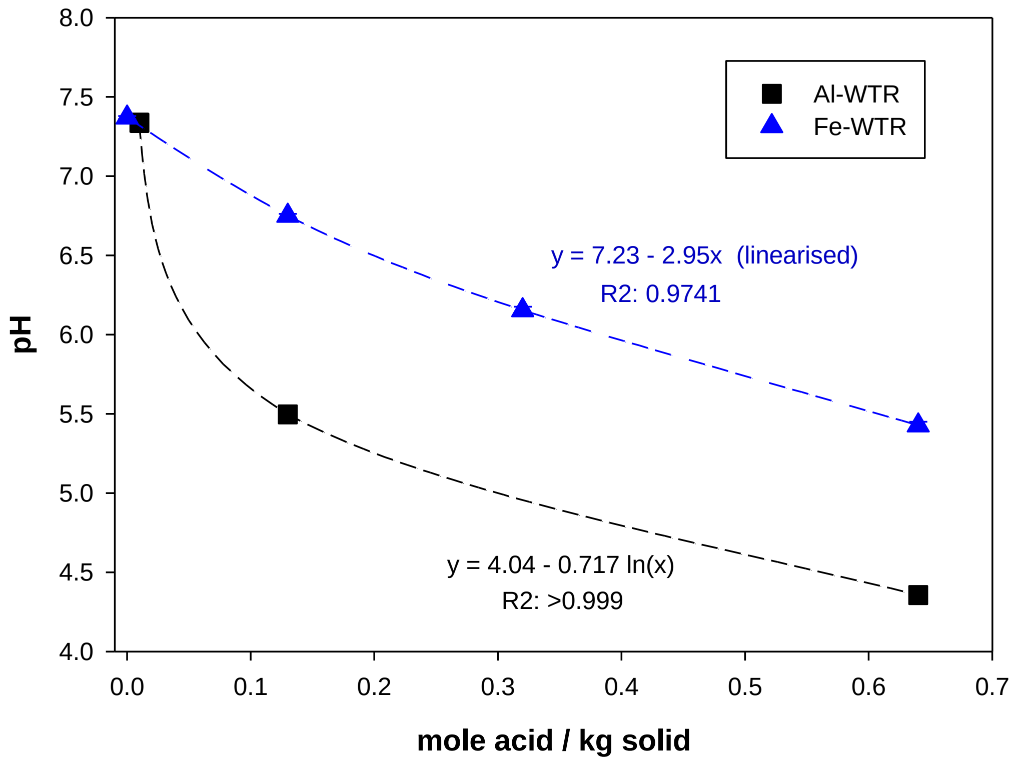


Figure 1. Applied acid vs pH plot for Al- and Fe- Water Treatment Residuals (WTR) in 0.001 M $CaCl_2$ extracts. Data points show mean (n =3) and error bars, where they exceed symbol margins, indicate standard error.

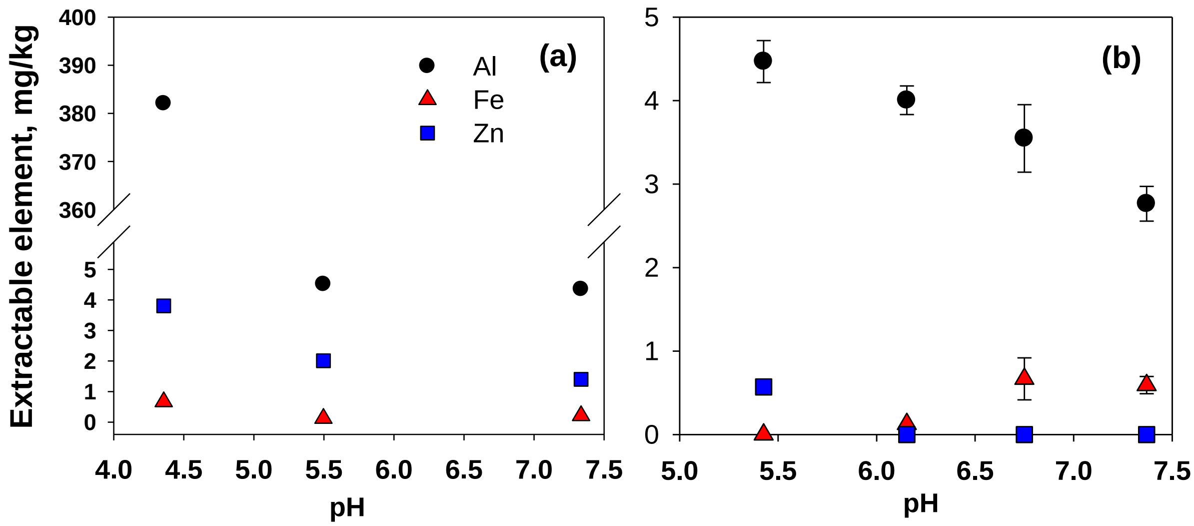


Figure 2. 0.001 M $CaCl_2$ extractable element concentrations in Al-WTR (a) and Fe-WTR (b) at varying solution pH (adjusted with HCl). Error bars indicate standard errors about mean, n=3 (note extractability of As, Cd, Cr and Pb was ≤1 mg/kg at all pH levels and so are not depicted).

