2nd Year Group Research Project (GEOG20009)
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The Avon Partner Project 2019

Project carried out in partnership with Malago Valley Conservation Group

Heavy Metal Contamination in Fruit Trees in Manor Woods Valley Orchard

49609, 52005, 54357, 50241, 51606

ABSTRACT

Heavy industry and its waste can contaminate soil, potentially contaminating fruit trees. Eighteen soil samples from the Manor Woods Valley Orchard, Bristol were tested for heavy metal (HM) contamination, along with five twigs and an apple. The impact of past brick and tile works was explored, and the possibility of planting more fruit trees reviewed. The soil was tested for HM levels, and soil characteristics known to affect HM uptake by plants - pH, carbonate content, organic matter (OM) content and grain size. The pH and clay content were not found to be in the range that affects HM uptake. However, the low OM may increase HM uptake into trees, while the high carbonate may inhibit it. All samples showed low HM concentrations suggesting no contamination. The resulting data led to the conclusion that fruit from this orchard is safe, and thus planting more fruit trees here can be recommended.

Word Count: 7852

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1. INTRO

The presence of industrial works and the waste left behind can have a huge impact on the health of the soil. Heavy metals such as lead and zinc can be found in the soils surrounding brickworks, which have the potential to be taken into the plants growing in these areas (Brumsack, 1977). Heavy metals are natural metallic elements of high density and atomic weight, and are toxic even at low concentrations (Tchounwou, 2012). Heavy metals are not degraded naturally and thus stay in soils for long temporal periods once they are polluted into the soils (Liu et al., 2013). They pose a wide range of risks to human health, depending on the metal in question. For example, cadmium is linked to kidney damage, mercury and lead to neurological damage, and arsenic to a variety of cancers, specifically skin cancer (Jarup, 2003).

Plants take up heavy metal from soils either from the solution phase, deposition of the contaminants through the air or via direct application (Liu et al., 2013). Heavy metal ions are then transported from the roots to other parts of the plant via xylem cell sap (Murtic, 2014). The relative mobility of heavy metals within the plants themselves is dependent on numerous factors including the metal type, its concentration, and the plant species (Murtic, 2014). When studying heavy metal contamination in soil in relation to plant uptake, it is essential to look at bioavailability rather than total heavy metal content solely. Bioavailability is a process driven by many different factors and can be divided into external and internal bioavailability - the former being the ability of the metals to go from the soil solution to the plant, and the latter being the ability of the metal to be mobile and exercise toxicological effects on the plant in question (Kim et al., 2015). Thus, in order to assess the safety of the fruit it is vital to analyse the plant's uptake of heavy metals, as well as total soil heavy metal content.

Metal uptake from soils into plants is influenced by soil pH, organic matter (OM) content, soil texture, soil carbonate and the plant species itself (Jung et al., 1996; Wang et al., 2015). Soil pH is negatively correlated with heavy metal concentrations in plants (Jung et al., 1996), such that in acidic soils, there is increased heavy metal transfer from the soil to the plant tissue (Wang et al., 2015). This is due to the fact that cationic metals become more soluble at lower pH levels, meaning that they are more likely to be incorporated into the plant tissue (USDA, 2000). Smith et al. (1994) found that at higher pH, cadmium exists as free metal ions which are highly soluble to plant tissues, and that the effect was considerable enough to argue for the adjustment of maximum cadmium safety levels based on soil pH.

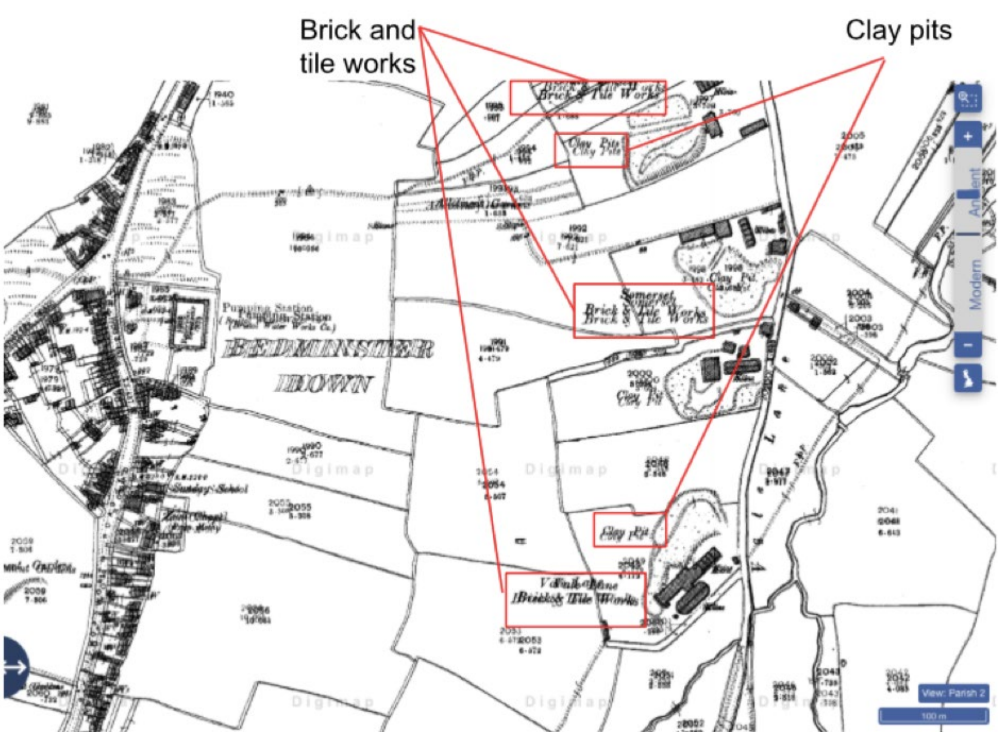
Carbonate has also been found to have an inhibiting effect on heavy metal uptake from soil into plants, thus high carbonate content in soil reduces heavy metal bioavailability (Wang et al., 2015). Wang et al. (2015) found that the bioaccumulation of nickel and cadmium in plants has a tipping point: if soil carbonate exceeded more than 1% then uptake of heavy metals was dependent primarily on pH and carbonate.

Heavy metal uptake is also influenced by clay content, which is known to absorb metal ions through attaching to the hydroxyl ions within their structure (Rieuwerts et al., 1998). Negatively charged clay particles are effective at removing positively charged heavy metal ions from soil solutions due to their high cation exchange capacity, surface area and pore volume (Uddin, 2017). Moreover, the clay also serves as a migration route for heavy metal ions through the soil, increasing their mobility (Rieuwerts et al., 1998).

The significance of OM on heavy metal bioavailability is related to the phase of the soil that heavy metal ions are in. OM is metal binding (Rieuwerts et al., 1998), due to its high absorption and cation exchange capacity, which results in the immobilization of heavy metal in the soil (Kwiatkowska-Malina, 2017). By forming simple and chelate compounds with heavy metal ions, OM becomes a natural barrier to the bioavailability of heavy metals to plants (Kwiatkowska-Malina, 2017). According to Qu et al. (2017), the levels of soil organic carbon have an important role in ecological restoration of areas after industrial practices.

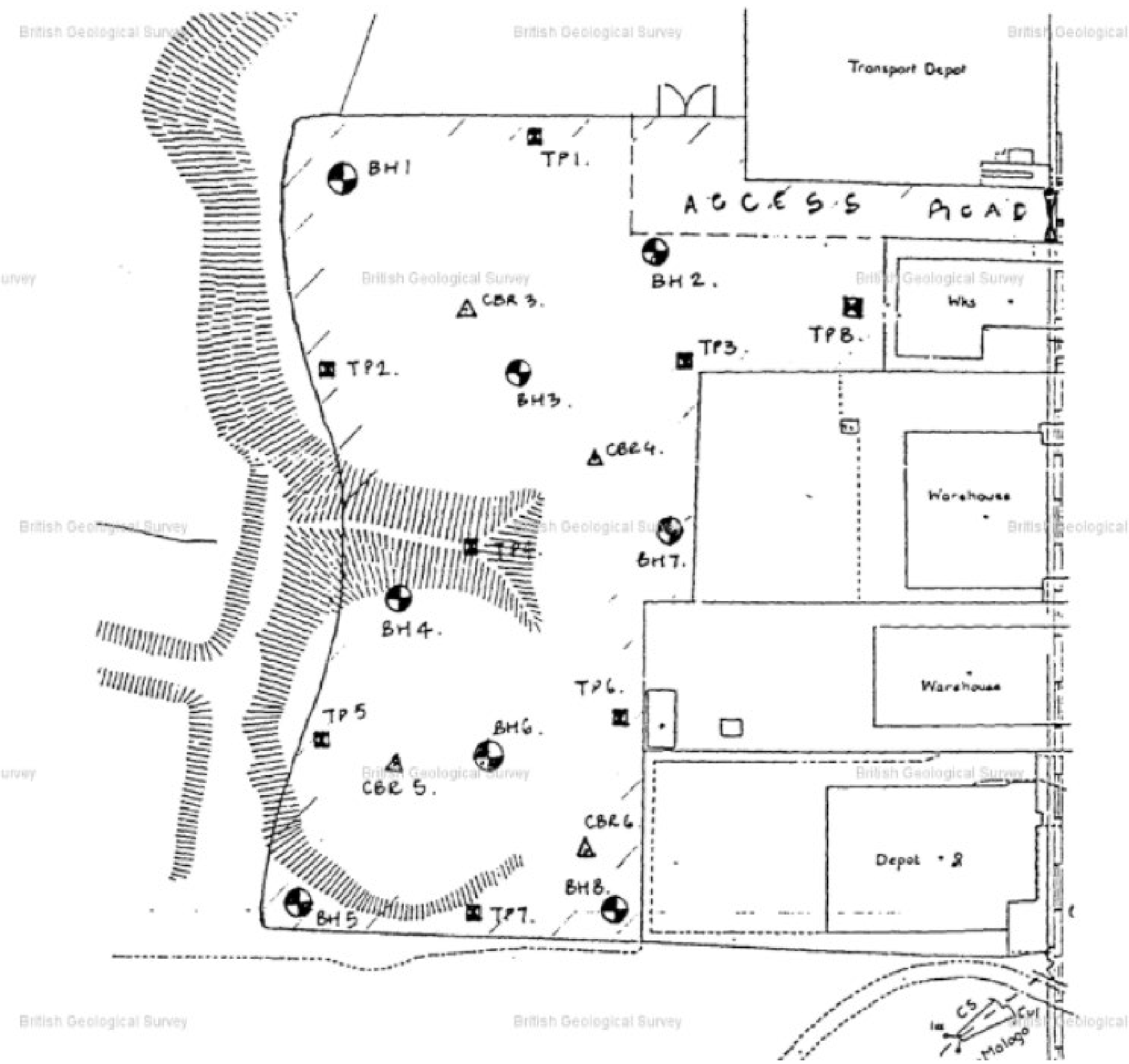
The metal species is also important - cadmium, nickel and zinc being of high mobility through soils and plant tissues comparably to copper, chromium and lead (Kim et al., 2015). Li et al. (2006) found cadmium to be more easily accumulated by plant root systems than other toxic metals. The key factors will also impact heavy metals relatively differently. Rieuwerts et al. (1998) argue that the evidence for OM content affecting uptake to plants is questionable, noting that the correlation between OM and heavy metal uptake is positive with zinc, negative with cadmium and no relationship with lead. This could be explained by the numerous interaction effects that impact heavy metal bioavailability. For example, the impact of OM on the fractionation of heavy metal in soil is pH dependent, with soluble organo-metallic complexes forming at a higher pH, yet this effect is inhibited by high carbonate soils (Walker et al., 2003). Notably, different plant species also have different heavy metal uptakes, with certain plant species being hyperaccumulators - able to accumulate high concentrations of heavy metals into their parts (Tosic et al., 2015). This all makes for a complex picture of interactions that becomes site, plant species, and metal dependent.

