The application of X-ray micro Computed Tomography imaging for tracing particle movement in soil

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Abstract

Despite significant advances in recent years in the application of sensing and imaging technologies to the study of the hydraulic behaviour of soils, our understanding of how solutes and particulate matter move through soil is still limited and often based on idealised soil structures. The aim of this work was to use an appropriate proxy material, in this case (Decabromodiphenyl ether (DBDE)), to represent 1-2 µm sized chemical particles, frequently used in agricultural practices, in order to trace their spatial and temporal movement through a soil column. X-ray Computed Tomography (CT) was employed to map the 3D pore geometry and facilitate visualization of the concentration distribution of the highly X-ray attenuating proxy material as it was applied and subsequently leached through the soil over a 5 hour period. Simultaneously the soil eluate was collected from the bottom of each column and the concentration of proxy material measured using Gas Chromatography to compare with the imaged data. The method developed for visualising the tracer material in 3D at the pore-scale was successful. The results demonstrated that the tracer material initially moved rapidly through the upper portion of the soil but subsequently became immobile, despite repeated elution with water as confirmed by imagery. Similar concentrations to those applied to the soil surface were not recorded at any point in time towards the bottom of the soil column. By visualising the DBDE distribution in 3D it was possible to correlate the highest concentrations of the tracer material with the location of the smaller size pores (i.e. mesopores). Dynamic X-ray CT imaging of tracer materials has considerable potential as a proxy for the visualization of particulate agricultural chemicals that will assist with understanding how their transport behaviour varies both spatially and temporally.
Introduction

The UK National Action Plan for Sustainable Use of Pesticides (Defra 2013) encourages the sustainable use of pesticides and highlights the importance of the life cycle of chemicals. As intensification of agriculture accelerates to meet increasing food demand, it is becoming increasingly important to understand precisely how agricultural chemicals interact with, and move through, soil (Carvalho 2006). Such agrochemicals are known to cause contamination in surrounding ecosystems when applied excessively (Gamon et al. 2003), therefore development of sustainable management practices are essential to achieve both increased crop production and decreased pollution. Improved understanding of agrochemical behaviour in soil will contribute not only to the alleviation of environmental impact but also to the possibility for improvement in controlled placement leading to increased bioefficacy and reduced use rates.

The movement of agrochemicals through soil is strongly influenced by the physical, chemical and biological characteristics of the soil and plants, as well as those of the chemical itself. This intricate combination of influences makes understanding how an agrochemical moves through soil both spatially and temporally extremely difficult. Traditional methods for examining solute flow through soil have focused on conventional bulk ex-situ chemical analysis of soil and eluate (Andreu and Picó 2004). Continuous soil column tests have been routinely used to produce breakthrough curves to characterise the behaviour of the chemical of interest (Brusseau et al. 1991). Such techniques have also been combined with
mathematical models to predict chemical movement e.g. Magga et al. (2012). In combination these approaches have been used to provide detailed information on the fate of chemicals applied to soil, however research in this area has been hindered by a lack of real time data, particularly from a three dimensional (3D) perspective. One technique that has shown promise in this regard is X-ray Computed Tomography (CT) which allows the visualization of the internal fabric of a porous media such as soil. With CT it is possible to non-destructively visualize the internal pore geometry of the structured media and, with repeating scanning, characterisation of the active processes in situ can be attempted assuming the solute/particulate material can be distinguished from the soil background. X-ray attenuation is linked to the material elemental composition; hence elements with a high atomic number have a higher X-ray attenuation and are potentially good candidates to act as a tracer of solute flow in a soil.

The use of tracers in X-ray CT imaging is routine in medical research to improve the contrast of blood vessels and tumours and a wide range of tracers have been applied such as gold nanoparticles (Nune et al. 2009) and iodinated agents (Lusic and Grinstaff 2012). There has been limited research into the use of tracers to visualise solute movement in soil (Anderson et al. 1992, Clausnitzer and Hopmans 2000, Anderson et al. 2003, Luo and Lin 2008). A key limitation is that X-ray CT is best suited to the visualisation of motionless objects (Luo and Lin 2008) as the scan process generates spatial data of the entire sample over the whole course of the scan, with any effect of change in the material morphology typically corrected at the end of the scan. However with the repeated scanning of the same sample over the shorter timescales that is now achievable (as low as 5 minutes), it is possible to include time as a variable providing the solute transport is not too rapid. Mooney (2002) used X-ray CT to
visualize soil water movement, identifying preferential flow paths, in soil columns by spatial subtraction of images pre- and post-wetting. Anderson et al. (2003) combined X-ray CT imaging with an iodide breakthrough experiment, and was able to compare the results with laboratory derived measurements of saturated hydraulic conductivity. Luo and Lin (2008) passed an iodide tracer through a soil column (30 cm) over a 23 hr period and repeatedly scanned the soil column to demonstrate the spatial variation in hydraulic behaviour of the soil.

