Final report

Geochemical lead contamination of cattle, sheep and free range chickens on UK farms

FSA Project FS241030

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Contents

Summary

This survey was carried out by the University of Bristol, assisted by Aberystwyth University, the Food and Environment Research Agency (Fera), The National Soil Institute (NSI) at Cranfield University, the Animal Health and Veterinary Laboratories Agency (AHVLA) and the British Geological Survey (BGS).

Three regions within England and Wales(Aberystwyth, Derbyshire and The Mendips), known to be areas with high levels of soil geochemical lead (Pb) were sampled for extensively reared sheep, cattle, laying hens and broilers (chickens reared for meat). For comparison, control samples of similarly reared animals were also collected from areas with low levels of soil geochemical Pb. Soil from pasture and ranging areas was also sampled and analysed for Pb, as were sources of feed and water available to the animals.

Levels of Pb in the blood, muscle and liver of the animals were measured as was Pb in the kidneys of cattle and sheep, and eggs from the laying hens. Levels of Pb in all tissues were increased in all animals from the regions of high geochemical Pb compared with low, however, in only one out of the 82 animals (a ewe) sampled from the high areas was the level above that of the current maximum level (ML) of 0.1 mg/kg for meat. In contrast, samples of liver and kidney in sheep were mostly above the ML of 0.5 mg/kg, as was kidney from cattle from these regions. Only a small number of cattle livers and hen livers were above the ML. ML's are generally only set for foods that contribute significantly to general dietary exposure. There is currently no ML set for lead in eggs.

A statistical modelling exercise was carried out to determine if levels of blood Pb could be used to predict the levels that would be found in consumed tissues. The models show promise, predicting levels of Pb within all of the specific tissue types tested to an accuracy that could be of use to regulators. The predictive power of the models was marginally improved if other variables such as age, the time of year of sampling and levels of Pb in local soils were included. Seasonal variation was only statistically significant for levels of Pb in sheep, with the highest levels seen in November.

A further analysis was carried out to test whether environmental variables alone could be used to predict levels of tissue Pb. Whilst the study had only a low power to investigate this relationship, as it had not been designed for this purpose, nonetheless, the results do suggest that there is predictive power from measurements of soil Pb alone. This is of importance, as it suggests that it should be possible to link data, already available, relating to the distribution of geochemical Pb across the UK as a whole, to data on the distribution of farmed animals across the UK as a whole. This would enable a determination of the risk of Pb exposure from animal sources for the UK population. Given that there is not thought to be a threshold for critical adverse Pb-induced effects, the larger areas within the UK with low to moderate levels of geochemical Pb could make an important contribution to population Pb exposure.

Introduction

Background

Both the Joint FAO/WHO Expert Committee on Food Additives - JECFA [1] and the European Food Standards Agency - EFSA [2], in the same year, concluded that the current provisional tolerable weekly intake (PTWI) for lead (Pb) of 25 µg/kg bodyweight (b.w.) was no longer appropriate, so it was withdrawn, as there is no evidence for a threshold for critical Pb-induced effects. EFSA concluded that in adults, children and infants, the margins of exposures were such that the possibility of an effect from Pb in some consumers, particularly in children from 1-7 years of age, could not be excluded. The JECFA report concluded that, for populations with a prolonged dietary exposure to Pb, measures should be taken to identify major contributing sources and foods. If found to be appropriate, methods of reducing dietary exposure, that are commensurate with the level of risk reduction, should then be sought so as to ensure adequate protection of the UK consumer.

The majority of on-farm Pb toxicity events are found to be due to point-source contamination e.g. animals ingesting metallic Pb from broken or badly stored batteries, Pb-based paints, bonfire ash, etc. However, the longer-term exposure to lower level contamination, arising from agricultural production in areas of naturally-occurring geochemical-Pb, is less easily discerned, and raises particular concern when the foods are intended for the human food-chain. Hence, JECFA has concluded that national governments should identify significant sources of dietary Pb, with a view to establishing control methods to reduce any potential routes of exposure, where the level of risk was found to be appropriate. Therefore, as part of the evidence gathering exercise required before any such activities are undertaken, there is a need to establish the extent to which geochemical-Pb contamination can enter the food-chain, specifically via beef animals, lamb, chickens and eggs.

State of Science in This Area

There is a significant body of work available within the literature relating to the uptake of heavy metals by animals ‘at grass’ from the environment. Much of this work is focussed on anthropogenically generated sources such as mine waste [3], aerial deposition from smelters [4], sewage sludge [5, 6, 7] and other sources [8], rather than naturally-occurring ‘geochemical’ sources. In addition to this, many of the studies have involved the use of a limited number of livestock species, produced under restricted conditions, and sampled within relatively small geographical areas. As a result, there is a paucity of data available upon which food safety agencies can make robust evaluations of the risks associated with animal production across the range of relevant species and husbandry practices. However, government agencies now hold nation-wide data relevant to Pb contamination in animal products in a form which is easily accessible and may be combined to provide a nation-wide overview of the complete risk posed within any given geochemical-Pb scenario.

Scientific and Technological Basis for the Work

Soil survey databases were used in conjunction with government animal holding databases to identify farms located in areas of high (≥1500 mg/kg), medium (< 1500 and ≥150 mg/kg) and low (<150 mg/kg) geochemical Pb. The categorisation of high, medium and low areas was agreed with FSA on the basis of the distribution of Pb levels seen throughout the UK. A map of Pb top soil levels in England and Wales derived from the NSI database is shown in Appendix 1:-

- Cranfield University have access to, and hold, national soil databases which contain detailed, systematically collected, information on soil properties, which includes soil-Pb concentrations. The main databases are the National Soil Inventory (NSI), a 5km resolution grid sampling across all England and Wales (http://www.landis.org.uk/data/nsi.cfm) and the Hutton database, a 10km resolution grid sampling across Scotland. Aberystwyth University hold farm specific data from numerous internal studies investigating areas high in geochemical Pb.

- The Animal Health and Veterinary Laboratories Agency (AHVLA) hold data which identify individual 'agricultural holdings', the geographic location, area, number and types of animals on them and additional information regarding the type of production system. Data from this database was combined to identify appropriate farms in areas of differing geochemical-Pb, which were approached to participate in the study.

- Authenticated, traceable samples of meat, offal, eggs, soil and herbage were taken from farms in areas of high geochemical-Pb and also from control areas.

Multilevel Modelling is a powerful statistical approach which has been specifically developed to facilitate the analysis of survey data [9]. In most surveys, data points are unavoidably 'clustered', with the result that correlations invariably exist between the data points. This means that they cannot be seen to be truly 'statistically independent' samples. In addition to this, clustering will often be at several, higher levels, e.g. within-farm animals; and within-geographic area and within farms. Multilevel Modelling provides a valid means of analysing these types of data structure and additionally is robust in coping with missing values and unbalanced 'fixed' effects. Therefore, the use of Multilevel Modelling provides a suitable means of analysing the data collected in this study, thereby maximising the use of the information held within it. The Centre for Multilevel Modelling (http://www.bristol.ac.uk/cmm/), the group which develops and maintains the most widely used software implementation of this statistical approach, is based within the University of Bristol.

Relevance to FSA Requirement C01R0021

The study was designed to provide FSA with the evidence it required for a preliminary study relating to levels of high geochemical Pb in cattle, sheep and free range chickens and eggs from UK farms. Research has shown that a major source of contamination is by means of ingested soil, for example, with soil intake in sheep of up to 40% of dry matter intake at certain times of year [10]. Further, as also listed in the requirements, the study provides an assessment of the use of blood-Pb levels to predict the burden of Pb within the associated animal tissue.

The study is also a proof of concept of an approach put forward in our original proposal. That is the ability to predict the contribution of soils to Pb in animal products produced on land in any geochemical-Pb area, not just 'high', and across the UK as a whole. This is important, as many of those soils which do not fall within the arbitrary category of 'high' used here, are likely to contribute a significant proportion of dietary-Pb exposure simply because of the larger areas of farmable land that these soils represent. This cut-down study provides evidence that a predictive model can be formulated, as per our original proposal, additionally providing data that could be used as part of an expanded study which would be required to more accurately define the relationship.

Materials and Methods

Sampling Regions

Using topsoil data from the National Soil Inventory (NSI), held by Cranfield, and data held from surveys carried out by the Department of Geography and Earth Science, Aberystwyth University, three sampling strata were identified within England and Wales; regions where geochemical levels of Pb were frequently found to be ≥ 1500 mg/kg (High), regions between 1499 to 150 mg/kg (Medium) and regions < 150 mg/kg (Low). Given the scale of the survey, three geographically distinct areas with high levels of geochemical Pb were identified; The Mendips, in Somerset, Aberystwyth, in Wales, and the Peak District in Derbyshire. Sampling of animals was divided between these three areas as evenly as possible to allow the survey to capture any differences in the risk of contamination between the varying soil types. Farm specific data from AHVLA and Aberystwyth University was used to target individual farms within these regions for livestock sampling. Local veterinary practices were also used to help identify farms that had experienced problems with Pb in the past and where the farmers were interested in assisting the survey.

Sampling Plan

The aim was to sample the number and type of animals from the High, Medium and Low regions as shown in Table 1, with animals from the High regions selected evenly from each of The Mendips, Aberystwyth and Peak District. As shown in Table 1, within sheep, both lambs and ewes, and within cattle, beef animals and cull dairy cows were sampled, as these types of animal enter the human food chain at very different ages and age was likely to be a factor correlated with the level of Pb contamination found in body tissues. The age of slaughter of poultry is more standardised, with the majority of commercially held hens culled at approximately 72 weeks of age and the majority of free range broilers slaughtered at youngest, at 56 days of age.

Table 1. Sampling plan of number and type of animal by regions of Low, Medium and High Pb.

Cattle	Low	3 young stock (beef), 3 mature animals (dairy cull)
	High	9 young stock (beef), 9 mature animals (dairy cull)
Sheep	Low	3 lamb, 3 ewe
	Medium	3 lamb, 3 ewe
	High	9 lamb, 9 ewe
Free Range Hens	Low	6 hens
	High	18 hens
Free Range Broilers	Low	6 broiler
	High	18 broiler

A further aim within the survey was to attempt to sample a cluster of 6 animals within a single farm to allow an estimate of within-farm variation. However, it was anticipated that whilst a cluster of 6 hens or broilers within a single farm would be possible, only a limited number of farms would be able to supply a cluster of 6 cattle (3 beef, 3 dairy culls) or 6 sheep (3 lambs, 3 ewes) to the survey. The collection of animals was planned to extend over a 12 month period to capture any seasonal variation in levels of Pb contamination.