The Manor Woods Valley Orchard has a history of heavy industry; there were brick and tile works present throughout the 20th century until the 1970s. Namely, the Vale Lane Brick and Tile Works and The Somerset Brick and Tile Works, with their associated clay pits, which were established at the northeast end of Manor Woods Valley by 1903 (Copas, 1989). Their expansion continued in the 20th century, whereby the works were extensive enough to be serviced by a small tramway. However, by 1972 these brickworks were subsequently demolished and replaced by industrial units. It is the historical presence of industrial activity in this site, which has led to concern as to whether the soil here, and therefore any plants grown in the orchard, may be contaminated with heavy metals. The plants of concern are 37 apple and pear trees, situated just to the west of where the site was (figure 1), which were believed to have grown from fruit cores left by the workers or to have been planted after the war.



Source: Ordnance Survey (1900)
Figure 1: Historic Digimap (1900) of Manor Woods Valley Orchard

There have been three borehole investigations here over the past 40 years to assess the content of the waste that was used to fill the hole left behind by industry. Firstly in 1978 by the Avon County, then in 1987 by a surveying company (BGS, 2019). The most detailed was an investigation done in 1995 by First Bus, to assess whether the site would be suitable to build a bus depot. Most of the boreholes showed very similar results - bricks, tiles, glass, tyres, leather, plastic, but there were three slightly more alarming results - the odours of diesel, hydrocarbons, and ammonia were found in boreholes 1, 3, and 6 respectively (Figure 2).



Source: BGS, 2019

Figure 2: Map of the 1995 borehole locations from the British Geological Survey borehole scans

This paper's first aim is to assess the impact of the past industry in regard to heavy metal contamination of the soil. Secondly the paper aims to determine whether any soil contamination, if present, is translated into the fruit trees. Finally, the paper will judge whether the fruit yielded from the trees in the orchard is safe for human consumption, to determine the value of the Malago Valley Conservation Group (MVCG) planting more fruit trees in the orchard. The sampling strategies and fieldworks techniques are described, followed by the laboratory methods. The results for each of the factors influencing heavy metal uptake in plants are then discussed, followed by the heavy metal content of the soil and the trees themselves.

2. METHODS

2.1 Tree GPS

The MVCG required accurate locations of all 37 fruit trees in the orchard on an ordnance survey map. Each tree in the orchard is tagged with a unique number, ranging from 0501 to 0537. Using a handheld GPS device, the British National Grid (BNG) reference of each tree was recorded in order to plot the map (figure 3). Following the day in the field, these BNG references were converted into easting and northing coordinates; then using ArcGIS the coordinates were plotted onto an ordnance survey map obtained from the Digimap website.

2.2 Sampling

A total of twenty-four samples were collected from the orchard in late January 2019; eighteen soil samples, five tree samples and one fruit sample. The spatial distribution of the chosen sampling sites ensured the inclusion of both the area of the orchard where trees are currently growing as well as the open area in the centre of the orchard where there is interest in planting more trees in the future. For the tree samples, new-growth twigs were taken from five individual fruit trees shown at sites 1, 2, 3, 4 and 7 in figure 3. These trees were chosen because of the availability of new growth twigs at accessible heights as well as the accessibility of the trees themselves, due to some trees in the west of the orchard being blocked by the presence of Japanese knotweed. In addition, an apple that had been left in the orchard from the previous harvest season was collected although unfortunately the exact tree from which the apple came was unknown due to all the apples having been previously collected into a single pile.

Around each of the five trees used for twig samples, two soil samples were taken at separate depths, 0-20cm and 20-40cm. Then, additional soil samples were collected from two more sample sites not associated with specific trees (sites 5 and 6 in figure 3), in the open area of the orchard where there is interest in planting trees in the future, again at two depths. At site 6 the soil sampling was undertaken three times in order to evaluate soil heterogeneity, giving a total of eighteen soil samples from the orchard. The soil samples were collected using a 20cm manual soil corer. The sample depths were chosen following an evaluation of the methodologies of previous soil contamination studies; investigations into the effects of industrial works on heavy metal absorption by plants studied the top 40cm of the soil profile (Ismail et al., 2012), whilst studies focussing on orchard soils and fruit tree uptake centred their investigation on the top 20cm (Li et al., 2006). Further, investigations into soil contamination in Bristol soils specifically studied the upper 15cm of the soil profile (Giusti, 2011), and tree root systems tend to be shallow but widespread, with 80-90% of the roots being in the top 69cm of soil (Crow, 2005). The original intention was to include a depth of 40-60cm in addition to the 0-20cm and 20-40cm depths, however the poor quality of the soil, specifically the presence of bricks and glass, made this impractical and so the study was limited to two depths.

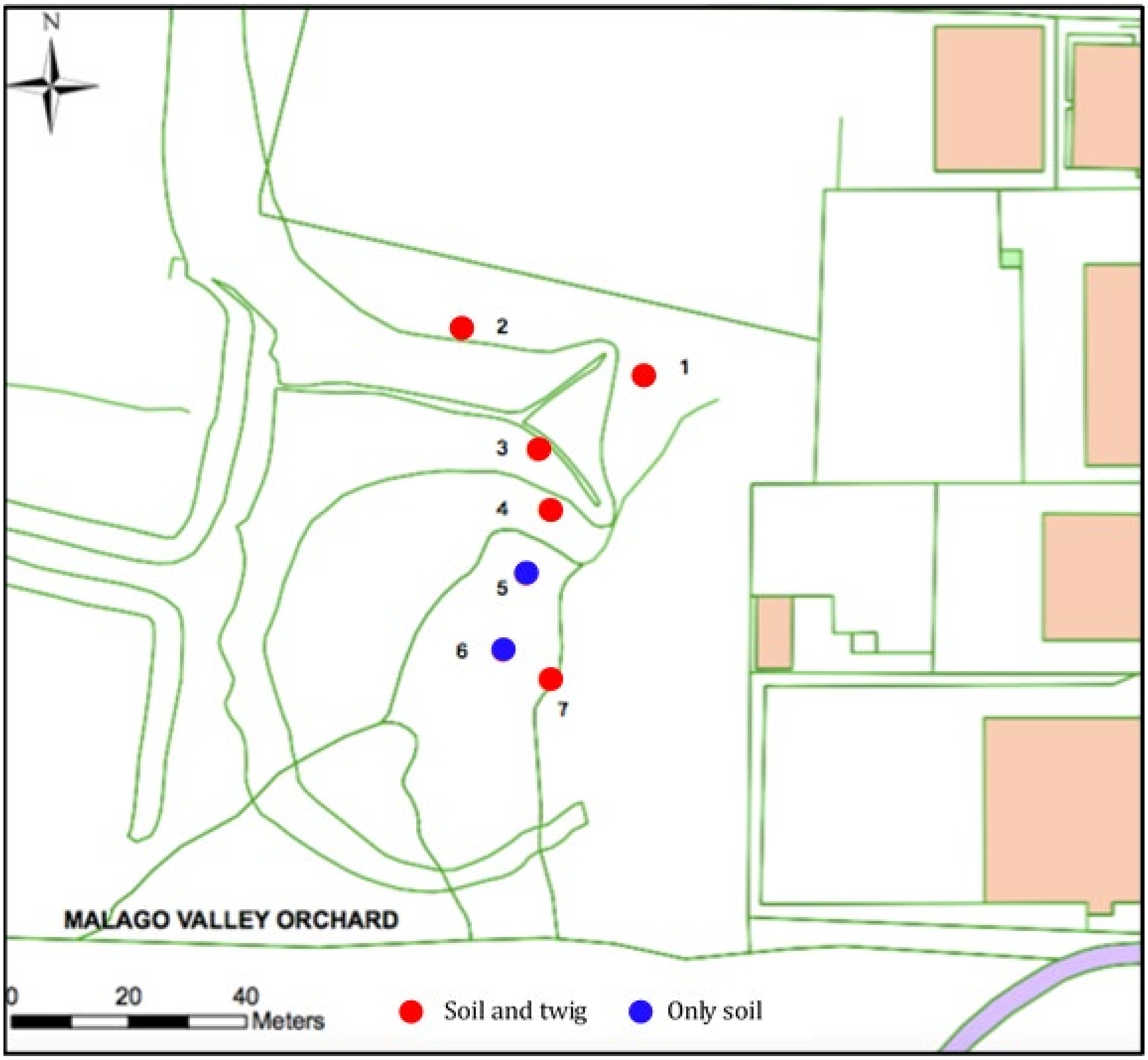


Figure 3: Sample sites

2.3 pH

pH is a measure of the hydronium ion, and the value determines if the soil is acidic (<6.5), neutral (6.5 - 7.5) or alkaline (>7.5) (INRA, 2008). It cannot be measured in-situ or directly from the soil, and so the samples must first be prepared for the analysis. The method used was based on the 1:1 water pH determination (Pansu & Gautheyrou, 2006): 5g of dried soil was weighed and put into a 50ml tube, 5ml of pure water was added and the tube was shaken vigorously for ten seconds before being left to stand for 10 minutes. A pH meter was then used to directly measure the pH of each sample within the solutions. Specifically, a glass pH electrode with temperature compensation was used; this is the most commonly used method for pH determination since the potential being measured by the electrode reaches equilibrium faster than other methods and has good reproducibility (Horiba, 2012), in addition to being more convenient to use and giving more accurate results than other available electrodes (Ali & Sharif, 2015).

2.4 Carbonate content

The carbonate mineral content of the soil was established using the vial calcium carbonate method. This method assesses carbonate content of soil samples via proxy through creating a carbonate calibration curve based off of the pressure gauge response when the sample is dissolved in hydrochloric acid, thus releasing CO_2 gas. Firstly, three calibration standards were created by measuring 200mg of powder into 100ml glass serum vials. Then 1g of 2mm sieved dry soil from each sample were ground down and placed in empty bottles. Then 2ml autosampler vials were filled with 1.5ml of 6M HCl and inserted upright into the vials. The bottles were then sealed with butyl rubber bungs and steel caps, then pierced with a syringe to release excess pressure. The pressure at time zero was measured with the gauge, then the bottles were turned over to release the acid and put on a shaking table for 30 minutes. The pressure was then measured again, with the carbonate determined via the calibration curve created earlier (Cobb, 2017).