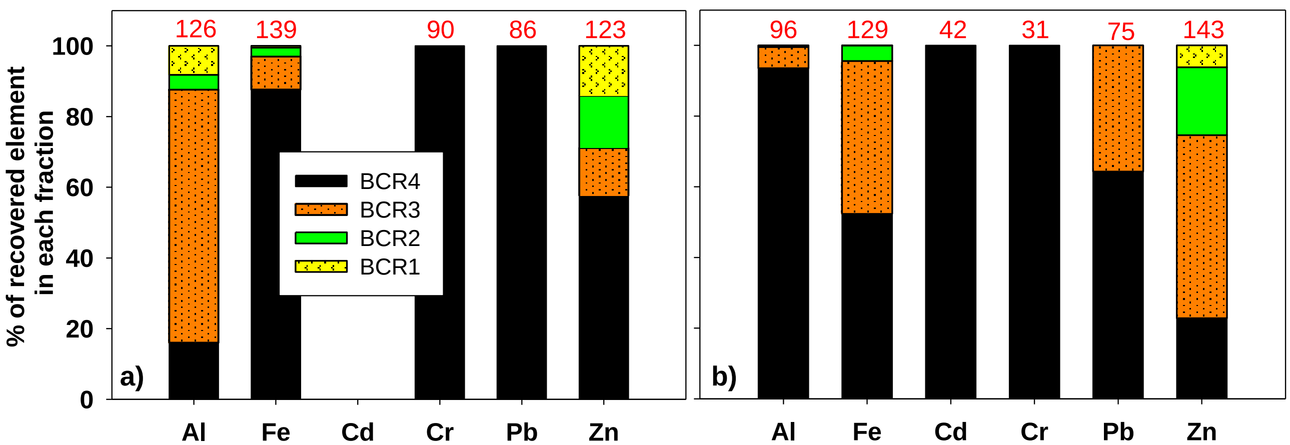


Figure 3. Element fractionation in aluminium (a) and iron (b) based water treatment residuals according to the BCR sequential extraction scheme (BCR 1= 0.11 M acetic acid; BCR 2= 0.1 M hydroxyl ammonium chloride at pH 2.0; BCR 3= hydrogen peroxide treatment followed by heating and then 1.0 M ammonium acetate at pH 2.0; BCR 4 = aqua-regia digestion of residues). Percentages for each fraction are relative to the sum of all fractions for a given element. The numeric values above the columns indicate the element mass balance, i.e. 100 x sum of BCR fractions/original total digest.

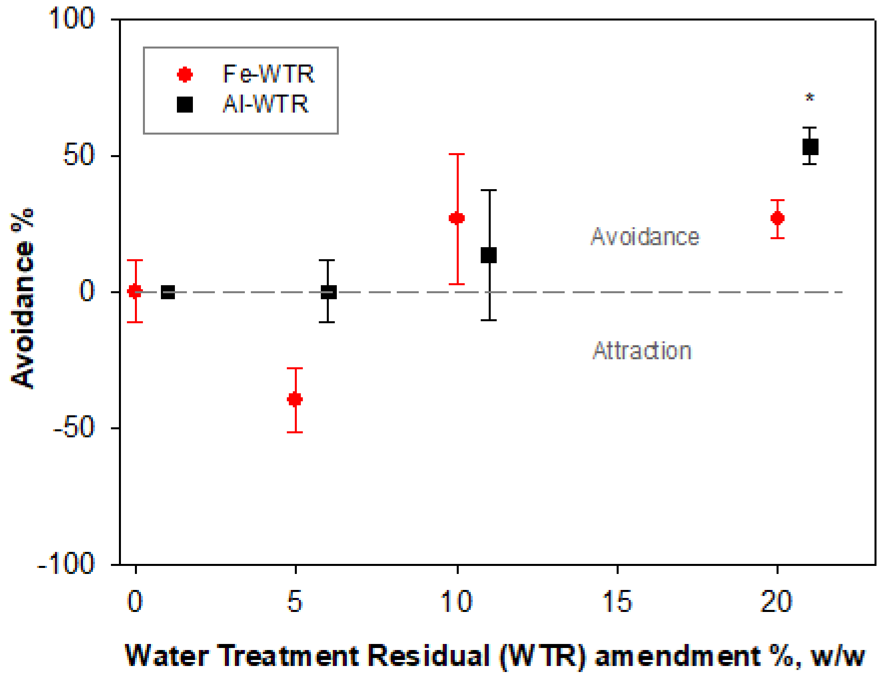
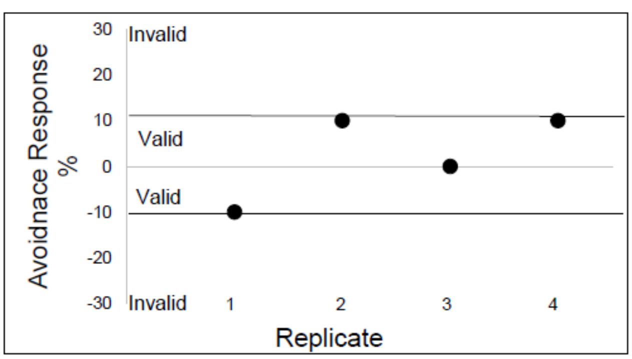