Farm Level Measurements

On every farm from which animals were obtained bulked soil samples were collected. Fields and areas identified by the farmer where the animals had been free to range/graze over recent months, for at least 4 weeks, were sampled by collecting at least 25 sub-samples at even intervals whilst following a 'W' shaped path across the area as a whole. A soil auger was used to a depth of 20cm and the top 5cm, containing grass and root, discarded. Sampling from waterlogged areas and spots contaminated with faeces was avoided. Post collection, samples were spread out on benches to air dry until little moisture remained, then manipulated to ensure a mixing of the sub-samples before being sent to Eurofins UK laboratory in Wolverhampton for analysis of Pb by ICP-MS, following acid extraction, and a 'simple' soil texture analysis using a hand texture chart (). Approximately one sample from each site was also analysed for Pb by ICP-MS following EDTA extraction. This was done to allow a comparison of the Pb levels from each method. It has been shown that EDTA extraction may more closely reflect the Pb available for uptake by an animal [11]. The details of the methodologies supplied by Eurofins UK are provided as electronic Supplement 4.

Where possible, on each site from which a bulked soil sample was obtained, a water sample was obtained from a source available to the animals and a bulked herbage sample was also collected at each sub-sampling point. The herbage was cut to a level similar to which it might be grazed (approximately 2.5cm) avoiding further contamination with soil, but retaining any already present. Samples of any other feed available to the animals were also obtained, such as preserved fodder (hay and silage), concentrates, etc. These were stored at -20°C, before being sent to the Food and Environment Research Agency (Fera) laboratories, York for analysis of Pb by ICP-MS. Samples were analysed as sent and any soil present included in the analysis to more closely match an animal's intake. Details of the analytical methods employed by Fera are given in Appendix 2.

Animal Level Measurements

Blood, muscle and liver samples were collected from all four types of animal. Additionally kidney was collected from cattle and sheep, and eggs collected from laying hens. Arrangements were made with the farmers whose cattle were identified as suitable for sampling for the sampling team to be alerted when the animals were to be sent for slaughter. Arrangements were then put in place with the Meat Hygiene Service (now FSA) operatives at the abattoirs to collect the required samples. Where a farmer would have normally sent animals to market the farmer was reimbursed for any price difference between the abattoir price and market price on the day. Sheep to be sampled were personally collected by the survey team and transported by trailer to the abattoir at the University of Bristol Veterinary School, where they were laired overnight for slaughter the next day. Similarly, all poultry were transported to the Veterinary School where broilers were slaughtered and sampled upon arrival and laying hens held overnight with feed and water available and only slaughtered and sampled the following day after they had laid an egg. Hens were housed individually overnight so that the egg could be matched to the individual bird. For all animals whole, untreated blood was collected into sample tubes at the time of slaughter. Whole breast muscle samples were obtained from poultry, diaphragm (skirt) and sterno-mandibularis/sterno-mastoideus muscle were obtained from sheep and sterno-mandibularis/sterno-mastoideus muscle obtained from cattle. Sterno-mandibularis and sterno-mastoideus are muscles within the neck and were sampled as their removal had least effect on the value of the carcasses. Samples were stored at -80°C before being sent to Fera laboratories, York for analysis of Pb by ICP-MS. Details of the analytical methods employed by Fera are given in Appendix 2. All analyses are reported on a fresh weight basis.

Isotope Ratio Analysis

Analysis of the differing proportions of the different isotopes of Pb within a sample allows samples to be approximately matched to the geographic source of the Pb. An analysis of the samples collected for the survey was carried out to check that Pb in the animal samples was of local geochemical origin and had not occurred from an alternate source. An analysis of the Pb isotope ratios found within each animal surveyed was carried out by Professor Jane Evan's laboratory at the British Geological Survey (BGS). As successful analysis is dependent upon the presence of a sufficient amount of Pb, to maximise the chance of a successful analysis different tissue types were analysed for each species: liver from sheep and laying hens, kidney from cattle and blood from broilers. The report of the analysis provided by BGS, which includes the method is given in Appendix 3. Note that the raw results in Appendix 3 are labelled using an internal laboratory code which does not match other codes in this report. However, another, indexed copy of the results is provided as an electronic Supplement 2 to this report, and the results are also analysed, reported and discussed in much fuller detail below.

Results

Data

The database of full results from the survey is included with this report as an electronic Excel file as Supplement 1 and the results of the isotope ratio analysis of selected tissue samples from each animal is included as an electronic Excel file as Supplement 2. FSA have also been supplied with a further Excel file (FS241030 - FSA reporting form - February 2014.xls) with the relevant survey results entered in to the standard FSA reporting form which uses the EFSA concise food classification system. All Pb levels are given as mg/kg in Supplements 1 and 2, and only these units are used in the analyses reported below. FSA had requested that blood Pb is reported in units of µmol/l and these units are included for blood only as a separate column in Supplement 1. However, only units of mg/kg are used in this report as mixing units between sample types would have made interpretation of the results more difficult. Units of mg/kg Pb may be converted to µmol/l Pb by dividing by 0.2072.

Soil and Water

A key aim of the survey was to target areas of high and low soil geochemical Pb. Figure 1 shows the maximum levels of Pb found in a bulked soil sample from the land associated with each animal sampled across the survey as a whole. It can be seen from Figure 1 that the success in achieving the very highest levels of Pb in the associated soil samples was dependent upon species, but a reasonable range was acquired for all species and some extremely high levels of soil Pb in bulked samples also acquired for all species. The distribution of the levels of Pb seen in Figure 1 reflects the husbandry associated with each of the animal types. The survey took place from February 2012 to June 2013. As the sample IDs shown in Figure 1 approximately reflect the chronological order in which samples were collected, and samples were collected at a reasonably steady rate, Figure 1 shows how sample collection progressed within species and how samples were clustered in time. Sample ID is also referenced within the electronic data file.

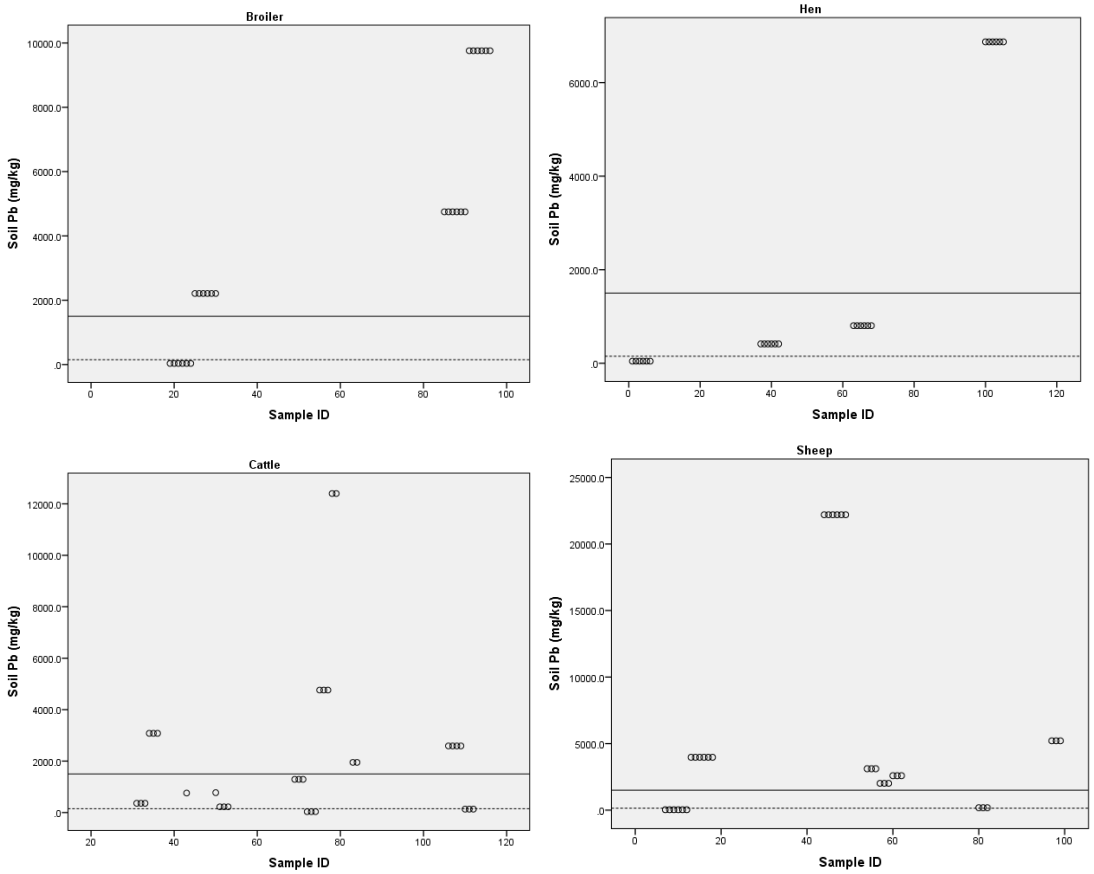


Figure 1. The maximum levels of Pb obtained from the bulked soil samples associated with each animal in the survey, broken down by species. Note that the scale of the vertical axes on each graph differ. The solid horizontal line on each graph is set at 1,500 mg/kg soil Pb and the broken line, at 150 mg/kg. Sample ID corresponds to Row ID in the electronic Supplement files (equivalent to Animal ID).

Only one commercial scale, free-range laying hen farm could be identified on land within the areas of high Pb, and upon the advice of their veterinarian, this farm declined to participate in the survey. The free-range hens from areas of geochemical Pb above 1,500 mg/kg were collected from small-scale free-range systems and these were always located in close proximity to the farm house. The age of birds required (at end of lay) further constrained the numbers available for the survey. No commercial free-range broiler systems could be identified on an area of land associated with the highest levels of geochemical Pb. This is because these areas are generally at higher altitude, more exposed and on relatively rougher terrain than areas low in geochemical Pb. However, free range runs and broilers of a commercial free-range strain were provided by the survey team to farms familiar with keeping poultry. In this way it was possible to target their placement on soil with the highest levels of Pb. One batch was reared in the Aberystwyth area and two batches were reared on different farms in the Mendips. Given the altitude and exposure of the areas of high geochemical Pb in Derbyshire it was not considered practicable to attempt to rear outdoor broilers in this area. Broilers were only reared for the survey in the spring and summer months, again because of the relative exposure in winter of land high in geochemical Pb.