This method, otherwise known as the pressure-calcimeter method, is a widely used method for soil carbonate determination (Kassim, 2014). Other methods include the acid neutralization method, acetic acid method, and titration method, yet comparable studies have found that the calcimeter method produces slightly lower carbonate estimates compared to these other methods (Kassim, 2014; Elfaki et al 2016), but has been found to have more rapid and accurate results than the titration method (Elfaki et al, 2016). Issues may arise when compounds that react with the acid and release gas, such as sulphides, can interfere with readings as the gas released cannot be attributed to carbonate content (Ashworth, 1997). Nonetheless it is a trusted method that yields somewhat reliable and reproducible results.

2.5 OM content

A loss on ignition was carried out in order to calculate the OM content of the soils to assess the effect it may have on the heavy metal uptake of the fruit trees. The soil samples were ground up using a pestle and mortar, sieved, and weighed. The samples were heated for 24 hours at 105℃ in order to remove all the moisture. They were then placed in the furnace at 850℃ for 30 minutes, and the ash was weighed, and the mass loss was assumed to be the mass of the OM content (Ball, 1964), as the carbon present in the OM combusts and oxidises to form CO_2 (Heiri et al, 2001). This was chosen over using a lower temperature of 375℃ for 16 hours as this method removes only 90% of the OM on average (Keeling, 1962).

2.6 Grain size

In order to carry out grain size analysis within soil samples (between 0.02µm and 2000µm) a mastersizer machine was used (Malvern Mastersizer 3000 in Bristol School of Geographical Sciences Laboratory). It works by passing a laser through the dispersion media containing suspended sample (in water). The dual wavelength laser measures small particles using the blue laser and larger ones with the red. This data is then converted using the Fraunhofer approximation and Mie theory to create a particle size distribution (Malvern, 2019).

This method uses residue from the loss on ignition analysis, as it is essential no OM is passed through the Mastersizer which may damage it. Using PC connected mastersizer software, the instrument was initialised, and the laser aligned. Firstly, a background reading was taken using a beaker of distilled water and no suspended sample to give an obscuration of 0%. It is essential to take a background reading, as obscuration values above 0% without addition of sample, would result in incorrect particle size analysis once sample was added. Small spatulas of the sample were then added to the beaker of water until obscuration reached 10-15%. Once obscuration had stabilised, five measurements of the sample were taken using the computer software. Once these measurements were taken the system was cleaned through to ensure future sample readings would not be contaminated from previous sample particles. The software provided a data sheet of grain size composition (μm), which was then exported into an excel file for analysis using a grain size classification triangle.

2.7 Heavy Metal Concentration in Soils

To measure the heavy metal content of the soil samples, 1g of each sample was weighed and placed into a test tube with 8ml of magnesium chloride with hydrochloric acid. Magnesium chloride is required to remove free metals available in the soils for analysis. The tubes were then placed in a shaker for 30 minutes and the centrifuge for 20 minutes to allow the soil material to settle at the bottom of the test tubes, leaving liquid sample suspended above. Using a pipette, the suspended liquid from each sample was removed and placed into a new test tube. Each sample was then filtered to remove any particles larger than 2mm. These samples were then sent off to a laboratory to test for the following heavy metals: calcium, cadmium, copper, manganese and lead.

2.8 Heavy Metal Concentration in Trees

In order to analyse heavy metals within the five twig samples and the apple sample, a wet oxidation was undertaken, based on a Khejdahl oxidation (heating the samples with sulphuric acid to decompose OM present) (Allen, 1989; Fleck, 1965).

1g of each twig sample was weighed and cut into small pieces to maximise surface area for digestion. This sample was put into a digestion tube and 4.4ml of digestion mixture (0.42g selenium powder, 14g lithium sulphate, 350 ml 30% hydrogen, 420ml of concentrated sulphuric acid). Each twig was allowed to digest at 360°C for 2 hours, until the solution had turned colourless. This mixture was then left to cool and 50 ml of distilled water was added until no more sediment would dissolve. The mixture was then left to cool again and then was filtered through into a 100 ml volumetric flask. The flask was made up to 100 ml with addition of more distilled water. As there is no volatilisation of heavy metals in this method, the solution was then analysed for heavy metals (cadmium, zinc, iron, nickel and chromium) (Cobb, 2017).

3. RESULTS AND DISCUSSION

3.1 Tree GPS

In terms of spatiality, the plotted locations of all 37 fruit trees in the orchard appear to be accurate, however issues with the accuracy of the handheld GPS devices used mean that there is no certainty around the validity of the proposed location of all the fruit trees. A high resolution map of the orchard has been produced, as shown by figure 4:

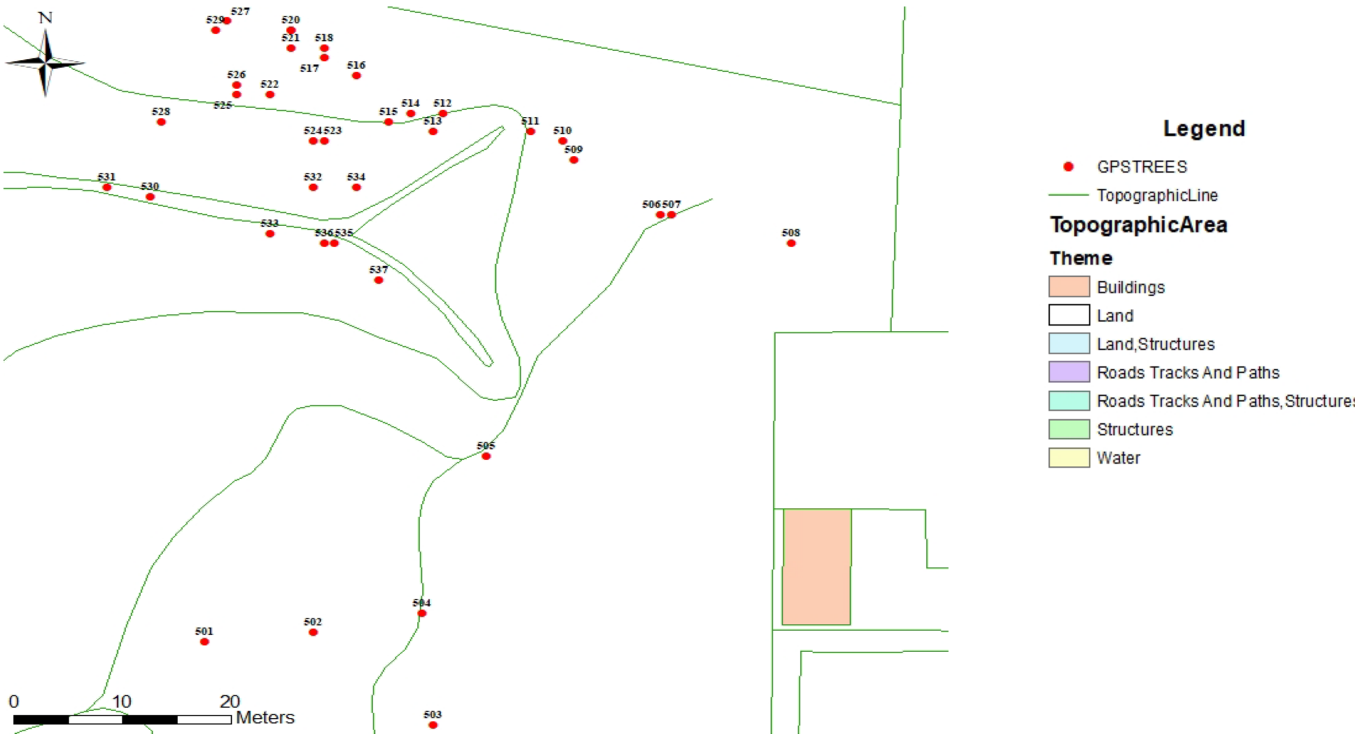


Figure 4: High resolution map of the Manor Woods Valley Orchard

The primary focus of this project is to investigate the potential heavy metal contamination of fruit and soil in the orchard, hence the remainder of this section will explore the results from the pH, carbonate, OM and heavy metal analyses', and discuss the implications of these results.

3.2 pH

The pH results show that the soil in the orchard is neutral, ranging from 6.4 to 7.4 (Figure 5). The findings are all similar regarding each site position and depth, demonstrating limited spatial and depth variability for pH. These results hold significance in relation to heavy metal contamination of the fruit trees as pH is an important factor controlling the uptake of heavy metals by vegetation from contaminated soils. Soils with low pH have increased heavy metal transfer from the soil to the plant tissue (Wang et al., 2015) as a result of the cationic metals becoming more soluble at lower pH levels (USDA, 2000). The orchard soil having a neutral pH means that the heavy metal ions are less soluble than they would be with a lower pH, hence heavy metal uptake by the fruit trees will not be enhanced by pH.

It is important to note that such measurements have limitations, specifically the methods are carried out on disturbed samples and soil suspensions, hence do not truly reflect in-situ conditions.