To date there has been limited research concerned with tracking the movement of particles or suspensions in soil using X-ray CT at the pore (µm-mm) and column (mm-cm) scales. In this work we tested a novel proxy for agrochemical suspension concentrates, Decabromodiphenyl ether (DBDE), chosen due to its X-ray attenuation properties and similar suspension behaviour compared to routinely used compounds such as Chlorothalonil, a broad spectrum fungicide which has an average particle size of 2 µm (Curry et al. 1998). The objectives of this investigation were to determine the efficacy of DBDE as a general proxy tracer for particulate agrochemical movement in soil via CT imagery and to investigate if X-ray attenuation can be used as a surrogate for tracer concentration over time and following elution by water.

**Materials and Methods**

**Soil column preparation**

Soil was obtained from the ‘18 acre’ field site at Syngenta Jealott’s Hill International Research Centre, Bracknell, UK. The soil was a Brown earth with a sandy clay loam with 49 % sand, 27 % silt, and 24 % clay. Soil was sieved to < 2 mm and wetted to 17 % moisture.
content; centrifuge tube filters were used as soil columns (23 mm diameter, 40 mm length), with holes at the bottom to allow free drainage of soil solution. The outer tube holder was used to hold the columns during scanning and to collect soil eluate. Six tubes, each containing different concentration of the tracer suspensions used for calibration during the image analysis process, were secured to the inside edge of each column, before the soil was packed into the columns at a bulk density of 1 g cm\(^{-3}\). To examine tracer movement through 40 mm of soil, triplicate columns were packed to heights of both 10 and 40 mm and the bottom 10 mm of each of these columns scanned to represent tracer movement at the top and bottom of a 40 mm soil section, hence six columns in total. Eluate was collected from the bottom of each section after each scan in a small container added to the bottom of the sample for this purpose; the columns were left to saturate overnight the day before scanning.

**Tracer material and calibration**

DBDE (from Sigma-Aldrich) was chosen as the tracer material due to its high X-ray attenuation capacity arising from the high electron density of bromine. It was milled using Yttria Stabilized Zirconia Beads (0.3 mm) to generate particles with a size range of 0.1-6 µm in diameter and a volume mean diameter (VMD) of 2-3 µm suspended in DI water with 5 % Soprophor® 3D33 (tristyrylphenol polyethoxyester phosphate ester, Solvay) as a dispersing agent. An initial test was undertaken to confirm that DBDE was detectable in soil and then to identify the appropriate concentration for application (cross-sectional X-ray images of the tracer and tracer embedded in soil are shown in Supplementary Figure 1). From this a DBDE suspension was prepared with a concentration of 200 mg ml\(^{-1}\) and sonicated for 1 hour before use. DBDE particle suspensions were then prepared with concentrations of 25, 50, 100 and 200 mg ml\(^{-1}\) and Kelzan S® (CP Kelco), a natural xanthan gum based thickener, was added.
to reduce settling of particles over time. The suspensions were pumped into rubber tubing (0.89 mm diameter) using a peristaltic pump after which the tubing was cut to 40 mm length and sealed with PVA glue, before being secured to the side of each column with adhesive tape.

X-ray CT scanning procedures

Each column was scanned using a Phoenix Nanotom® X-ray μCT scanner (GE Measurement & Control Solutions, Wunstorf, Germany). Discrete regions of interest (ROI) only were scanned at settings of 120 kV and 90 μA with a 0.1 mm copper filter. Projections (n = 1200) were captured over a 30 minute time period for each column, with a pixel resolution of 12.5 μm, as such we do not resolve individual particulate material below this. Each column was positioned so that only the base of the column (10 mm) was scanned i.e. the region 0-10mm was scanned for half the treatments and the region 30-40mm for the other half. This approach allowed us to directly relate the associated physical and chemical measurements of these regions to the obtained X-ray images. Each of the six columns was scanned before, and after, the application of 1 ml of DBDE (200 mg ml⁻¹) and then scanned a further 10 times continuously over a 5 hr period. At the beginning of each post-DBDE scan 1 ml of water was added to the top of the column and the soil eluate collected from the tube holder at the end of each scan. A total of 72 CT scans were undertaken.