Sheep tended to be grazed upon the most contaminated pastures as these were at the higher altitudes. Cattle are less able to stand the extremes of weather at altitude and tended to be grazed on the lower, richer, but less contaminated pastures of the farms in the areas of high geochemical Pb and this is reflected to some extent in the levels of Pb seen in the bulked soil samples associated with the two

species (Figure 1). For example, the bulked soil sample Pb for the one bovine obtained from the farm with the highest bulked soil Pb for sheep (22,200 mg/kg) was 770 mg/kg.

Only one bulked soil sample was collected for each batch of poultry as each batch was confined to a single, fenced area. The number of bulked samples collected for sheep and cattle ranged from one to five and was dependent upon the extent that the animals were moved between pastures and also upon the farmers' ability to recall the areas recently grazed by animals. The simple texture analysis identified four soil types (clay loam, silty clay loam, sandy silt loam and silty loam. These were not used in the analyses reported below as they were confounded with other variables.

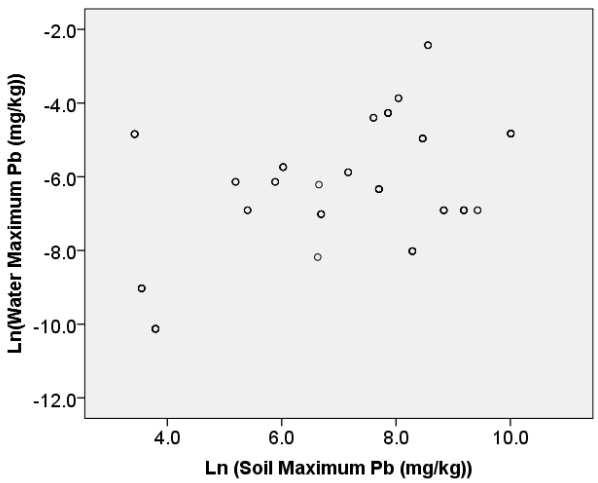
A small scale investigation of the heterogeneity to be found in levels of geochemical Pb in spot soil samples within a locality (three fields) was carried out with the help of Aberystwyth University on one of the survey farms, an upland farm in Aberystwyth, known to have high levels of Pb. The study is discussed below and the results shown in Appendix 4. Spot samples across the three fields ranged in value from 177 to 2061 mg/kg Pb as determined by portable X-ray fluorescent (pXRF) analyser. A comparison of measurements of soil Pb by ICP-MS and portable X-ray fluorescent (pXRF) analyser is also provided in Appendix 5.

An analysis of the relationship between the levels of Pb measured in soil using either acid extraction or EDTA extraction, followed by ICP-MS, is described in Appendix 6. EDTA extraction consistently gave levels of lead at 50% the value of those given by acid extraction. Because of the very close linear relationship between the two methods, only the values measured using acid extraction are used in this report.

Water samples were collected from drinking sources for all animals, but for two groups of three cattle. It can be seen from Figure 2 that there was a relatively weak relationship between the maximum levels of Pb in bulked soil samples from a site and maximum levels of Pb found in the water, and this was especially poor if the two samples with least Pb are ignored.

Figure 2. A plot of the maximum level of Pb found in a bulked soil sample and the maximum Pb level found in the associated water sample. Note that the natural log values are used in the plot.

Levels of Pb in bulked soil samples from control (low) sites ranged from 30.8 to 222 mg/kg and from 360 to 22,200 mg/kg from high Pb sites. The respective values for water were 0.00004 to 0.00789 mg/kg low, and 0.00030 to 0.0880 kg/kg, high (the current EU maximum guideline for Pb in drinking water is 0.010 mg/kg [12]).



Animals

The actual number of animals sampled within each of the target categories is given in Table 2, with the target number in brackets. Control, mature cattle were three over target, high, beef cattle six over target and high, mature cattle two under target. With sheep, medium Pb level lamb were three under target, and high, three over target for both lamb and ewe, with the remaining categories of sheep and all poultry on target. In terms of total animals sampled the overall number was 112 against a target total of 102. Cattle in the high category were collected from across 9 farms and from 3 farms for the low category, the respective numbers of farms for sheep were 6 and 1, and 1 for medium. For poultry, animals were collected in groups of 6 from 4 farms for layers and 4 farms for the broilers.

Table 2. Number and type of animal obtained in the survey by regions of Low, Medium and High Pb with target number in brackets.

Cattle	Low	3 (3) young stock (beef), 6 (3) mature animals (dairy cull)
	High	15 (9) young stock (beef), 7 (9) mature animals (dairy cull)
Sheep	Low	3 (3) lamb, 3 (3) ewe
	Medium	0 (3) lamb, 3 (3) ewe
	High	12 (9) lamb, 12 (9) ewe
Free Range Hens	Low	6 (6) hens
	High	18 (18) hens
Free Range Broilers	Low	6 (6) broiler
	High	18 (18) broiler

Animal Tissues

Summary Statistics and Comparison between Areas

All tissue samples were collected and analysed per animal as planned, bar the blood samples from two cull cows that were slaughtered before the sampling team at the abattoir were prepared to take samples. The approximate age ranges of animals sampled were: 16 to 130 months for cattle, 6 to 72 months sheep, 9 to 26 months laying hens and 2 months of age for broilers.

The isotope ratio analysis provided a means of checking that the Pb seen in tissue samples was of local geochemical origin and not from point contamination from, for example, Pb from a car battery. One tissue sample from each animal in the survey was analysed for isotope ratios. A sample with ratios at variance with other samples from the same geographic area, combined with an unusually high level of Pb in that sample or another tissue sample from the same animal would have led to the exclusion of the animal from the survey results. Following inspection of the isotope ratios and the levels of tissue Pb no sample was excluded. Inspection of the isotope ratios was by means of a dynamic, moving plot in which the seven ratios are projected in 2-dimensions whilst being rotated in 7 -dimensions. A video showing an example of the exploration of the isotope ratios is provided as electronic Supplement 3 and a snapshot summarising the general conclusions of the data exploration is shown in Appendix 7 together with a description of the approach to the graphical, statistical analysis. The clustering in the isotope ratios by geographic location is readily apparent from the snapshot in Appendix 7 with Pb from Aberystwyth very distinct from that of The Mendips and Derbyshire, and a marginal overlap between the ratios from The Mendips and Derbyshire, whilst the control samples are again distinct, but widely dispersed. A paper for submission to a refereed

journal discussing the isotope ratio results has been prepared and a draft is currently with FSA for approval (Evans et al, 2014).

The distribution of the levels of Pb from the animal tissues sampled across the survey are shown in Figures 3 and 4 as a series of boxplots broken down by tissue type and then animal type within tissue. Figure 3 shows only samples from the areas classified as high in geochemical Pb and Figure 4 shows only samples from the control, low geochemical Pb area samples. The Pb levels in control tissue samples were an order of magnitude less than those from areas of high Pb and so the vertical axes in Figure 4 have been up-scaled by an order of magnitude. It should also be kept in mind when making comparisons across the Figures that approximately only one third the number of samples contributed to each of the histograms from the control areas in Figure 4. The box in a boxplot encompasses all data points between the 25 and 75 per cent quartiles, whilst the bar in the centre of the box shows the median value (50 percentile). The whiskers extend to 1.5 times the height of the box or, if no case/row has a value in that range, to the minimum or maximum values. The circles outside the whiskers are outlying values, whilst the asterisks show extreme points that have values more than three times the height of the boxes.

From Figure 3, on areas of high geochemical Pb, there appear to be distinct patterns in the levels of Pb that are dependant both upon type of animal and type of tissue. These patterns appear to be mirrored, but to a less marked extent, in the animals from areas of low Pb (Figure 4). The boxplots show the raw values found in the tissues, not adjusted for actual Pb concentrations found in the bulked soil samples from land the animals were known to have grazed, but as such they are probably a better reflection of the true situation found across farms located on soils high in geochemical Pb. The liver (L) and kidney (K) of sheep (S) accumulated the highest levels of Pb (Median values L = 1.603, K = 1.748 mg/kg) with lower levels of Pb found in the liver of broilers (B), cattle (C) and hens (H) (Median values B = 0.140, C = 0.284, H = 0.271 mg/kg). However, the highest concentrations of Pb in cattle was seen in kidney (Median 0.725 mg/kg). Kidney from broilers and hens was not included in the survey as the kidneys are small and are not consumed on any scale.

The levels of Pb found in blood (Median values B = 0.209, C = 0.070, H = 0.377, S = 0.111 mg/kg) were generally an order of magnitude less than those found in liver and kidney for sheep and cattle, but with higher levels to be found in the blood of both broilers and hens.. The levels of Pb found in muscle tissue (Median values B = 0.005, C = 0.005, H = 0.009, S = 0.021 mg/kg) was two orders of magnitude less than those found in liver and kidney, with the overall shape of the distributions appearing a close match to those found in liver across animal type.