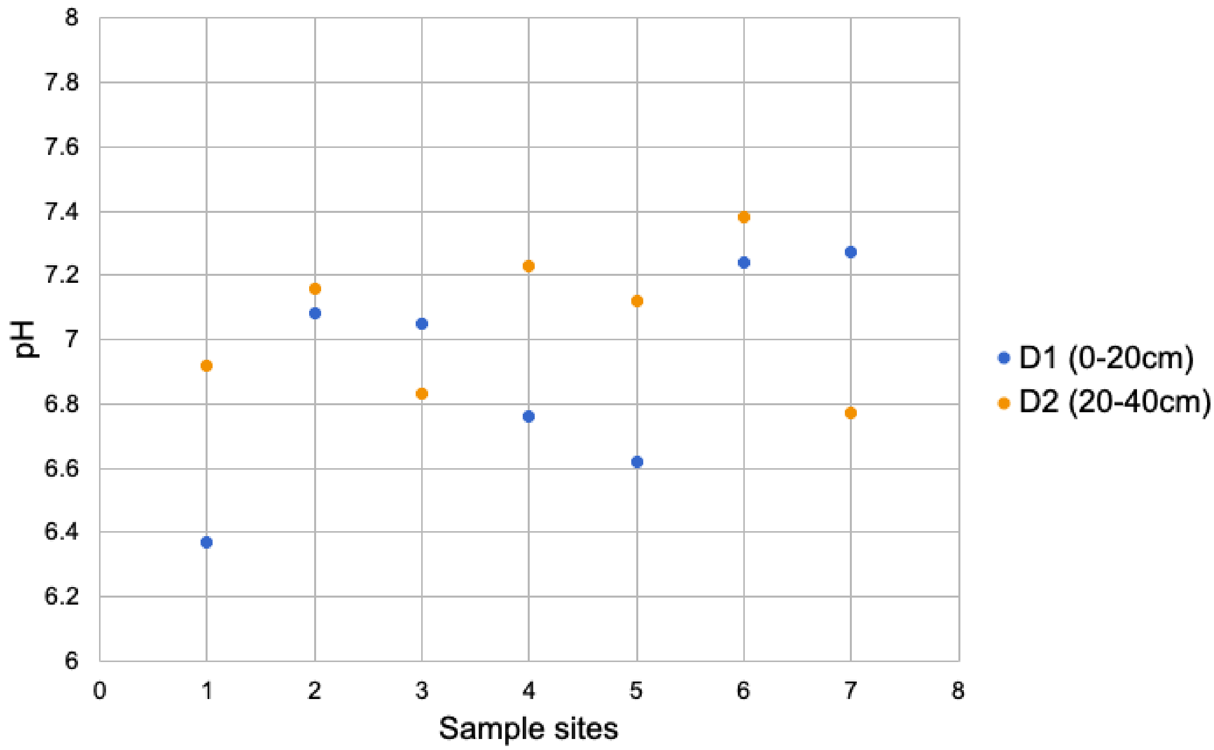


Figure 5: Scatter plot of the soil pH levels at the Manor Woods Valley Orchard

3.3 Carbonate content

The carbonate readings at the site were varied, with an average carbonate content percentage of 10.241+/- 5.91. The large standard deviation is an indicator of the wide amount of variance within the data. The values covered a range of 0.457 - 20.015% carbonate in the soil samples of the site, as illustrated by figure 6. It can also be seen that in sites 3, 4 and 5, the carbonate was higher at depth 2 (20cm-40cm), which meant depth 2 had a slightly higher average percentage carbonate (10.48%), compared to depth 1(0cm-20cm) (9.98%). Areas of high carbonate are potentially elated to concrete being present in the samples. For soil 1 depth 2, and soil 3 depth 2, the pressure measured by the instrument was too high to be recorded, and thus they had to be repeated with 0.25g and 0.5g of soil instead of 1g. Once redone, they returned very low carbonate values (0.457%), which indicates that high values are a result of the presence of chunks of brick or concrete. The triplicate samples at site 6 for both depths further illustrated how heterogenous the site was. Depth 1 ranged from 9.40-12.82% and depth 2 ranged from 0.99-19.21% carbonate. This further emphasises how mixed the site was and that the carbonate result is largely dependent on the sample taken tested (whether concrete was present). Though this does indicate anthropogenic sources of carbonate in the site, it brings into question the robustness and reproducibility of the data. Sources of error in the calcium carbonate determination in soil methods are the presence of clay minerals and OM (Kassim, 2014), but since the site had low levels of clay minerals and OM, it can be assumed that this did not have an impact on the outcome, though other sources of error and uncertainty are present.

The Manor Woods Valley Orchard data has been compared to two other Bristol sites (Dundry Hill and Fenswood Farm) for contextual purposes, using data collected and analysed with identical methodology (RMPG 1, 2018; RMPG 2, 2018). Table **1** illustrates that Manor Wood Valley soil's average percentage carbonate is considerably higher than that of both Dundry Hill and Fenswood Farm. Notably, Dundry Hill soil overlays a bedrock of oolitic limestone, thus making the soil there naturally higher in carbonate, and yet Manor Wood Valley soil carbonate content is still the highest, further indicating the anthropogenic sources of the carbonate. Manor Wood Valley and Fenswood Farm both overlie Mercia Mudstone bedrock, which has lower percentage carbonate than Dundry Hill, so you would expect Manor Wood Valley to potentially have similar percentage carbonate to Fenswood Farm without anthropogenic influence.

As indicated previously in the literature, studies have found that if carbonate concentration is over 1% in the soil, then uptake is dependent primarily on pH and carbonate (Wang et al., 2015). Here, the carbonate was considerably over 1% across the site, though mixed, so heavy metal uptake is likely to be strongly influenced by these two factors. Since the pH was neutral, and carbonate was high, an inhibiting effect due to carbonate is likely present in regard to the migration of heavy metal from soil to the trees in the orchard. However, this may be hard to determine due to the large amount of variability at the site.

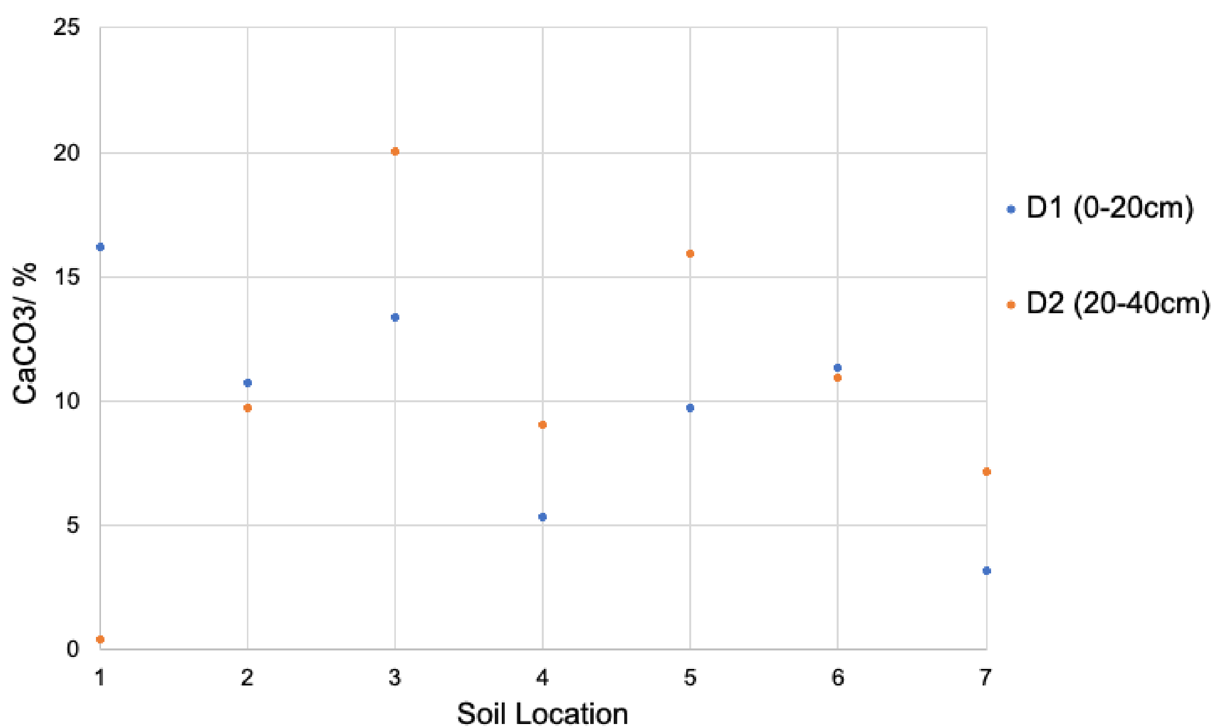


Figure 6: Scatter plot of the percentage carbonate in the soil samples from Manor Woods Valley Orchard

Site	Average percentage carbonate (%)
Dundry Hill	6.349 +- 1.19
Fenswood Farm	0.919 +- 0.21
Manor Woods Valley Orchard	10.241 +-5.91

Table 1: Table showing the average percentage carbonate at three sites in Bristol

3.4 OM Content

The loss on ignition investigation showed the OM content of the soil in the orchard, and it seems to be consistently low, with none of the samples having a percentage OM content of more than 8% (figure 7). The level of OM content required for a soil to be considered healthy has very little consensus (Oldfield et al 2015), but this site is lower than other nearby sites (table 2) The lowest value was found at site 7 in the lower depth, which was found to be 4% OM. Soils from depth 1 (0cm-20cm) look to have slightly higher organic content than the lower soils, which could be accounted for by the presence of grass, leaves, and fruit on the soil surface which would decompose to add OM to the upper layer of the soil. The site 6 repeats show the sample variability to be fairly low (appendix 1). When compared to the other sites in Bristol (table 2), which were analysed in an identical way, you can see it is much lower than what potentially could have been its natural state before the industry was put on the site. As the underlying bedrock at Fenswood Farm is the same as the orchard (RMPG 2, 2018), so the soils should be quite similar. There has never been additional OM was added at the orchard as fertiliser, therefore it can be assumed that the OM content is too low to have a protective impact to heavy metal uptake.

The higher temperature loss on ignition was chosen, however has a major limitation - the loss of structural water from clay and the loss of CO_2 from calcium carbonate occurs in synchrony with the OM loss (Ball, 1964). The clay content of the soils, however, is so low that the loss of structural water would account for less than 1.3% of the weight loss (Ball, 1964). Carbonate levels appear to be very high in some of the soil samples, and according to Salehi et al. (2011), higher temperatures used in loss on ignition investigations, above 500°C cause a significant loss in calcium carbonate, so the organic content may be even lower than figure 7 suggests due to calcium carbonate breakdown causing mass loss.

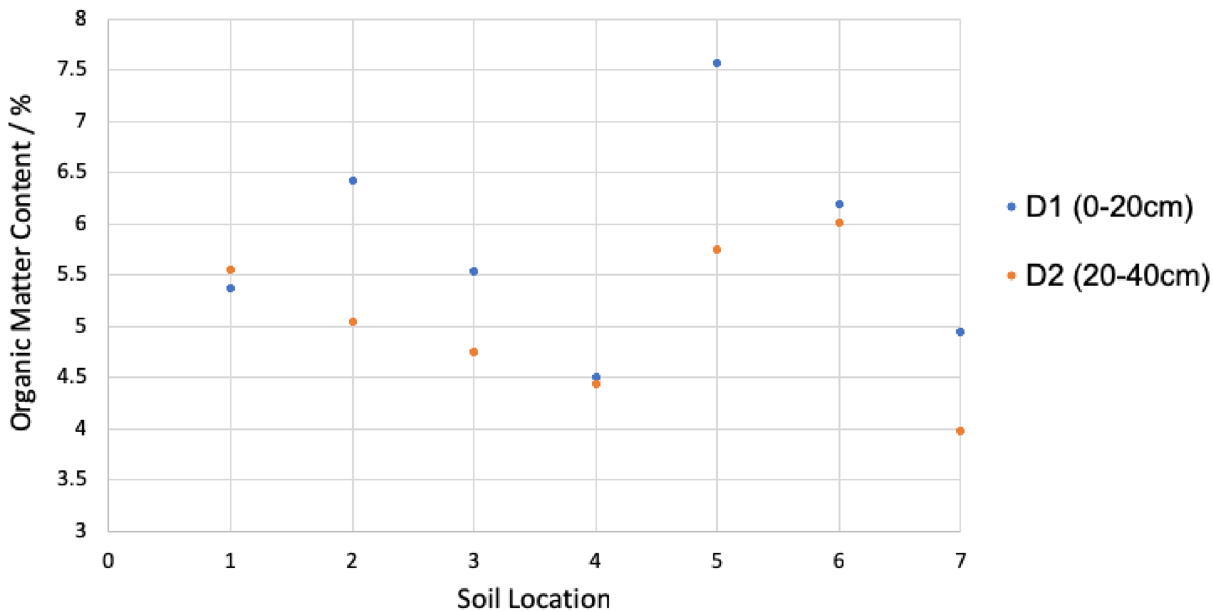


Figure 7: Scatter plot of the OM content in the soil samples from the Manor Woods Valley Orchard

Site	Average percentage OM content (%)
Dundry Hill	29.66 +- 2.50
Fenswood Farm	7.24 +- 1.81
Manor Woods Valley Orchard	5.44 +- 0.90

Table 2: Table showing the average percentage OM are three sites in Bristol

3.5 Grain size

Figure 8 shows the results for grain size composition of each site in the orchard, an average of depth 1 and 2 per site, as there was minimal inter-depth variation (appendix 3; appendix 4), displaying percentage clay content. Each pie chart shows similar findings regarding each soil position and depth for grain size composition, in which the largest proportion was sand and silt, followed by gravel and finally clay. This was expected, as brick usually contains 50-60% silica (sand) and the site contains industrial infill of bricks and tiles from the works established west of the orchard (Punmia et al., 2004).