Chemical analysis of DBDE

Gas chromatography was used to determine the DBDE concentration in the eluate collected from the bottom of each soil column after each scan (i.e. 30 min intervals). The aqueous soil eluate samples containing DBDE particles were dried using a Genevac HT-4X centrifugal
vacuum evaporator. Toluene was added to the dried deposit to dissolve the DBDE after which the samples were centrifuged to remove soil particles. Samples were diluted further in toluene as required before analysis by gas chromatography using an Agilent 6890 Series GC System, equipped with cool on column injection system, a fused silica DB-5 column (15 m x 0.25 mm id; 0.25 µm film thickness) (Agilent J&W, Agilent Technologies) and an electron capture detector. The peak area for DBDE was used to calculate the DBDE concentration of the sample, with reference to a calibration standard. By determining the concentration of DBDE collected from the eluate of each section it was possible to construct a chemical breakthrough curve for each section of the soil column by plotting % recovery over time.

Image processing and analysis

The radiographs of each scan were reconstructed in 32 bit format to avoid compression of the greyscale histogram, which is especially important when attempting segmentation of materials with similar attenuation. After reconstruction each image volume of 10 mm x 23 mm diameter was imported into Volume Graphics (VG) StudioMAX® 2.0 and the images cropped to a cylindrical shape with a height of 7.76 mm and diameter of 2.3 mm. A median filter of 3 pixels was applied to correct for any residual noise.

Suspension concentration calculations

A region within each tracer calibration tube was manually selected and the average greyscale value within it was calculated using the volume analyser tool in VG StudioMAX® 2.0. The average greyscale values along with the known DBDE concentrations were used to calibrate each scan to calculate the concentration of the tracer. It was important to undertake this process for each individual scan even though time consuming as brightness (and hence
greyscale value) can vary between CT scans due to ageing of the X-ray tube filament thus influencing the image segmenting process.

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The calibration tool in VG StudioMAX® 2.0 facilitates a thresholding technique that can be used to create ROIs based on pre-selected greyscale values chosen to represent the background and the material. We observed three clear ROIs for soil, solute-filled pores and pores filled with only highly attenuating tracer particles (seen as the white particles in Figure 1B-D). The distinction between solute filled pores and tracer filled pores was the former, which we attribute to the added solution that contains DBDE and tracer filled pores, had a significantly lower X-ray attenuation than the latter which we attribute to aggregated, settled-out DBDE particulate material. This was confirmed in the elution studies were the location of solute filled pores varied over time and the tracer filled pores did not. It was not necessary by this approach to segment the air-filled pores though this would have been straightforward if necessary. Figure 1 illustrates the method used for creating ROIs for the total tracer filled pores (C), essentially the combination of ROIs for solute filled pores (A) and deposited tracer filled pores (B). The volume analyser tool was used to provide the average greyscale value and the volume of each ROI, as well as an individual greyscale histogram. The average greyscale value of the final ROI was then used to derive the relative tracer concentration of the tracer in each scan. To ensure that no residual soil mineral material was included in the calculation of the deposited tracer filled pores, soil mineral content was segmented using the automatic threshold option in VG StudioMax and then the histogram frequency data of the solute filled pores and the mineral portions were aligned and the frequencies subtracted. The
average greyscale value was then recalculated using the new frequency list and the resulting concentration determined.

Pore size analysis

In order to examine the relationship between soil pore size and tracer concentration, the images of the tracer filled pores only i.e. settled out DBDE as described above were imported into the image processing software ImageJ 1.46r. Here it was possible to separate the pores that were filled with highly attenuating deposited tracer material into two classes based on size, in this case pore diameter. We used the size classes as described by Brewer (1988); meso-pores 30 – 75 µm and macro-pores > 75 µm to separate the pores within each image. Image stacks of these pore sizes were imported back into VG StudioMAX® 2.0 to obtain the average greyscale value data and hence concentration of DBDE.