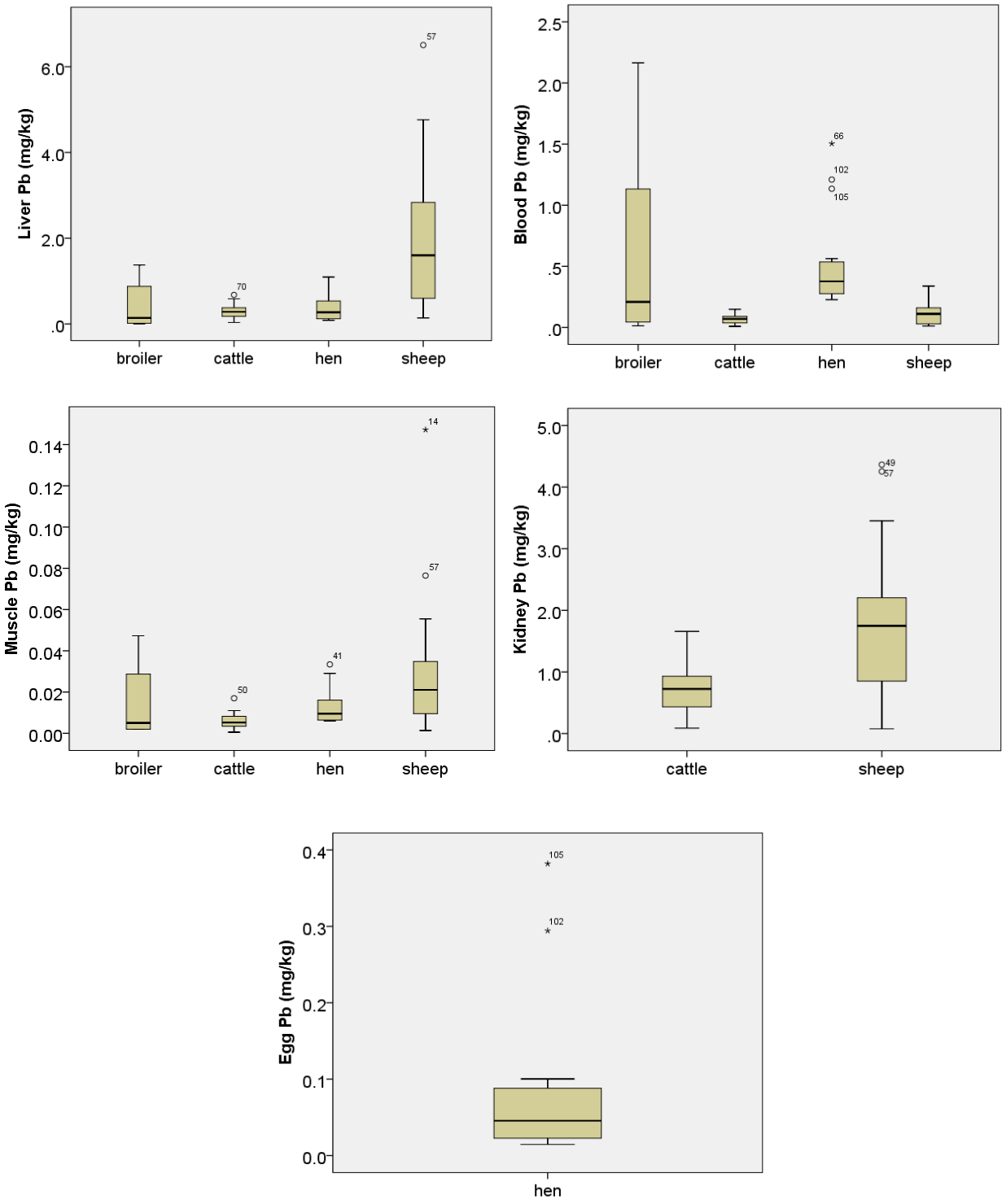


Figure 3. Boxplots showing the distribution of Pb from the five tissue types sampled from areas of high geochemical Pb only, broken down by type of tissue and animal. Note that the vertical axis scale differs between tissue type. The labels for distant values and outliers correspond to the Row ID (animal number) in the Supplement 1 Data file.

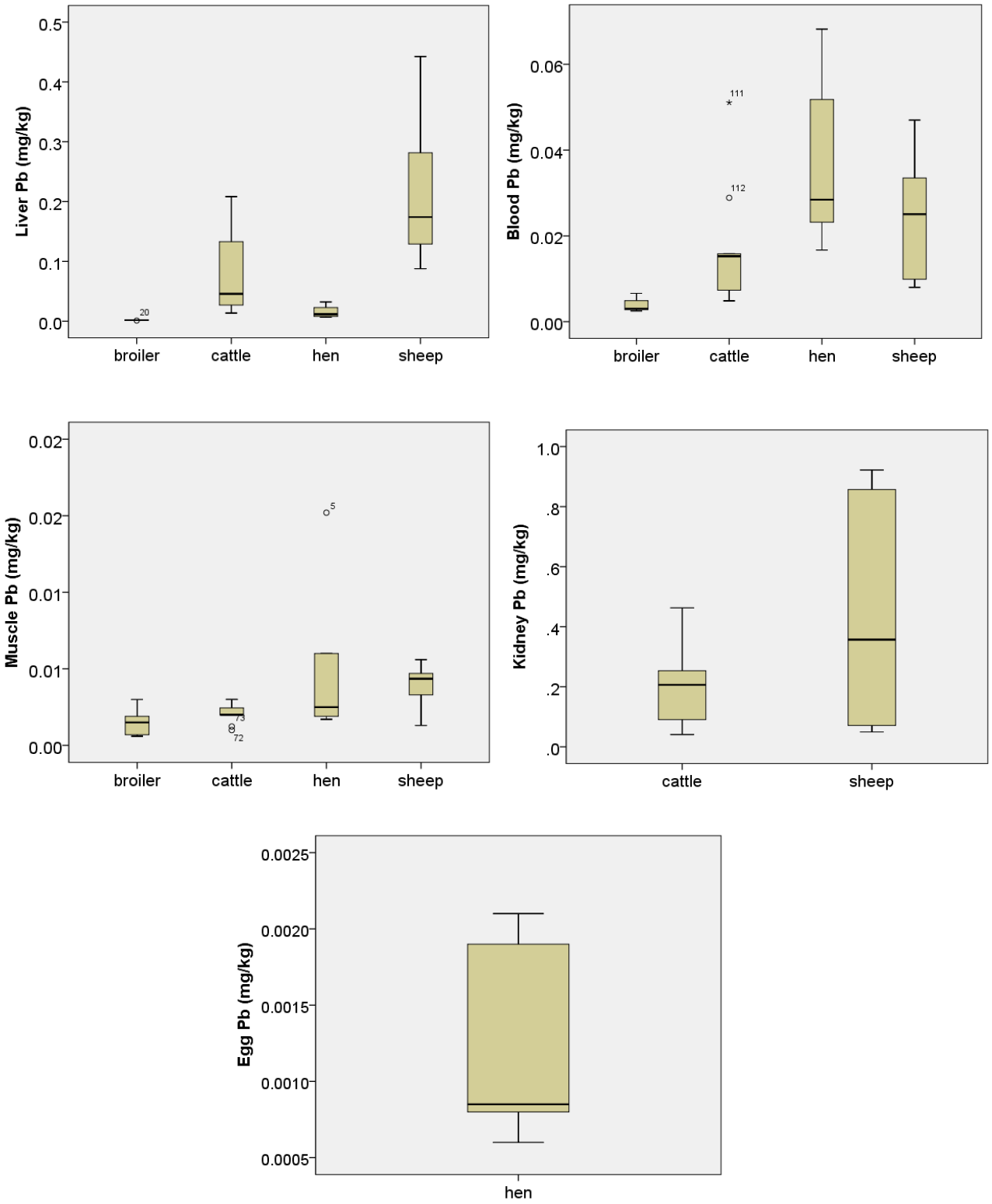


Figure 4. Boxplots showing the distribution of Pb from the five tissue types sampled from areas of low geochemical Pb only, broken down by type of tissue and animal. Note that the vertical axis scale differs between tissue type. The labels for distant values and outliers correspond to the Row ID (animal number) in the Supplement 1 Data file.

The Pb levels found in egg showed a similar distribution to those in the blood from the laying hens, but approximately four times lower. The outlying values seen in the boxplot for hens in Figure 3 were from the birds kept on the soil with very high levels of geochemical Pb (> 6000 mg/kg (Figure 1)).

Paired t-tests were carried out within animal type to formally test for overall differences in Pb levels between the tissue types; liver – kidney, kidney – blood and liver – blood. All comparisons showed the Pb levels in the paired tissue types to be significantly different ($p < 0.001$) apart from liver – kidney in sheep ($p = 0.688$). The analyses were carried out on the natural log transformed data to meet the assumptions required for this parametric test. Table 3 shows the mean ratios of tissue Pb to blood Pb within animal type together with the 95% confidence interval for each estimate. A ratio was calculated, for each animal, within tissue type within animal type, using the raw, untransformed values. As the distributions of the ratios were generally right skewed they were then natural log transformed for calculation of the mean and a 95% confidence interval. The back transformed values for the mean and 95% confidence interval are shown in the Table.

Table 3. The ratios of the untransformed Pb values of blood Pb to tissue Pb within each animal type (i.e. tissue Pb/blood Pb). The mean value is shown together with an upper and lower 95% confidence interval (ci).

Ratio		Broiler			Cattle			Hen			Sheep	
	Mean	lci	uci	Mean	lci	uci	Mean	lci	uci	Mean	lci	uci
Liver:Blood	0.524	0.453	0.606	4.30	3.56	5.20	0.587	0.480	0.718	13.4	10.9	16.6
Kidney:Blood	-	-	-	11.3	9.42	13.5	-	-	-	14.0	11.3	17.3
Muscle:Blood	0.064	0.038	0.106	0.105	0.080	0.139	0.037	0.025	0.054	0.207	0.168	0.255
Egg:Blood	-	-	-	-	-	-	0.085	0.062	0.118	-	-	-

Prediction of Pb Levels in Consumed Tissues

Statistical models were constructed to test the power of Pb levels in blood alone to predict levels of Pb in each of the other tissues. A second set of models was then constructed to test the predictive power when additional variables such as age and time of year of sampling were available. A further, third set of models was then constructed to test the predictive power when additional environmental variables such as levels of Pb in the soil grazed, Pb levels in nearby water supplies, and levels of Pb in herbage and other feed were available.

Statistical models were fitted at the level of animal type, the aim being to produce a single equation that would predict the Pb levels within the various tissue types within that animal. This process was repeated through the three steps described above, by introducing and testing additional predictor variables to give one predictive equation for each animal type, for each of the three steps. A Chi-square test of the change in log likelihood was used to determine which terms were retained in a model.

Models were constructed using the hierarchical modelling statistical software MLwiN which allowed the underlying structure of the data and internal correlations to be properly taken into account. A three level model was specified; tissue type, within individual animal, within farm. For all of these analyses, the levels of all Pb measurements (tissue, soil, water and feed) were natural log transformed to accommodate the assumptions required; of normally distributed residuals and homogeneity of their variance.

Prediction from Blood Pb Alone

In step one, for prediction from levels of blood Pb alone, tissue type, blood Pb and their interaction were entered as predictor variables into the models. A squared term for blood Pb was also tested in all models to investigate the possibility of a non-linear relationship. The fitted models for each of the animal types are shown in Table 4 and their graphical representations are shown in Figure 5 together with the raw data upon which the models were based. In these models and below, overall terms were retained in the model at $p \leq 0.05$ based on a Chi-square test of the magnitude of the change in log likelihood. Note that when the overall inclusion of a fixed factor is significant all of the levels of that factor will be represented in the predictive equation but individual levels may not be significantly different from the level tied to the constant.

For readers not familiar with the formulation, interpretation and use of these type of statistical model, in Appendix 8 the parameter estimates for cattle from Table 4 are used as an example of how to construct the predictive equation and how to use it to predict Pb levels in muscle, liver and kidney.