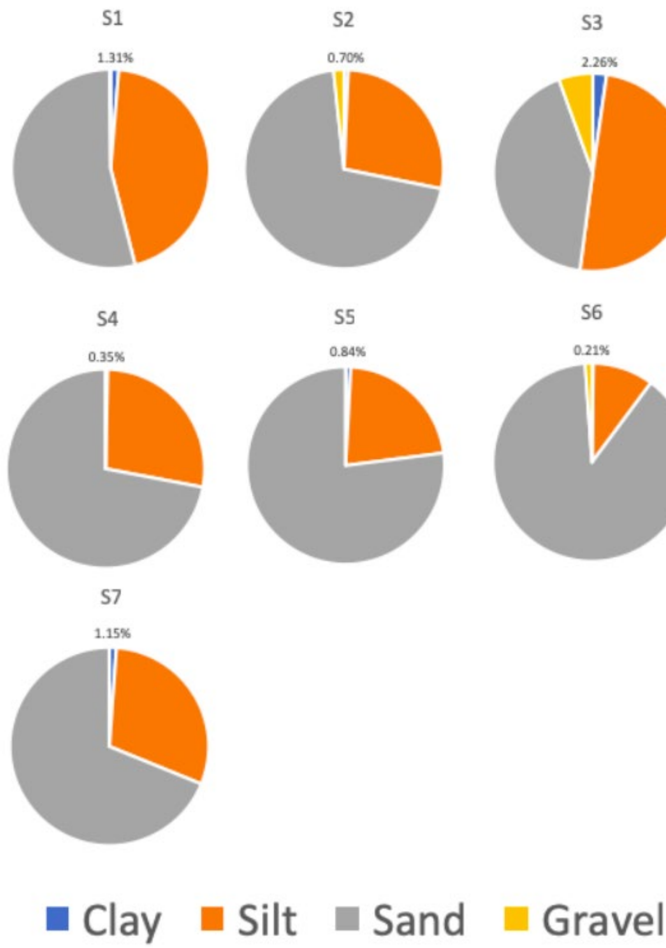


Figure 8: Pie charts showing the soil grain size composition at each sample site

Clay content of soils ranging from only 0.096 - 2.368% shown in figure 8, demonstrates an overall low proportion of clay in samples (appendix 5). As a point of reference in order to confirm the validity of low clay content in samples, it was compared against the same secondary data - Dundry Hill and Fenswood Farm, which have no previous history of industrial activity (table 3). The samples from these sites underwent identical methodology and mastersizer analysis to the samples collected within the orchard, to facilitate valid inter-site comparison. Table 3 shows that the clay content for Fenswood Farm and Dundry Hill were 24.27% +-6.57 (RMPG 2, 2018) and 25.69% +-9.07 (RMPG 1, 2019) respectively, two values greater than the average clay content of the Manor Woods orchard (0.98% +- 0.70).

Site	Average Clay content (%)
Dundry Hill	25.60 +- 9.07
Fenswood Farm	24.27 +- 6.57
Manor Woods Valley Orchard	0.98 +- 0.70

Table 3: Table showing the average clay percentage at three sites in Bristol

The clay proportion was the focus of the mastersizer data because of its link to heavy metal uptake in plants. Clay particles are negatively charged which results in attenuative properties, to facilitate retention of heavy metals, reducing available uptake to plants (Uddin, 2017). This links to the project's second aim which is to assess whether any soil contamination present assimilates into plant material.

It is, however, important to underpin the limitations of the mastersizer methodology in grain size analysis. As the machine uses only a small spatula of soil sample to analyse grain size, the issue arises as to whether a sample of this size is representative to the whole site, whereby soils are known to have heterogeneous physical, chemical, and biological properties (Jackson et al., 1993). These results, however, were generally homogenous in their description of soil composition in a broader context, demonstrating clay is consistently the smallest fraction of soil type within the samples.

3.6 Heavy Metal Concentration in Soils

Figure 9 shows the measured heavy metal soil concentrations at all 7 sites in the Manor Woods Valley Orchard. The highest concentration of any isotope of each heavy metal, shown as points, are compared against their respective EU guideline values (Tòth et al. 2015), shown as horizontal lines, on a logarithmic scale. These results distinctly show that the measured heavy metal concentrations at all 7 sites fall far below their respective EU guideline values, shown in table 4. For cadmium, the average value at the orchard site was 0.0053 mg kg^{-1}, for copper, the average value on site was 0.0308 mg kg^{-1}, for manganese 2.1096 mg kg^{-1}, and finally, for lead 0.2194 mg kg^{-1}.

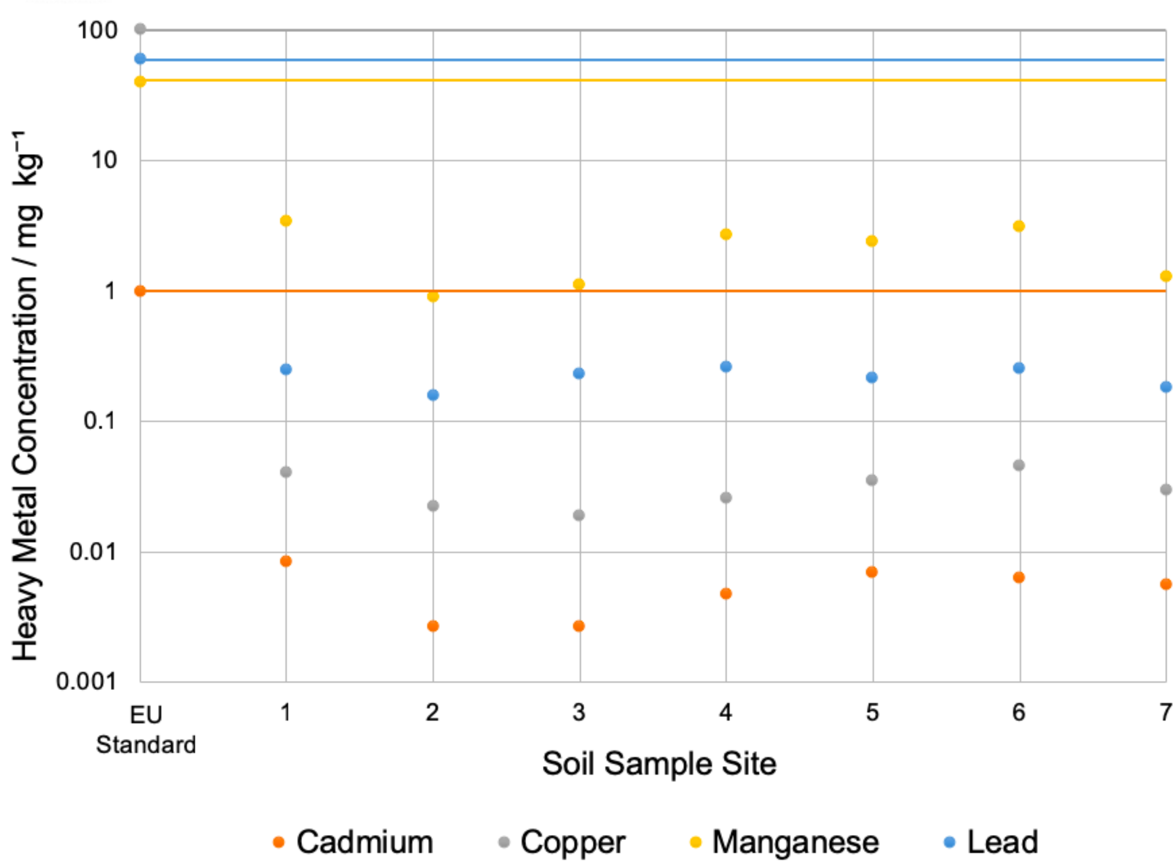


Figure 9: Graph showing soil heavy metal concentrations, compared against EU guideline values

Metal	EU guideline value (mg kg^{-1})
Cadmium	1
Copper	100
Manganese	40
Lead	60

Table 4: Table showing recommended EU guidelines for heavy metal concentrations in topsoils (Tòth et al. 2015)

Given that there is a history of industrial activity in the area where the Manor Woods Valley Orchard now sits, it was expected that there would be elevated concentrations of heavy metals in the soil. As this was not the case, it is important to speculate upon possible reasons as to why heavy metal concentrations in the surface soil are so low. Firstly, it must be considered that the boreholes containing the industrial debris are up to 14 m in depth; hence, any contaminated debris or contamination hotspots may be located far below the surface, much deeper than the 40 cm core that was extracted. Secondly, any contaminated material that is situated deep below the surface may be inert or insoluble, or unavailable to the plants due to being situated so far below the tree root system. Therefore, any contaminants containing heavy metals may be unable mobilise and leach up towards the shallower soils where the investigation was based.

The results of this analysis suggest that the MVCG should not be concerned about possible heavy metal contamination in the orchard. However, as a point of reference, it is also necessary to compare this site's data against secondary data collected in the Bristol region.

One such example of secondary data that can be used for comparison is a study undertaken by students at the University of Bristol in the Dame Emily Park (Group 21, 2019), which was previously host to a coal mine. Soil samples were taken near the concrete pit that now conceals the mine, and the group tested for the presence of any heavy metals using the same laboratory method as in this investigation. The group analysed the soil for similar heavy metals, namely copper and cadmium (appendix 5). For copper, the measured concentration was marginally higher in Dame Emily Park compared to the Manor Woods Valley Orchard, with an average of 0.0537 mg kg^{-1} compared to 0.0308 mg kg^{-1} respectively. Whilst for cadmium, there was only one reading of 0.0029 mg kg^{-1} available for the Dame Emily Park site, since the other site values were below the detection threshold; this value is lower than the average concentration of 0.0053 mg kg^{-1} at the Manor Woods Valley Orchard site.