Figure 4. Earthworm avoidance test (48h) at 0, 5, 10 and 20% WTR addition rate (note slight off-set for ease of viewing). The asterisk (*) indicates significantly different avoidance value in the 20% Al-WTR treatment compared with the dual control (i.e. un-amended soil on each side of test vessel). Error bars indicate standard error about mean (n=3).

SUPPLEMENTARY INFORMATION



SI Figure 1. Preliminary experiment verifying that the avoidance test setup met all validity criteria in terms of no avoidance or attraction bias.

SI Table 1. BCR element fractionation (mg/kg, mean ± standard error SE) and mass balances (sum of fractions / total digest)

Al-WTR	Al	SE	Cd	SE	Cr	SE	Fe	SE	Pb	SE	Zn	SE
BCR1	11998	173	b/d	-	b/d	-	65	1	b/d	-	14.7	1.6
BCR2	6173	73	b/d	-	b/d	-	317	3	b/d	-	15.3	0.4
BCR3	105251	743	b/d	-	b/d	-	1189	56	b/d	-	14.1	0.4
BCR4	23488	903	b/d	-	9.3	0.3	11121	360	5.00	0.19	59.8	2.2
Fraction Sum	146909			-	9.3		12692		5.00		104.0	
Total digest	116400			-	10.3		9110		5.80		84.2	
Sum/total	1.26				0.90		1.39		0.86		1.23	

Fe-WTR	Al	SE	Cd	SE	Cr	SE	Fe	SE	Pb	SE	Zn	SE
BCR1	6.6	0.4	b/d	-	b/d	-	65	1	0	0	13	0.8
BCR2	25.0	0.4	b/d	-	b/d	-	9980	203	0	0	40	0.3
BCR3	410	6.6	b/d	-	b/d	-	98730	751	9.0	0.10	109	8.2
BCR4	6416	1483	12.4	0.9	1.53	0.72	119389	5003	16.3	0.83	48	2.1
Fraction Sum	6857		12.4		1.53		228165		25.3		210	
Total digest	7135		29.3		4.87		176850		34.0		147	
Sum/total	0.96		0.42		0.31		1.29		0.74		1.4	

SI Table 2. Element concentrations in *Eisenia fetida* earthworm tissues converted to dry mass equivalents (mg/kg ± standard error)

	Al	**Cd**	**Cr**	**Fe**	**Mg**	**Mn**	**Ni**	**Pb**	**Zn**	**P**
Control	43.0±5.5	0.75±0.05	2.6±0.3	273.2±27.1	46.6±1.2	46.6±3.3	2.4±0.2	1.21±0.12	80.4±0.8	516.1±7.0
5% Fe	44.7±4.0	0.63±0.05	2.5±0.3	460.8±24.9	46.1±0.6	64.1±5.7	2.5±0.2	1.24±0.13	76.3±3.9	468.5±12.9
10% Fe	26.0±6.9	0.57±0.05	1.9±0.4	406.2±128.7	32.4±7.0	57.9±12.6	2.1±0.4	0.94±0.20	71.6±3.3	365.3±97.6
20% Fe	36.1±4.4	0.66±0.03	2.4±0.1	836.6±66.3	39.6±1.5	86.2±4.5	2.8±0.1	1.30±0.05	72.6±1.8	484.8±8.0
5% Al	71.9±20.0	0.70±0.02	1.9±0.4	180.7±46.5	39.2±5.6	47.9±5.9	1.9±0.2	0.97±0.12	71.4±3.2	415.9±58.4
10% Al	212.2±58.1	0.66±0.04	2.0±0.4	172.6±39.7	42.9±4.1	52.7±8.0	2.1±0.3	1.37±0.26	72.7±5.4	471.7±37.6
20% Al	142.0±41.8	0.60±0.08	1.6±0.1	132.7±10.0	33.5±6.0	42.8±1.8	1.6±0.0	1.05±0.05	69.1±6.3	405.0±87.7