Table 4. The parameter estimates (β), their standard error (se) and p value, for the statistical models of levels of Pb in consumed tissues as predicted by levels of blood Pb alone. Four models were constructed, one for each animal type. All Pb levels are natural log transformed for the models. The predicted values from these models are shown graphically in Figure 5.

Predictor Variable	Broiler			Cattle			Layer			Sheep		
	β	se	p	β	se	p	β	se	p	β	se	p
Constant (Liver)	-0.465	0.178	0.009 **	1.3	0.388	<0.001 ***	-0.289	0.171	0.091	2.537	0.309	<0.001 ***
Muscle	-3.554	0.155	<0.001 ***	-5.107	0.285	<0.001 ***	-3.867	0.219	<0.001 ***	-4.173	0.096	<0.001 ***
Kidney	-	-	-	0.592	0.285	0.038 *	-	-	-	0.039	0.092	0.672
Egg	-	-	-	-	-	-	-1.528	0.219	<0.001 ***	-	-	-
Blood	1.07	0.05	<0.001 ***	0.943	0.112	<0.001 ***	1.162	0.088	<0.001 ***	0.983	0.092	<0.001 ***
Muscle x Blood	-0.559	0.045	<0.001 ***	-0.421	0.083	<0.001 ***	-0.732	0.112	<0.001 ***	-	-	-
Kidney x Blood	-	-	-	-0.113	0.083	0.173	-	-	-	-	-	-
Egg x Blood	-	-	-	-	-	-	0.264	0.112	0.018 *	-	-	-

* p < 0.05, ** p < 0.01, *** p < 0.001

Overall, from Table 4 and Figure 5 it can be seen that the models provide a reasonable prediction of levels of Pb in consumed tissues from measurement of an animal's blood Pb alone. The majority of parameter estimates are highly significant and the raw data in the graphs matches the prediction lines reasonably well. In all cases, as blood Pb increases so does the predicted level of tissue Pb. Within sheep the rate of change with blood Pb was the same for all tissues, and levels in liver and kidney were predicted to be the same at any given level of blood Pb. The rate of increase of muscle Pb with blood Pb was lower than for the other tissues in broilers, layers and cattle. For cattle the difference in predicted Pb between liver and kidney for a given level of blood Pb did reach significance, unlike sheep, as illustrated in the respective graphs.

Prediction by Blood, Age and Day of Year (Season)

In step two the animal level variables age (in months) and 'day of the year the samples were taken' were tested as additional predictors in the models derived in step one. The day of year was converted to a seasonal effect, that is, it was converted into a separate sin term and a cosine term which when both

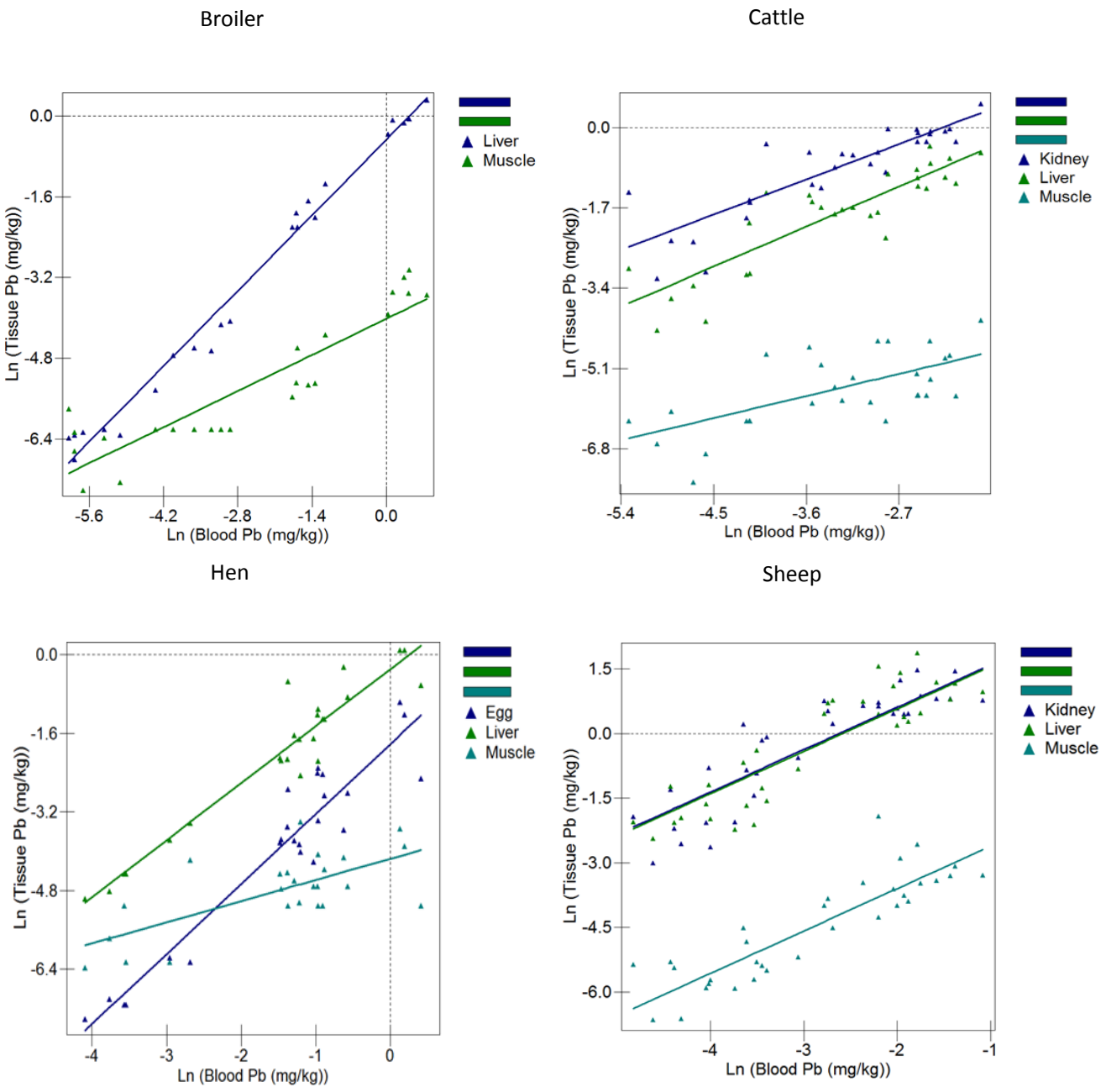


Figure 5. A graphical view of the statistical models predicting levels of tissue Pb from blood Pb alone. Each graph shows the model for one type of animal with the predicted values for a tissue type shown by a line. The actual data upon which the models are based are shown by the points. All Pb values are on a natural log scale.

entered into the model allowed a single cycle of a sinusoidal form to be fitted to the outcome variable - adjusted for amplitude and shifted along the x-axis to provide a best fit. Given the relatively small sample size for each animal only a subset of possible models were tested; age and also its interaction with type of tissue were tested plus the sin and cosine variables as main effects only. The parameter estimates, their standard errors and p values for these models are shown in Table 5.

Table 5. The parameter estimates (β), their standard error(se) and p value, for the statistical models of levels of Pb in consumed tissues as predicted by levels of blood Pb, age and 'day of the year the sample was collected'. Four models were constructed, one for each animal type. All Pb levels were natural log transformed for use in the models. The Sin and Cos terms allow a seasonal effect to be modelled, as described in the main text.

Predictor Variable		Broiler			Cattle			Layer			Sheep	
	β	se	p	β	se	p	β	se	p	β	se	p
Constant (Liver)	2.027	0.783	0.010 *	1.1961	0.328	<0.001 ***	-0.289	0.171	0.091	2.862	0.235	<0.001 ***
Muscle	-3.554	0.155	<0.001 ***	-5.107	0.285	<0.001 ***	-3.867	0.219	<0.001 ***	-4.446	0.146	<0.001 ***
Kidney	-	-	-	0.5916	0.285	0.038 *	-	-	-	0.221	0.146	0.130
Egg	-	-	-	-	-	-	-1.528	0.219	<0.001 ***	-	-	-
Blood	1.105	0.082	<0.001 ***	1.0499	0.099	<0.001 ***	1.162	0.088	<0.001 ***	1.192	0.081	<0.001 ***
Blood x Muscle	-0.559	0.045	<0.001 ***	-0.421	0.083	<0.001 ***	-0.732	0.112	<0.001 ***	-	-	-
Blood x Kidney	-	-	-	-0.113	0.083	0.172	-	-	-	-	-	-
Egg x Blood	-	-	-	-	-	-	0.264	0.112	0.018 *	-	-	-
Age (Mnths)	-	-	-	0.0087	0.003	0.004 **	-	-	-	0.008	0.004	0.024 *
Age x Muscle	-	-	-	-	-	-	-	-	-	0.007	0.003	0.024 *
Age x Kidney	-	-	-	-	-	-	-	-	-	-0.005	0.003	0.129
Sin	-1.363	0.611	0.026 *	-	-	-	-	-	-	-0.23	0.089	0.010 **
Cos	2.218	0.713	0.002 **	-	-	-	-	-	-	0.154	0.139	0.268

* p < 0.05, ** p < 0.01, *** p < 0.001

Age was not tested within the broiler model as all birds were sampled at approximately the same age. There was a highly significant seasonal effect within the broiler model; however, as there were only four sampling times it is not possible to determine if this was a real effect of season or simply spurious over-modelling of the data. In the cattle model increased age was associated with increasing Pb in all the consumed tissues. There was no seasonal effect detectable within the cattle data. Within the predictive model for laying hens, there was no detectable effect of age. There was a significant effect of season, however, this was not retained in the model as given the number of predictors already fitted and the small sample size it could easily have been due to over-fitting. Within the predictive model for sheep, there was a small but significant increase in tissue Pb with increasing age which was approximately doubled in rate within muscle on a natural log scale. There was a significant seasonal effect on levels of Pb in tissues with a minimum in May rising to a maximum in November. If this effect was real, it could reflect the faster growing spring grass and slow growing, potentially muddy grass in winter, but equally could also be due to the seasonal nature of lamb production.

Prediction by Blood, Age, Soil and Feed

In step three, variables associated with the environment in which the animals were reared were tested as additional predictors in the models derived in step two. The maximum level of Pb found in a bulked soil sample, a water sample, a fresh herbage sample, a preserved herbage sample, a concentrate feed sample and the maximum Pb level found in any of the feed sources, where applicable, were tested as variables within the models from step 2. All of these variables were natural log transformed. Again, given the relatively small sample size of the study each of the variables was simply added as a main effect; polynomials and interactions with other variables were not tested.