Another group of students from the University of Bristol collected heavy metal data in the Bishopsworth and Malago Conservation Area (Group 23, 2019). This secondary data was collected in the meadow area of the conservation site, which runs adjacent to the Manor Woods Valley Orchard, and shows similar concentration values to this investigations results (appendix 6). Firstly, in regard to cadmium, this group recorded an average value of 0.0084 mg kg^{-1} across the meadow, which is greater than the average value of 0.0053 mg kg^{-1} recorded in the orchard. With regard to copper, the group recorded an average value of 0.0191 mg kg^{-1}, which is lower than the average value of 0.0308 mg kg^{-1} in the orchard. Although these values are slightly different, they are uniform in the sense that the mean values for both the meadow area and the orchard fall far below the respective guideline soil values recommended by the EU for copper and cadmium.

Furthermore, the average heavy metal concentrations can be compared against ambient background values for UK soils. Based on a survey, undertaken between 1978 and 1983, of 5,651 soil samples across England and Wales, ambient background heavy metal concentrations in soils were calculated. The mean value for cadmium was 0.8 mg kg^{-1}; the mean value for copper was 23.1 mg kg^{-1}, whilst the mean value for lead was 74.0 mg kg^{-1} (McGrath & Zhao, 2006). These ambient values are substantially higher than the concentrations found in the orchard, further supporting the conclusion that contamination is not a cause for concern.

It is also important to highlight any limitations of the laboratory or fieldwork methods used to test for heavy metal contamination in the soil. Firstly, in the heavy metal analysis, it was not possible to test for specific contaminants such as mercury and arsenic - this is significant since consumption of elevated concentrations of such heavy metals can lead to serious health implications. Elevated consumption of inorganic arsenic, for example, is known to be a human carcinogenic, and may also lead to cardiac disorders and damage to the reproductive system (Hong, Song and Chung, 2014). Furthermore, the laboratory method used for testing for heavy metals within the twigs and apple removes only bioavailable metals, as opposed to the standard method testing for aggregate heavy metals (total digest) which usually yields greater numerical values for heavy metal concentrations. This means concentrations assumed in the results may not be directly comparable against guideline values from literature.

3.7 Heavy Metal Concentrations in Trees

Figure 10 shows results for measured heavy metal concentrations in 5 twig samples collected from 5 different fruit trees from within the orchard, and one apple sample. The graph shows measured concentrations of cadmium, chromium, iron, nickel and zinc. The highest concentration of any isotope of each heavy metal, shown as points, are compared against their respective daily guideline consumption values, shown as horizontal lines, on a logarithmic scale. These results show that the measured heavy metal concentrations in all 5 twigs and apple fall far below their respective guideline daily consumption values (table 5). For cadmium, the average value at the orchard site was 0.0004 mg kg^{-1}, for chromium 0.0474 mg kg^{-1}, for iron 0.1547 mg kg^{-1}, for nickel 0.0273 mg kg^{-1}, and finally for zinc 0.4225 mg kg^{-1}.

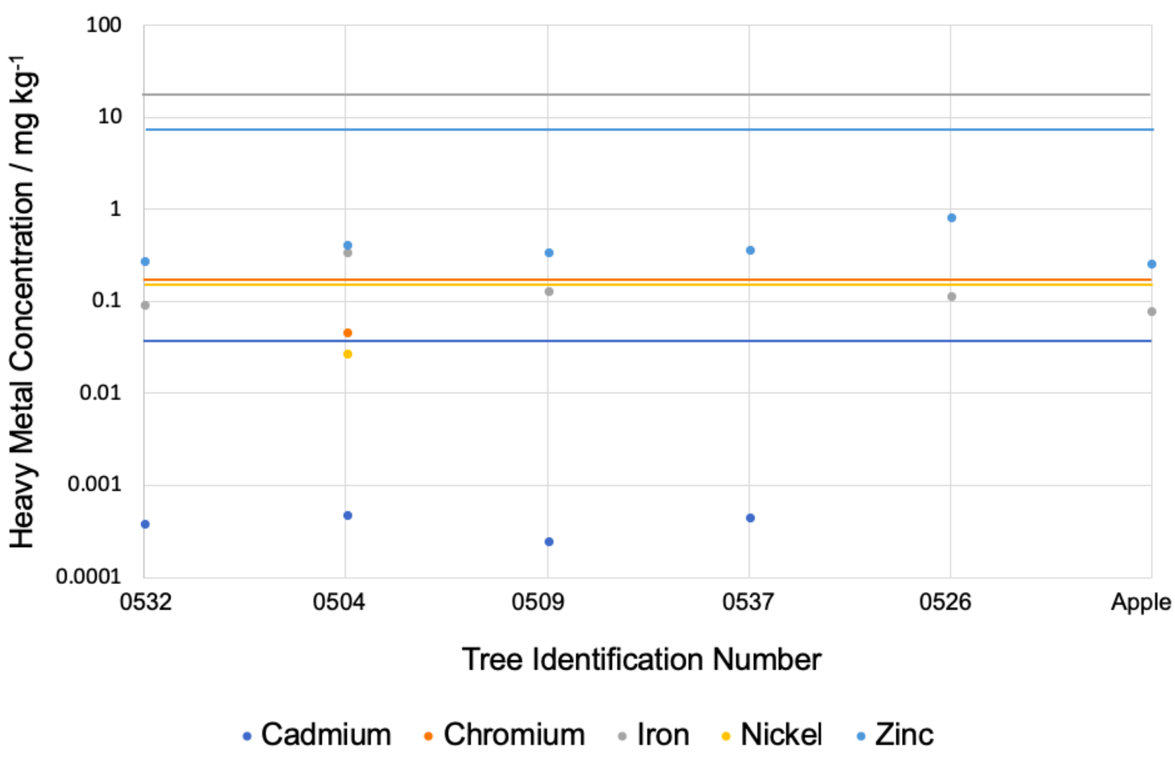


Figure 10: Graph showing twig and apple heavy metal concentrations (shown as points), compared against guideline daily amounts for human consumption of each metal (shown as horizontal lines)

Metal	GDA for an average human (Mg day $_{-1}$)	Source
Cadmium	0.06	(Commission regulation (EC), 2006)
Zinc	9.5	(Mayoclinic, 2017)
Iron	11.75	(NHS, 2017)
Nickel	0.196	(EFSA, 2015)
Chromium	0.2	(National Research Council, 1989)

Table 5: Table showing the guideline daily amounts for the average human for each heavy metal tested for

These results concur with the data for heavy metals in soils, which showed heavy metal concentrations significantly below their respective EU guideline values. It is therefore unsurprising the values for heavy metals within the twigs and apples were low, as low heavy metal concentrations in soils results in little available heavy metals for plant uptake and assimilation into fruits. Additionally, translocation is highly variable within fruit trees and a complex process dependent upon heavy metal type, concentration, structure and ability of a shoot to form a storage organ. This means that even if there were to be elevated heavy metal concentrations within the soil, this doesn't necessarily equate to their translocation into tissue and thus fruit and twigs (Murtic, 2014). Specifically relating heavy metal uptake analysis in apple trees, Tosic et al. (2015) concluded that, as a species, apple trees have successful mechanisms to preventing assimilation of toxically high concentrations of heavy metals into their fruit. The concentration of heavy metals translocated with an apple tree decrease in the following order: root > leaves > branches and twigs > fruit, supporting the findings of very low concentrations of heavy metals within twig samples and one fruit from the orchard (Tosic et al., 2015).This is furthermore supported by table 6 which shows the results of investigations into heavy metal concentrations of a site, also contaminated by industrial activity (metallurgical factory), analysing the same metals within this study (soils and twigs/apple: cadmium, zinc, nickel, iron, copper, manganese, lead). Tokaliogˇlu et al. (2001) find heavy metal concentrations within plant tissues increase in the order of fruit, twig, root and leaf. This consolidates the findings from this investigation, that even if heavy metal contamination is present in soils, the fruits of trees consistently demonstrate successful mechanisms to protecting against accumulation into its fruits.

Element	Fruit		Leaf		Twig		Root		Control fruit samples	Typical plant tissue contents
	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
Lead	7.1	4.7-11.0	100	21.7-284	11.2	3.1-29.3	52	2.88-196	0.12	0.05-3
Zinc	30	17-40.7	292	49.4-724	54	5.16-119	184	6.46-1750	23.9	15-100
Cadmium	0.3	0.05-0.4	1.69	0.2-3.74	0.49	0.09-2.15	2	0.05-12.2	0.05	0.01-0.3
Iron	30	16-63	398	183-783	54	23.0-111	435	35.3-1667	181	40-500
Nickel	3.2	1.8-4.8	5.75	1.72-11.8	2.34	0.27-6.88	3.98	0.20-14.8	0.4	0.5-5
Copper	4.2	2.7-6.0	6.39	1.9-17.4	6.62	1.62-27.2	5.84	2.18-13.2	2.72	2.5-25
Manganes e	6.5	4.2-8.7	166	67-415	70	15.4-184	60	16.9-151	2.4	50-1000

Table 6: Table showing mean concentrations of metals in plant tissue samples (pg/g ***dry*** weight, n = 22) in research conducted by Tokaliogˇlu et al. (2001)

The results of these heavy metal analyses answer the final two project aims. The first being that there are negligible heavy metal contaminants from the soil present in the fruit trees, due to both low heavy metal concentrations within these soils (when compared to EU guideline thresholds for soils), and to the protective mechanisms in apple trees to prevent assimilation of toxically high heavy metal concentrations into its fruit. These results support that the MVCG should not be concerned about possible heavy metal contamination in the orchard. The second being that the level of contamination in the fruit is safe for human consumption, as the heavy metal concentrations in the five twigs and one apple sample were significantly below the daily guideline amounts for human consumption of the metals tested (table 5).

However, there are limitations to this laboratory work. Firstly, likewise for the heavy metal analysis in soils, it was not possible to test for mercury and arsenic, two carcinogenic heavy metal contaminants which bear serious health damage to the cardiac and reproductive if consumed (Hong, Song and Chung, 2014). Furthermore, the laboratory method used for testing for heavy metals within the twigs and apple again only removes free (bioavailable) metals, which only yields very approximate estimations of available trace element status of the soils (Tokaliogˇlu et al, 2001). This contrasts the alternative total digest method, which usually yields greater numerical values for heavy metal concentrations. This means concentrations assumed in the results may not be directly comparable against guideline values from literature.

It was also not possible to compare concentrations of copper, cadmium, manganese and lead tested within the soils to tree samples, as concentrations within the twigs were not high enough to be considered non-background levels. Only cadmium, which was measured in soils, was present in high enough concentrations within the twigs to be detected in heavy metal analysis. This does however consolidate the findings that fruits from the trees are safe for human consumption, where there has been negligible uptake of heavy metals from the soils.

Moreover, as we were not able to determine which specific tree the apple that we sampled fell from, we were unable to analyse exactly what proportion of the heavy metal content of the soil made its way into the fruit. Had we known the source tree, we could have sampled the soil around the tree, to explore how the heavy metals are absorbed from the soil, into the new growth twigs, and finally through to the fruit.