Results

Figure 2A shows a typical greyscale X-Y image of soil produced from an X-ray CT scan. The composition of the soil material is displayed in a range of grey shades that are visible due to differing levels of X-ray attenuation. The average greyscale values were determined via the calibration tubes that were placed on the outside of the soil column during the scanning process to represent concentrations of 25, 50, 100 and 200 mg ml⁻¹ respectively (Supplementary Figure 1). There was a strong relationship between DBDE concentration and X-ray attenuation (R² = 0.98), which can be seen by eye, as concentration increases the material within each tube appears lighter in colour. Figure 2B shows a scan taken from the same location as Figure 2A, after the addition of 1 ml of the DBDE tracer (200 mg ml⁻¹) with the highly attenuating tracer visible in the soil pores. The final binarised image (Figure 2C)
highlights just the tracer filled pores (in white) after removal of the surrounding soil and mineral grains. Using the average greyscale value of the tracer-filled pores from each image, a value for DBDE concentration could be derived from the calibration graph.

By repeated washing through of the DBDE with water, and subsequent re-scanning, a tracer breakthrough curve (Figure 3) was obtained for each of the 10 mm soil sections. The mean concentration of tracer in the uppermost 10 mm section immediately following application of DBDE was 80 mg ml\(^{-1}\) which declined rapidly over a 3 hour period to 42 mg ml\(^{-1}\). The concentration continued to decline over the next 2 hours, albeit at a slower rate, and reached 28 mg ml\(^{-1}\) at the end of the experiment. This incomplete breakthrough is supported by the low concentration of tracer in the lowermost 10 mm section (30-40 mm depth in Figure 3) where the concentration showed a slight increase over time but no defined peak breakthrough.

The same trends were observed in the Gas Chromatography recovery of DBDE (Figure 4). The eluate collected from the bottom of the 0-10 mm section had an initial peak recovery of 15.7% of the total added DBDE, which rapidly declined after the first 1 hour 30 minutes to 4.5%, before decreasing to <1% by the end of the 5 hour period. The recovery from the 30-40 mm section was different; after the addition of the DBDE the recovery was <1% which increased to 3.6% by 90 minutes declining to 2.6% by the end of the experiment. After 3 hours the recovery from the eluate collected from both sections was broadly similar.

To understand more about the effect of pore size on tracer transport, the tracer filled pores category were separated into two groups defined by diameter, meso-pores, 30 µm-75 µm and
macro-pores, >75 µm. Figure 5A demonstrates how the distribution of the tracer changed over time in the uppermost 10 mm section of the soil column. Initially the soil was very wet, with only 16 % of the pores of the total pore volume air filled (as derived by imagery hence limited to the resolution used in this study). As the tracer infiltrated the soil, the proportion of air-filled pores increased due to drainage despite the repeated washing through of water, and the proportion of tracer-filled pores decreased in both the macro-pores and meso-pores classes. The volume in the macro-pores remained higher than that of the meso-pores throughout (mesopore volume was <15% of the total pore volume by the imaging approach and classification used here). Figure 5B illustrates the link between pore size and concentration, only for the tracer-filled pore category, showing that while the concentration of the tracer in both the meso- and macro-pores decreased, it remained consistently higher in the meso-pores despite this only accounting for a small part of the total pore volume. The relationship between the concentration of solute and tracer filled pores (derived by the calibration from the X-ray images) and pore size is visualised in 3D over time in Figure 6. Tracer movement is revealed to be less rapid than the associated redistribution of water through the repeated washing through process; in addition, the higher concentration of DBDE in the smaller sized pores is clearly visible.