In terms of environmental variables, for broilers, herbage was only obtained from three of the four sites as there was insufficient to sample when the birds were collected, and two of the four concentrate rations fed had the same level of Pb. Thus, given that there were only four sites across the 24 birds sampled there was little power within the study to investigate environmental variables and with data from only four

farms there is a likelihood that any significant variables would be significant simply because of over-fitting the model to too few data points. The size of the study means that it had little actual power to identify the effect of environmental variables in determining levels of tissue Pb in broilers. The above constraint also held for laying hens as these were also collected from only four farms, thus models for broilers and hens were not tested.

Within the predictive model for cattle none of the environmental variables were significant in adding any further predictive power, although soil Pb did approach significance, an increase in soil Pb concentration being associated with an increase in tissue Pb concentration. As with broilers, because the environmental variables are farm level variables, rather than animal level variables (as with blood), there is a lack of power to determine an effect. Further weakening the power of the analyses, there were many missing values amongst the individual variables for herbage, preserved herbage and concentrate because different batches of animals had tended to be fed only one type of feed at the time of sampling.

The seasonal effect was first removed from the model for sheep obtained in step two, for the same reason given above, before the environmental variables were tested in the model. Neither maximum levels of soil nor water Pb were statistically significant. The sample size was reduced for the variables herbage, preserved herbage and concentrate as different batches of animals had tended to be fed on only one feed type at the time they were obtained. None of these variables were significant within the model. However, there were no missing values for the variable 'maximum Pb level across all feed sources' and this variable was significant ($p = 0.008$) within the model, predicting an increase in tissue Ln(Pb mg/Kg) of 0.133 (se 0.049) for every 1.0 increase of Ln(Pb mg/kg) in a feed, after tissue Pb had been adjusted for all the other variables in the equation.

Example Prediction Intervals for Blood Models and Blood, Age, Season Models

To give a more objective sense of the accuracy of the models produced for prediction from blood alone and the models using blood, age and season as predictors, Table 6 shows a predicted level of Pb in liver for each of the animal types for each model, together with a 95% confidence interval for that estimate. The prediction is made at the 'centre' of the data used to produce the model, that is at the mean level of blood Pb in the data, the mean age of the animals and a standardised time of year, all of which are constant within type of animal but which will vary across type of animal.

Table 6. The predicted level of Pb in liver, and its upper and lower 95% cent confidence interval (ci), at the 'centre' of the models using blood alone as a predictor (Table 4) and blood, age and season (Table 5). The mean level of blood Pb, the level at which the prediction is made, is also shown. All values are in units of mg/kg Pb fresh weight.

		Blood Pb	Liver Pb	lci	uci
Broiler	Blood alone	0.075	0.039	0.031	0.050
	Blood, age, season	0.075	0.039	0.034	0.046
Cattle	Blood alone	0.028	0.127	0.101	0.160
	Blood, age, season	0.028	0.124	0.104	0.148
Hen	Blood alone	0.221	0.130	0.105	0.159
	Blood, age, season	0.221	0.130	0.105	0.159
Sheep	Blood alone	0.054	0.717	0.557	0.923
	Blood, age, season	0.054	0.726	0.613	0.854

Prediction by Age and Environmental Variables Alone

As can be seen from Figure 5, an animal's blood Pb appears to be a good predictor of levels of Pb in its consumed tissues. However, it would be of use to be able to predict tissue Pb from simply the type and age of animals and the environment in which they are kept, without the requirement of first taking a blood sample. For this reason a model for each type of animal was tested with tissue type, age and the maximum level of Pb found in a bulked soil sample', a water sample', a fresh herbage sample', a preserved herbage sample', a concentrate feed sample and the maximum Pb level found in any of these feed sources, where applicable, as predictor variables. An interaction effect between the environmental variable and tissue type was tested but age was only tested as a main effect. Given the relatively small size of the data sets, each environmental variable was tested only individually within a model, and no polynomial terms were tested. The parameter estimates for the final models, with their standard error and p value, are given in Table 7.

Table 7. The parameter estimates (β), their standard error (se) and p value, for the statistical models of levels of Pb in consumed tissues as predicted by animal type, tissue type, age and soil Pb. Four models were constructed, one for each animal type. All Pb levels were natural log transformed for use in the models.

Predictor Variable		Broiler			Cattle			Layer			Sheep	
	β	se	p	β	se	p	β	se	p	β	se	p
Constant (Liver)	-7.956	2.522	0.002 **	-4.392	0.901	<0.001 ***	-6.272	0.954	<0.001 ***	-2.654	1.269	0.036 *
Muscle	0.961	0.701	0.17	-2.256	0.33	<0.001 ***	-0.156	0.59	0.791	-3.56	0.311	<0.001 ***
Kidney	-	-	-	1.259	0.33	<0.001 ***				0.341	0.311	0.273
Egg	-	-	-	-	-	-	-3.274	0.59	<0.001 ***	-	-	-
Soil	0.653	0.334	0.051	0.358	0.122	0.003 **	0.668	0.145	<0.001 ***	0.364	0.163	0.026 *
Soil x Muscle	-0.425	0.093	<0.001 ***	-0.216	0.048	<0.001 ***	-0.411	0.09	<0.001 ***	-0.084	0.041	0.040 *
Soil x Kidney	-	-	-	-0.047	0.048	0.327	-	-	-	-0.041	0.041	0.317
Soil x Egg	-	-	-	-	-	-	0.213	0.09	0.018 *	-	-	-
Age (Mnths)	-	-	-	0.004	0.005	0.424	0.602	0.112	<0.001 ***	-0.009	0.004	0.024 *

* p < 0.05, ** p < 0.01, *** p < 0.001

When tested individually within the broiler model, both maximum soil Pb and maximum water Pb were highly significant predictors of tissue Pb, with water being the slightly stronger. However, soil was the most consistent predictor across all the different types of animal taken together so the model with soil Pb is shown for broilers in Table 7. For the remaining animal types, soil Pb was the strongest predictor of tissue Pb. Water and other of the feed variables were variously statistically significant as predictors of tissue Pb, but only the models for soil are shown in Table 7. Although not significant, age was retained in the model for cattle as it had been shown to be highly significant in a previous model (Table 5).

The relationships shown in Table 7 between the levels of tissue Pb for each of the animal type and the levels of maximum soil Pb are shown in graphical form in Figure 6. The lines in the graphs show the model of the predicted values for each tissue type and the points show the actual data upon which the predictive models are based. The form of the relationships are very similar to those seen in Figure 5 where the levels of blood Pb were the predictor. This, of course, is because a strong correlation existed between levels of Pb in the soil and the levels found in the blood. As would be expected, the models in Figure 5 using blood as a predictor of tissue Pb show a tighter clustering of the raw data around the prediction lines than those in Figure 6 which use soil Pb. The models shown in Figure 6 are reasonable and are in agreement with those in Figure 5.

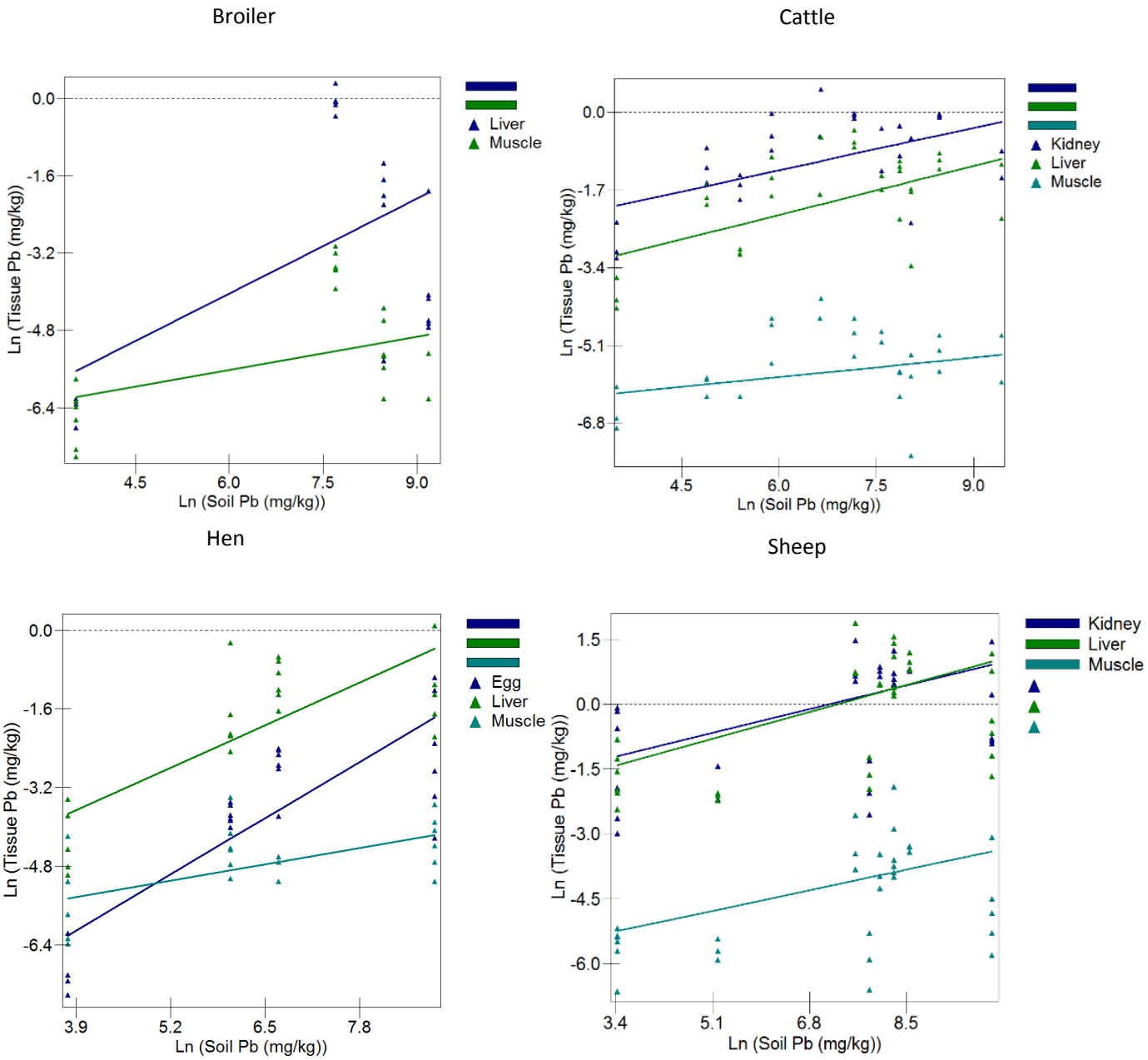


Figure 6. A graphical view of the statistical models predicting levels of tissue Pb from the maximum levels of Pb found in a bulked soil sample. Each graph shows the model for one type of animal with the predicted values for a tissue type shown by a line. The actual data upon which the models are based are shown by the points. All Pb values are on a natural log scale.