This research focuses upon heavy metal concentrations falling below threshold values for safe human consumption, whereby fruits are consumed by humans as opposed to the twigs. This underpins a limitation to the fieldwork methodology, that collection of the samples fell seasonally when there were minimal apples available for collection and analysis, meaning only one apple could be analysed for heavy metals. Analysis of only one sample means no statistical analysis may be performed on the data due to small sample size and thus limits the validity of conclusions drawn from results.

4. Future Work

There are several avenues to explore in terms of further research that could be carried out on the orchard site. Firstly, having conducted this particular investigation in January, it proved difficult to locate apples for analysis - only one apple was extracted from the site for analysis, however it was severely decomposed. Hence, if further analysis into potential heavy metal uptake by trees into fruit is to be conducted, fieldwork should be carried out during the apple harvesting season, when there is greater availability of fruit on site.

Secondly, the borehole data from the 1995 study, undertaken by First Bus, reported the possible presence of hydrocarbons, ammonia and diesel in the soil. It was not possible to test for these in this investigation - hence, another area of research could be to assess whether the health of fruit trees in the orchard are being affected by these contaminants as well as heavy metals.

Thirdly, the tree location map appears to be slightly skewed due to the accuracy of the handheld GPS devices used. Handheld GPS devices may not be adequate for this job since they can display error margins of up 5 to 10 metres under normal atmospheric conditions (Garmin, 2019). Tree locations could instead be recorded using a more accurate differential GPS device, which has an error margin of approximately 10 centimetres. Alternatively, a tree survey could also be carried out using an automatic level.

In addition, due to limitations in the coring equipment used in this investigation, only the first 40 cm of soil depth were analysed. This was due to the fact that the coring equipment used was unable to break through debris in the ground and hence it was not possible to core beyond this depth. Given that 80-90% of the tree root system is in the top 69 cm of the soil profile, use of a deeper corer could an area of further research could be to use a deeper corer that will enable analysis of the soil down the tips of the trees' roots. Using a deeper corer will also limit the potential for any cross-contamination of material between different depths since the coring device will only enter the ground once.

Finally, this investigation only carried out 20 samples due to time constraints, meaning that it was not possible to carry out a reliable standard deviation calculation of the site to gauge the variability of the soil characteristics and the precision of the results. Therefore, future investigations, with the capability to take more samples across the site, should be able to carry out standard deviation calculations with a minimum of 30 samples - this will provide a clearer picture of spatial variability across the site.

5. Conclusions

For all the heavy metals tested for in the soil samples from the Manor Woods Valley, the concentrations were observed to be lower than EU guidelines for safe soil levels. The pH, OM content, and clay content were thought not to affect any heavy metal uptake by the plants. However, the high carbonate levels may have enhanced heavy metal uptake into the fruit trees, but it was so variable over the site that it is hard to conclude. Nevertheless, the fruit trees sampled in the orchard, and the fruit produced all showed heavy metal levels lower than the guideline daily amount for safe human consumption, so the results suggest the fruit is safe to eat.

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Appendices

Appendix 1 - Soil pH measurements

Sample	D1	D2
1	6.37	6.92
2	7.08	7.16
3	7.05	6.83
4	6.76	7.23
5	6.62	7.12
6	7.24	7.38
7	7.27	6.77

Appendix 2 - Percentage mass loss from each soil sample after the loss on ignition (assumed to be percentage OM in each soil sample)

Sample	Percentage mass loss (%)	
	D1	**D2**
1	5.375	5.549
2	6.424	5.052
3	5.546	4.756
4	4.502	4.436
5	7.578	5.746
6.1	6.728	5.598
6.2	6.585	5.674
6.3	5.274	6.791
7	4.95	3.977

Appendix 3 - data from the mastersizer for every soil sample

S1D1

	1	2	3	4	5	avg
Clay (%)	1	1.18	1.31	1.41	1.52	1.284
Silt (%)	36.9	40.36	42.31	43.51	45.38	41.692
Sand (%)	62.1	58.24	55.88	54.51	52.81	56.708
Gravel (%)	0	0.2	0.47	0.53	0.27	0.294

S1D2

	1	2	3	4	5	avg
Clay (%)	1.07	1.19	1.29	1.57	1.57	1.338
Silt (%)	42.65	44.8	46.52	53.01	51.59	47.714
Sand (%)	56.25	53.99	52.21	45.43	46.88	50.952
Gravel (%)	0	0	0	0	0	0

S2D1

	1	2	3	4	5	avg
Clay (%)	0.53	0.62	0.69	0.74	0.79	0.674
Silt (%)	25.76	28.53	30.37	31.38	32.09	29.626
Sand (%)	73.7	70.84	68.93	67.91	67.13	69.702
Gravel (%)	0	0	0	0	0	0

S2D2

	1	2	3	4	5	avg
Clay (%)	0.6	0.69	0.66	0.76	0.88	0.718
Silt (%)	22.91	25.64	23.8	26.67	27.5	25.304
Sand (%)	73.41	70.77	71.91	69.11	67.88	70.616
Gravel (%)	3.04	2.9	3.66	3.49	3.73	3.364

S3D1

	1	2	3	4	5	avg
Clay (%)	0.6	0.69	0.66	0.76	0.88	0.718
Silt (%)	22.91	25.64	23.8	26.67	27.5	25.304
Sand (%)	73.41	70.77	71.91	69.11	67.88	70.616
Gravel (%)	3.04	2.9	3.66	3.49	3.73	3.364

S3D2

	1	2	3	4	5	avg
Clay (%)	2.73	2.28	2.2	2.25	2.38	2.368
Silt (%)	64.54	53.91	51.91	52.85	55.3	55.702
Sand (%)	32.74	38.34	39.34	38.9	37.27	37.334
Gravel (%)	0	5.35	6.51	5.98	5.06	4.58

S4D1

	1	2	3	4	5	avg
Clay (%)	0.07	0.18	0.3	0.37	0.39	0.262
Silt (%)	11.17	12.8	15.04	16.8	17.05	14.572
Sand (%)	88.74	87.01	84.66	82.82	82.54	85.154
Gravel (%)	0	0	0	0	0	0

S4D2

	1	2	3	4	5	avg
Clay (%)	0.09	0.25	0.45	0.62	0.74	0.43
Silt (%)	34.67	39.04	41.53	43.74	44.49	40.694
Sand (%)	62.25	60.71	58.04	55.65	54,8	58.89
Gravel (%)	0	0	0	0	0	0

S5D1

	1	2	3	4	5	avg
Clay (%)	00.51	0.55	0.67	0.74	0.77	0.648
Silt (%)	15.72	15.74	17.86	18.96	18.71	17.398
Sand (%)	83.76	83.73	81.44	80.28	80.53	81.948
Gravel (%)	0	0	0	0	0	0

S5D2

	1	2	3	4	5	avg
Clay (%)	0.74	1.07	0.96	1.16	1.19	1.024
Silt (%)	23.13	28.7	25.14	28.78	28.5	28.85
Sand (%)	76.15	70.22	73.91	70.07	70.27	72.124
Gravel (%)	0	0	0	0	0	0

S6D1

	1	2	3	4	5	avg
Clay (%)	0.16	0.26	0.33	0.35	0.49	0.318
Silt (%)	9.99	11,66	13.91	13.16	15.28	12.8
Sand (%)	89.82	88.09	85.77	86.5	84.18	88.872
Gravel (%)	0	0	0	0	0	0

S6D2

	1	2	3	4	5	avg
Clay (%)	0	0	0.07	0.15	0.26	0.096
Silt (%)	4.7	6.12	7.37	8.05	9.42	7.132
Sand (%)	93.1	91.44	90.94	89.2	88.13	90.562
Gravel (%)	2.22	2.41	1.62	2.63	2.18	2.212

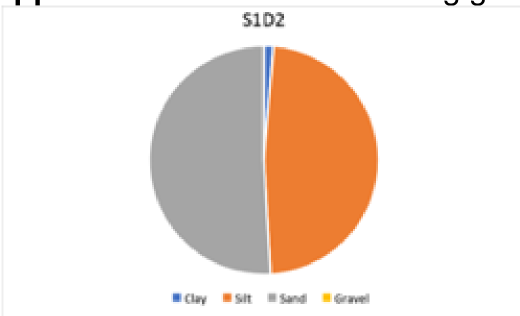
S7D1

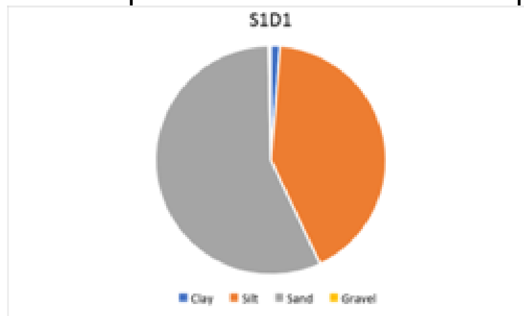
	1	2	3	4	5	avg
Clay (%)	1.02	1.28	1.85	1.99	2.08	1.644
Silt (%)	21.05	24.29	31.47	31.82	31.5	28.026
Sand (%)	77.91	74.39	66.66	66.2	66.42	70.316
Gravel (%)	0	0	0	0	0	0

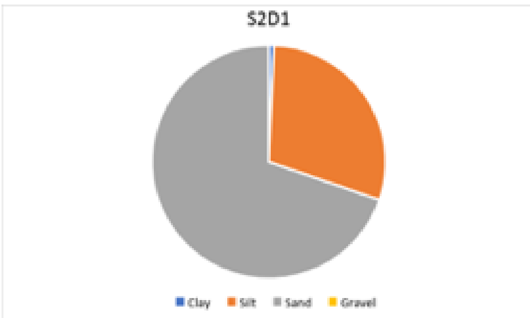
S7D2

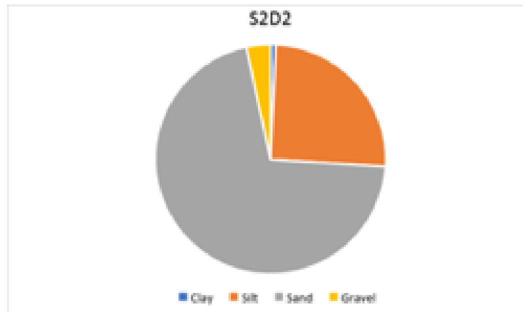
	1	2	3	4	5	avg
Clay (%)	0.21	0.46	0.67	0.85	1.05	0.648
Silt (%)	24.33	30.49	32.75	35.7	36.99	32.052
Sand (%)	75.43	69.1	66.58	63.49	61.97	67.314
Gravel (%)	0	0	0	0	0	0

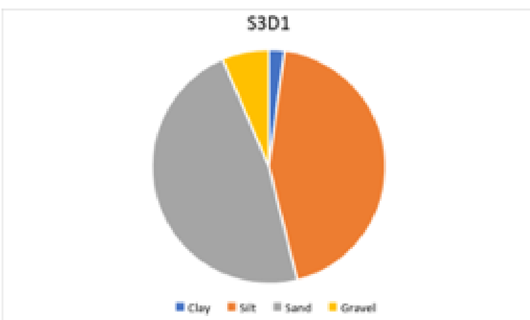
Appendix 4 - Pie charts showing grain size composition from each soil sample