**Discussion**

DBDE in suspension has been shown to be a useful particulate tracer material from an attenuation perspective, which highlights its potential to act as a proxy for active ingredients used in agrochemicals. The use of calibration standards for each sample/scan enabled an accurate construction of the DBDE tracer breakthrough helping to remove the influence of any fluctuations caused by X-ray CT parameters (Clausnitzer and Hopmans 2000). However
we identified issues with its mobility beyond a shallow layer of soil. The breakthrough results clearly demonstrated low particle breakthrough in the soil column at 40 mm depth (Figure 3). This lack of movement occurred despite the addition of water every 30 minutes. This contrasts with the results in the upper 10 mm where significant movement occurred in the early part of the experiment before rapidly declining suggesting that particles of DBDE were immobilised within the soil column. These data were confirmed by the recovery analysis in the eluate. Similar studies have also observed this for non-soluble particulate agricultural chemicals i.e. following application there is initial, limited movement but when leaching stops (Noling 2012) further movement only occurs if particles are dispersed (Paradelo et al. 2013). This is highly relevant to the development of new particulate morphological arrangements for agrochemicals that might have better mobility than the DBDE used here, though noting that there are advantages, such as increased residency, associated with the reduced mobility of compounds such as pesticides in soils. The extent to which mobility could be enhanced via a reduced particulate size or use of surfactants would be worth further research. As would the assessment of whether mobility of the tracer could be improved beyond the time period of elution in this study.

The pore morphometric data suggests the majority of the pores were filled following the tracer application and as water was applied, the volume of tracer filled pores decreased over the first 3 h of the experiment. After this stage the tracer particles were likely to have settled out of suspension illustrated by the lack of concentration change in each section after 2.5 h. Figure 5 shows that the concentration of the tracer in the meso-pores was continually higher than in the macro-pores, indicating that the particles collected in smaller pores and did not re-suspend easily, preventing complete breakthrough. Paradelo et al. (2013) made similar
observations when examining the transport of Cu bound to soil particles, using particle breakthrough curves combined with X-ray CT, indicating if particles did re-suspend, they might only move short distances and then reclog pores. Meso-pores occupy a size range within soil referred to as the ‘storage pore’ size range; 0.5-50 µm. These are often pores located within soil aggregates where water is held strongly and drainage is unlikely (Cornejo et al. 2004). One possibility for future studies is to consider that the combination of particle size and soil pore size used here was sub-optimal for enhanced particulate transport and that soil larger pores and/or smaller particles might have been more mobile.

The linking of soil pore characteristics to tracer movement highlights new capabilities for the use of X-ray CT in soil hydrology studies. Clausnitzer and Hopmans (2000) stressed the need for higher resolution in order to improve the accuracy of breakthrough experiments. Here we obtained a resolution of 12.5 µm, significantly higher than most comparable studies e.g. 86 µm (Clausnitzer and Hopmans 2000), 105µm (Luo and Lin 2008) and 2mm (Anderson et al. 2003). The benefits of higher resolution and repetitive scanning is the temporal pore-scale view (Figure 6), illustrating the difference in concentration between the tracer filled pores, in addition to the movement of DBDE. Despite the improvement in resolution, visualization of individual tracer particles was not possible here however via synchrotron imagery this would be possible albeit on smaller samples than considered here. Using our approach the overall particle movement can be derived from the movement of the suspension. The majority of the previous research that account for water flow mechanisms in soil have considered solute tracers rather than particulate material. However there has been widening research into particle movement in soil, in particular relating to chemical fate where natural occurring particles can either enable or prohibit the transport of adsorbed chemical
species (Kretzschmar et al. 1999, de Jonge et al. 2004, Sen et al. 2004) which highlights the importance of this area for further study.

**Conclusions**

X-ray CT offers a previously unachievable method for non-destructive 3-D visualization of tracer material embedded within soil through time. The method developed here not only illustrates movement of tracer in the soil but also the concentration. The behaviour of the tracer can then also be directly linked to the soil pore architecture. In this research we have demonstrated that the tracer, DBDE, was mobile only for a short time period (up to 3 hrs) after which it became immobile despite repeated elution of the soil column. From an agro-chemical perspective this would have advantages and disadvantages. By imaging the pore structure it was possible to show that the pore size in particular has a significant influence on the retention and concentration of the tracer. Whilst definitive conclusions regarding specific agricultural chemical particulate behaviour cannot be drawn from this experiment alone, DBDE quantified by X-ray CT has shown potential as a proxy for the visualization of particulate agricultural chemicals to help assist management decisions concerned with their fate in the rootzone and wider environment though further work is required across a range of particulate sizes and soil pore size/type combinations. Such studies could also be used to direct future research into optimising the morphological design of the particulate material used in future agrochemicals.

**Acknowledgements**

The authors thank Orla Sheehan for performing the GC analysis. We thank Marion Colin and Celina Love for preparing the DBDE suspension concentrates and eluate samples for
This work was funded by Syngenta Limited and the University of Nottingham via a studentship awarded to KG.

References