Example Prediction Intervals for Models Using Environmental Variables and Age

Similarly to Table 6, to give a better feel for the accuracy of the prediction, Table 8 shows the predicted level of Pb in the liver for each of the animal types from the model using soil and age as predictors alone, together with a 95% confidence interval for that estimate. Again the prediction is made at the 'centre' of the model/data where the interval will be at its narrowest. The mean soil Pb concentration for the animal type is also shown. All values are given on the original, untransformed, measurement scale.

Table 8. The predicted level of Pb in liver, and its upper and lower 95% confidence interval (ci), at the 'centre' of a model using soil and age alone as predictors (Table 7). The mean level of soil Pb, the level at which the prediction is made, is also shown. All values are in units of mg/kg Pb fresh weight.

	Soil Pb	Liver Pb	lci	uci
Broiler	1,375	0.040	0.009	0.177
Cattle	841	0.169	0.115	0.244
Hen	565	0.130	0.099	0.171
Sheep	1,536	0.735	0.393	1.361

Discussion

The survey was successful in obtaining samples from farms with some of the highest levels of geochemical Pb to be found in England and Wales (>1500 mg Pb/kg). This was only possible because FSA allowed the survey to be carried out anonymously. Nonetheless, a number of farmers did still decline to participate, but given the extreme levels of geochemical Pb to be seen in Figure 1 it is not thought that this has led to a bias in the survey results. All farmers were aware of the elevated levels of Pb on their land and most were cautious in cooperating with the survey. The majority took special measures to protect their stock, for example, by rotating animals across fields and keeping more susceptible animals from the fields with higher levels of Pb enrichment at the worst times of year.

The survey failed to meet target numbers for cull cows (7 instead of 9), but was well over-target for other cattle and overall animal numbers. The three lambs collected for the 'medium' Pb range, as identified from the NSI database, came from a farm at the foot of the Mendips. However, when the bulked soil analysis was returned at the close of the survey it brought them into the high Pb category. Nonetheless, a reasonable range of soil Pb was obtained for sheep (Figure 1). Dairy cattle were the most problematic of the animals to obtain. The greater majority of farm animals grazing land with the highest levels of soil Pb are sheep rather than cattle, and where cattle are grazed these are mostly animals reared for beef. This combined with the greater age that dairy culls are slaughtered meant that cull cows only infrequently became available.

The husbandry of the sheep, cattle and laying hens from the high soil Pb farms is reflected in the levels of Pb in the bulked soil samples taken from the land on which they were known to have recently been kept. Sheep were generally exposed to the highest levels on these farms (being grazed at higher altitudes where Pb levels were greater), with the bulked soil samples for cattle tending to have lower levels, and the hens being exposed to whatever level happened to be present in their range area close to the farmhouse. The placement of the broilers was dictated by the survey team and so could be deliberately targeted at areas with the highest levels.

The difficulty of obtaining an accurate measure of the exposure risk to animals on land with these very high levels of soil Pb is shown by the small scale study of local variation reported in Appendix 4. Within this small area the Pb concentrations in spot samples could differ by up to almost two orders of magnitude. The majority of the areas having naturally high soil Pb have also at some time been worked by man, and even if this was many hundreds of years ago, it is likely to have increased the variability within the area, producing 'hot-spots' which may not necessarily be picked up, even within a bulked sample. On

land where the source of the geochemical lead is from sediment deposited from contaminated upstream areas by flooding, (most notably on the grazing land nearer the foot of the rivers surrounding Aberystwyth), the deposition is likely to be more even, but probably with a demarcation within fields associated with the extent of any flooding. However, this type of land forms only a smaller part of the areas of high geochemical Pb in England and Wales.

A portable X-ray fluorescence spectrometer (pXRF) was used to carry out the assessment of variability within fields reported in Appendix 4. This instrument would have been of great benefit to the survey in confirming levels of geochemical lead on farms and in specific fields before sampling took place. As it was, farms were selected from historic records, the results of the soil analyses only becoming available weeks after a visit and after animals had been sampled. Whilst this did work reasonably well it would have been very convenient to be able to double check the farm as a whole, and once on farm to target sampling of animals currently grazing the fields with greater levels of soil Pb. This would have been possible as each pXRF reading took only 70 seconds. However, at over £20,000 for a single instrument, it was outside of budget. We are most grateful for the loan of the instrument from Aberystwyth University for the study reported in Appendix 4

ICP-MS and XRF measurements of Pb in soil have been shown to be in good agreement when a static, lab based XRF is used. Our study of agreement between the pXRF and the laboratory ICP-MS measurements of soil Pb showed the pXRF measurements to be a constant proportion of the measurement from the ICP-MS and showed, as would be expected, how the variability in the errors also increases in proportion to the magnitude of the soil Pb values. It is not fully understood why the two methodologies were systematically different, however, as the difference between the two methods of measurement were consistent, it is a simple matter to convert from one to the other.

Twenty five of the bulked soil samples were analysed using both acid and EDTA extraction as it was thought that EDTA extraction might give results that more closely measured the availability of the Pb to animals from the different types of ingested soil. However, surprisingly, the two extraction methods gave results that were very highly correlated, with the EDTA extraction systematically showing 50 per cent the levels of Pb found following an acid extraction (Appendix 6).

An analysis of the isotope ratios of the Pb found within each animal was carried out as a check that the Pb measured was locally derived and not a point contamination from a discarded, manufactured product containing lead. The Pb found in most contemporary products originates from other regions in the world, Australia in particular, and would have a set of ratios distinct from those found in Pb from the UK. The results obtained showed that all survey samples appeared to come from a local source of mineralisation, but also demonstrated that this approach appears to be very sensitive and able to attribute the source down to the level of specific regions of the UK with high precision (electronic supplement 3 and Appendix 7). It would be an interesting exercise to evaluate the exact precision that this approach affords. The results obtained here suggest that it may be possible to determine a source down to a very local level, perhaps even the level of a farm, or collection of farms.

The number of samples of consumed tissues that were above current maximum levels (ML) for Pb were not reported directly in the Results section, above. This is because the MLs are essentially arbitrary limits and, as discussed in the Introduction, contemporary thinking is that there is no real threshold for critical Pb-induced adverse effects. However, they are still a useful comparator against historic data. The current ML for Pb in meat is 0.1 mg/kg, and in liver and kidney 0.5 mg/kg. Of the muscle samples collected from all 82 animals on the ‘high’ geochemical Pb regions only one sample exceeded the ML for meat. The

sample, from a ewe, was particularly unusual as at 0.147 mg/kg it was 3 times greater than the next highest level in muscle from the same group of matched ewes sampled from the farm. Despite the high Pb in the muscle sample, the isotope ratios and Pb levels in all the other tissues from this ewe were otherwise similar to the rest of her group. This sample was retested, and the result verified.

None of the consumed tissue samples from the broilers were above the MLs. Five out of 18 livers from laying hens from the high farms were above the ML, there is currently no ML set for eggs. For sheep from the high category farms, 7 out of 12 ewe livers and 7 out of 12 ewe kidneys were above the ML, whilst for lamb the numbers were 12 out of 12 livers and 11 out of 12 kidneys. Generally, if a sheep was above the ML for liver so it was for kidney. For cattle from the high category farms 0 out of 7 cull cow livers and 3 out of 7 cull cow kidneys were above the ML, whilst for beef animals the numbers were 3 out of 15 livers and 13 out of 15 kidneys. As can be seen from the statistical models, and graphically in Figure 5, whilst Pb levels in liver and kidney within a single sheep tended to be very similar, within cattle the kidneys tended to accumulate a higher level of Pb than the liver.

Interestingly, three of the lambs from a control farm had levels of kidney Pb above the ML, but unlike those described above, their liver Pb was below the ML. The animals were from the South Downs, which are chalk, so it is most unlikely that the Pb had originated from local soils. A water sample from one of their troughs showed very high levels of Pb, comparable with the maximum found within the survey as a whole. It is likely that the source of contamination would have been from this trough, perhaps from old Pb farm piping. Apart from these three samples all other samples from animals from control farms were low, and below the MLs.

Whilst there are many studies in the literature that have measured levels of Pb in the different tissues of livestock, none could be found that had either sufficient sample size to test predictive models, or had attempted to model the relationships using anything more than simple correlations or tests of mean differences (Based upon a literature search of the Thompson ISI Web of Science database using the search terms ‘Pb’, ‘Soil’ and ‘Livestock’, which yielded 147 results). The models predicting Pb in the consumed tissues from blood alone fitted to the data here appear to be very promising. We have not included prediction intervals as this would overly complicate the graphs, however, a visual assessment of the fitted lines against the raw data (Figure 5) is sufficient to show the goodness of fit, and to allow some comparison across models. The confidence intervals given in Table 6 show the confidence interval for liver Pb at the point at which a model is most accurate. The increase in accuracy that the additional predictor variables afford can also be seen. That the data were collected from different geographic regions within the UK, across a variety of farms and at different times of year indicates that the models should be robust and broadly applicable to similar livestock throughout, at least, England and Wales. Although, because of the structure of the survey, there was only limited power to test models that used the environmental variables as predictors of tissue Pb, the results obtained appear promising with reasonably tight confidence intervals for the estimates of liver Pb (Table 8). Given the relatively small number of farms sampled, compared with the number of animals, the level of Pb in the soil proved statistically significant in the models and the parameter estimates appeared to show a sensible relationship with the predicted values. There was some evidence for a seasonal effect on Pb levels from the models fitted, but only for sheep, in which the levels of Pb appeared to peak within November. This is in line with the findings of Smith et al [4] whose work identified maximal soil intake in sheep as taking place in winter.