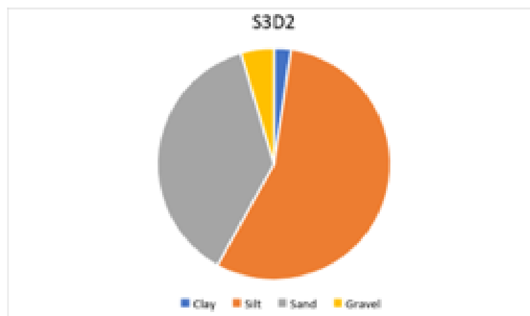


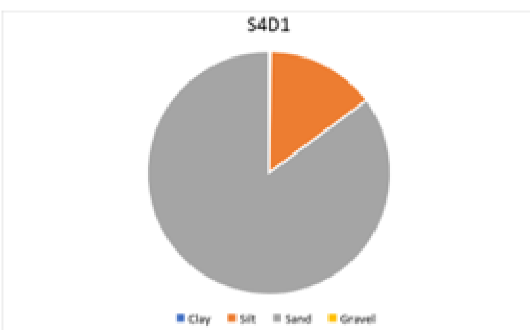


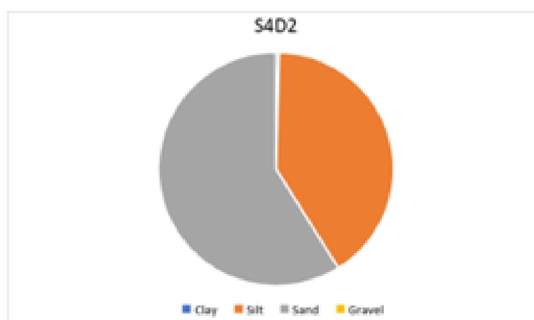


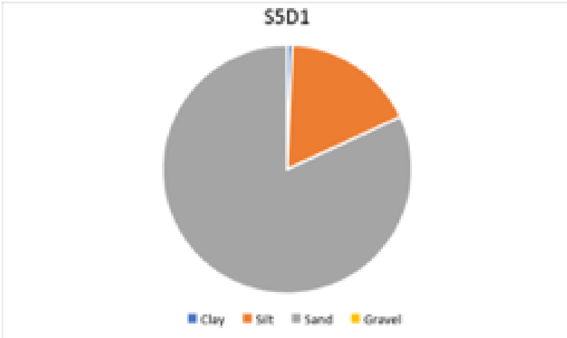


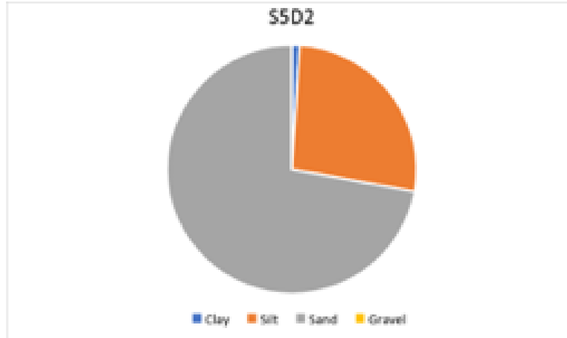


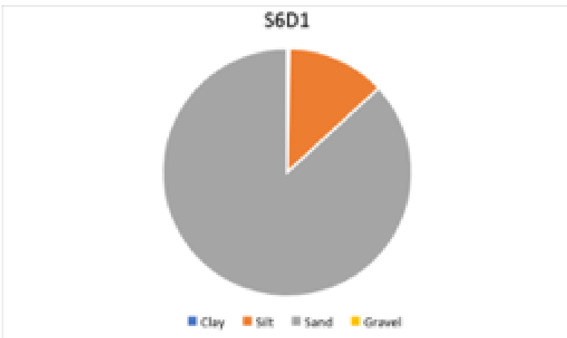


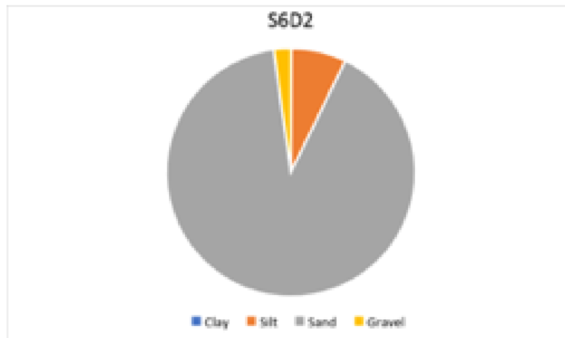


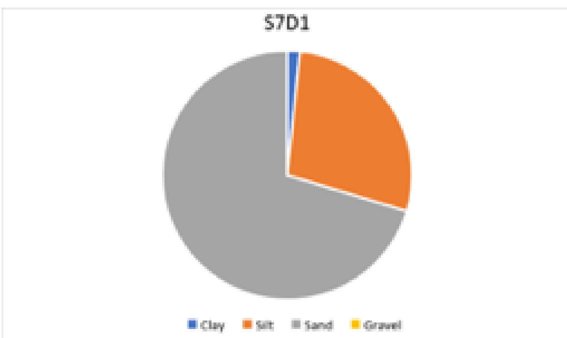


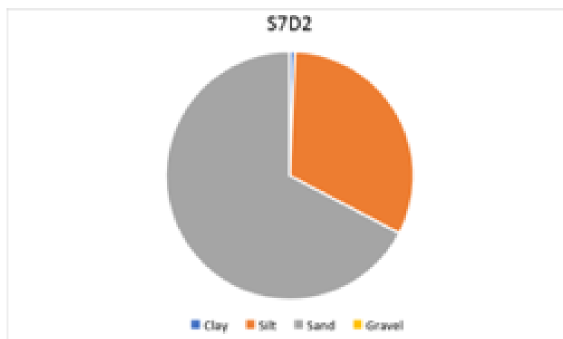

S5D1
Clay
Silt
Sand
Gravel


S5D2
Clay
Silt
Sand
Gravel


S6D1
Clay
Silt
Sand
Gravel


S6D2
Clay
Silt
Sand
Gravel


S7D1
Clay
Silt
Sand
Gravel


S7D2
Clay
Silt
Sand
Gravel

Appendix 5 - Clay content in soil sample

Site	Clay Content (%)	
	D1	D2
1	1.284	1.338
2	0.674	0.718
3	2.152	2.368
4	0.262	0.43
5	0.648	1.024
6	0.318	0.096
7	1.644	0.648

Appendix 6 - Heavy metal data from Group 21 (2019)

Sample	Cadmium (mg kg^{-1})	Copper (mg kg^{-1})
1	0.0029	0.0282
2		0.0489
3		0.1503
4		0.0191
5		0.0222

Appendix 7 - Heavy metal data from Group 23 (2019)

Sample	Cadmium (mg kg^{-1})	Copper (mg kg^{-1})
1	0.0126	0.0325
2	0.0186	0.0117
4	0.0054	0.0359
5	0.0063	0.0124
6	0.0044	0.0358
7	0.0029	0.0154
8	0.0187	0.0129
10	0.073	0.0252
11	0.0048	0.0076
12	0.0056	0.0121
13	0.0043	0.0148
14	0.0094	0.0127

Appendix 8 - Carbonate content of the soil samples

Site	Carbonate Content (%)	
	D1	**D2**
1	16.2	0.6
2	10.76	9.71
3	13.34	20.02
4	5.34	9.08
5	9.72	15.93
6.1	12.82	0.99
6.2	9.4	19.21
6.3	11.85	12.64
7	3.17	7.2

Appendix 9 - Average heavy metal concentration in soils at each site (using highest value from each site)

Site	**Calcium (Mg Kg^{-1})**	**Cadmium (Mg Kg^{-1})**	**Copper (Mg Kg^{-1})**	**Manganese (Mg Kg^{-1})**	**Lead (Mg Kg^{-1})**
1	305.06	0.0083	0.0409	3.365	0.246
2	189.9	0.0026	0.0223	0.888	0.1574
3	281.67	0.0026	0.0187	1.12	0.2277
4	336.39	0.0047	0.0249	2.669	0.2574
5	406.74	0.0068	0.0346	2.343	0.2177
6	397.62	0.0062	0.045	3.134	0.2506
7	276.76	0.0056	0.0293	1.248	0.179

Appendix 10 - Heavy metal concentration in trees

Tree identification number	Cadmium (Mg Kg^{-1})	Chromium (Mg Kg^{-1})	Iron (Mg Kg^{-1})	Nickel (Mg Kg^{-1})	Zinc (Mg Kg^{-1})	Weight (Mg Kg^{-1})
0532	0.00038113		0.0930920		0.27818961	0.2099
0504	0.00047416	0.0474158	0.3546705	0.0272831	0.42374585	0.2109
0509	00045188				0.37850881	0.2213
0537			0.11661832		0.84348943	0.2413
0526			0.0781802		0.25973498	0.2264
Apple	0.00025295		0.13111298		0.35132378	0.2372

Appendix 11: Bristol School of Geographical Sciences Undergraduate Ethics Form

		YES	NO	Action
1.	Does your research involve living human subjects?		NO	If NO, go to Q.3,11,12,13 & '*Declaration'*
2.	Does your research involve ONLY the analysis of large, secondary and anonymised datasets?		NO	If YES, go to Q.3,11,12,13 & '*Declaration'*
3.	Do/will others hold copyright or other rights over the information or data you collect?		NO	If YES please provide further details below
4.	Will you give your informants a written and/or verbal summary of your research and its uses?		NO	If NO, please provide further details below.
5.	Does your research involve covert surveillance (for example, participant observation)?		NO	If YES, please provide further details below
6.	Will your informants *automatically* be anonymised in your research?	YES		If NO, please provide further details below.
7.	Will you explicitly give *all* your informants the right to remain anonymous?	YES		If NO, please provide further details below.

8.	Will monitoring/recording devices be used openly and only with the permission of informants?	YES		If NO, why not? – give details below.
9.	Have you considered the implications of your research intervention on informants?	N/A		Please provide details below.
10.	Will data/information be encrypted/secured, and stored separately from identification material to maintain confidentiality?	YES		If NO, why not? – give details below.
11.	Will your informants be provided with a summary of your research findings?	YES		If NO, please provide further details below.
12.	Will there be restrictions on your research being available through the university data archive (e.g. by the sponsoring authorities)?		NO	Please provide details below.
13.	Have you identified other potential ethical issues arising from this research?	YES		The project looks at potential pollutant contamination e.g. of heavy metals such as lead into soils of the Manor Wood Orchard and assesses the likelihood the apple trees here have up taken these pollutants into the fruits which are available for the public to eat. If high levels of potentially harmful or toxic pollutants are found within the apples this bears ethical

				issues regarding the health of the public who have previously eaten these fruits over several years.