Pb in river sediment, and sometimes Pb in water, provide a useful summary measure of the degree of local and upstream soil Pb. Although water Pb was a significant predictor of tissue Pb within some of the

models, it was not as consistently good a predictor as soil Pb. This is most probably because the water samples collected within the survey were taken from the water available to the animals and this was very often water from the mains supply. In retrospect, it would have been useful to have also obtained a water sample from the nearest stream or river, regardless of whether the water was available to the animals. Further, in future studies it would be sensible to obtain a bulked river/stream sediment sample as this could provide a more accurate overall measure of the level of soil Pb as a whole in the surrounding area than samples from fields.

This survey has only looked at the contribution that areas of extremely high soil Pb have in determining the levels of Pb found in the tissues of livestock. It has also identified that the relationship between soil Pb and Pb levels in tissues appears to be a linear relationship on the natural log scale. It likely that, in terms of the overall intake of Pb in the human diet, larger areas of the UK where soils have medium to low soil Pb concentrations would contribute a greater part of the Pb load. If accurate equations linking levels of soil Pb to Pb in consumed animal tissues can be developed, it should be possible, with the combined use of current national livestock and national soil databases, to calculate the risk of Pb exposure for the UK population from livestock produced across the UK as a whole.

Conclusions

The survey has provided an estimate of the levels of Pb to be found in the blood and consumed tissues of cattle, sheep, laying hens and broilers extensively reared on regions with high levels of geochemical Pb across England and Wales. The study has demonstrated differences in the level of Pb accumulation which depend upon both the type of animal and the tissue type within an animal. The levels of Pb found in the consumed tissues could be predicted from the levels of Pb found in the blood of an animal. Blood Pb was a very strong predictor of tissue Pb in all tissues tested, and the predictive equations were often marginally improved with the inclusion of other variables such as the time of year a sample was taken, the age of an animal and the level of Pb found in the local environment (i.e. soil and water). Seasonal variation was only statistically significant for levels of Pb in sheep with the highest levels seen in November.

Prediction of the level of Pb in the consumed tissues from environmental variables alone appeared to be promising. Were predictive equations to be developed from a larger data gathering exercise, covering more farms and less animals per farm, in conjunction with national soil and animal databases, they could provide a useful estimate of overall population exposure to Pb from animal livestock tissues from UK production as a whole.

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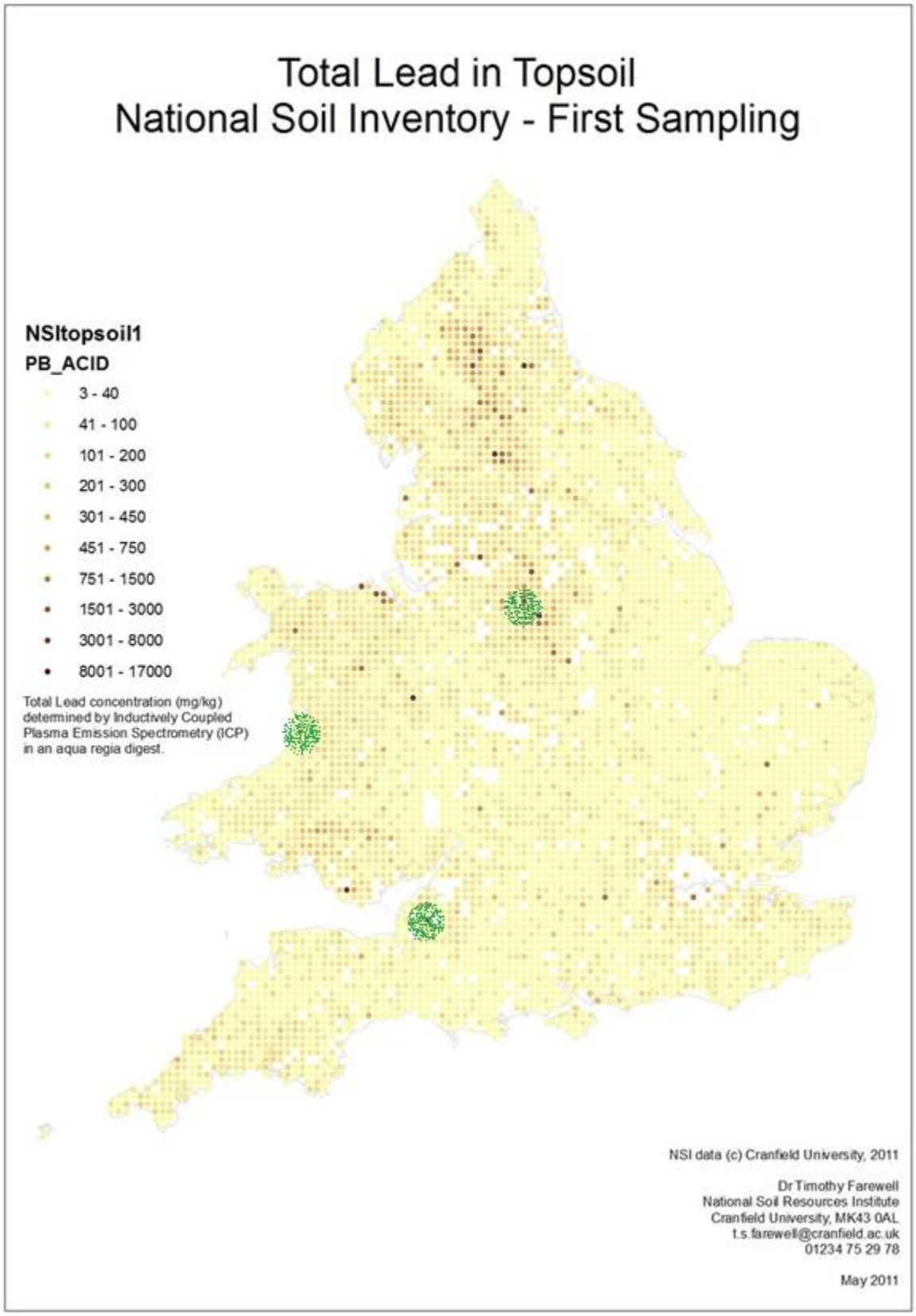
Appendix 1 – NSI derived Pb in Topsoil Map of England and Wales – with general sampling areas identified in green.



Appendix 2 – Fera Analytical Methods for Pb

Geochemical lead contamination of cattle, sheep, free range and organic chickens on UK farms

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1. INTRODUCTION

Lead (Pb) is a toxic metal that is present in the environment naturally and as a result of human activities. The main source of exposure to Pb for the general population is through the diet. Adverse effects of long-term cumulative exposure to Pb include damage to the kidneys and cardiovascular systems. However, it has also been demonstrated that low level exposure to Pb can affect the infant brain, leading to impaired intelligence. Both EFSA and JECFA have recently concluded that since it has not been possible to demonstrate a threshold level of exposure below which adverse effects on the infant brain are absent, the current PTWI for Pb of 25 µg/kg bw is no longer appropriate. JECFA has recommended that national governments should identify significant sources of dietary Pb, with a view to establishing control methods to reduce any potential routes of exposure, where the level of risk was found to be appropriate [1,2].

The majority of on-farm Pb toxicity events are found to be due to point-source contamination e.g., animals ingesting metallic Pb from broken or badly stored batteries, Pb-based paints, bonfire ash, etc. However, little is known about the longer-term exposure to lower level contaminationarising from agricultural production in areas of naturally occurring geochemical Pb. Therefore, there is a need to establish the extent to which geochemical Pb contamination can enter the food-chain.

This study, led by the University of Bristol, was designed to provide the FSA with data on the relationship between high geochemical Pb and Pb in the foodchain; specifically in cattle, sheep, free-range and organic chickens and eggs from UK farms. The findings are described in the main body of the University of Bristol's report. This appendix describes the determination of Pb in the animal tissue, herbage, feed and water samples collected for the study.

2. SAMPLES

A total of 504 samples were received during February 2012 and July 2013. These comprised samples of blood (110), muscle (112), liver (112), kidney (64), egg (24), water (30), herbage (31) and feed (21). The samples were collected from farms with either high, medium or low soil-Pb. Each sample was labelled with a unique identification code at the time of sampling. Upon receipt, the eggs were stored at +4°C, the feed at ambient temperature and all other samples were stored at -20°C.

3. SAMPLE HOMOGENISATION

Prior to analysis, meat, offal and feed samples were homogenized using a Buchi-400 blender. The egg samples (yolks and whites) were transferred to acid clean pots and shaken vigourously to mix, whilst the herbage was cut into small pieces using ceramic scissors or a ceramic knife. The whole blood samples were blended using a mini-turrex.

4. SAMPLE DIGESTION

To minimize Pb background, Milli-Q water, analytical grade reagents and acid cleaned plasticware were used throughout. Aliquots of each homogenised sample (approx 1 g meat/offal/blood, 2 g herbage, 0.5 g feed) were weighed into test tubes and 2 ml of concentrated nitric acid was added to each tube. The samples were then solubilised under high temperature and pressure using an Ultrawave Single Reaction Chamber microwave digestor system (Milestone). The digest solutions were cooled and transferred to polystyrene test tubes and made up to 10 ml with Milli-Q water.

5. Pb DETERMINATION BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY (ICP-MS)

Each sample digest was diluted 5-fold with internal standard, rhodium, and measured either on an Agilent 7700x or an Agilent 7500ce ICP-MS. Calibration standards were prepared using the same acid composition and commercially available Pb standard solution. ICP-MS conditions were as follows:

Mass:	208Pb
Cell mode:	'No gas'
Dwell time:	100ms
Cones:	Nickel
Nebulizer:	Glass concentric
Flow rate:	1 l/min
Tuning oxides:	< 1%

6. QUALITY ASSURANCE (QA)

All stages of sample handling, processing, analysis, data assessment and reporting were performed using UKAS-accredited (ISO17025) procedures. A 10 % audit (in duplicate) was carried out within the study to provide an indication of precision. Each analytical batch contained certified reference materials (CRMs), a freeze-dried liver in-house reference material, procedural blanks and spiked reagent blanks/samples for recovery estimate purposes. The criteria used to assess data are summarised below.

6.1 LIMITS OF DETECTION AND QUANTIFICATION (LOD and LOQ)

The LOD was defined as three times the standard deviation of the signal from procedural blanks when corrected for sample weight and dilution. The LOQ was defined as ten times the standard deviation of the signal from procedural blanks when subsequently corrected for sample weight and dilution.

6.2 INSTRUMENT STABILITY

Analyses included re-measurement of a calibration standard at set intervals during each ICP-MS run. In order to pass, the re-measured standards had to be within ± 20% of the initial value.

6.3 SPIKE RECOVERY

Data were accepted if the recovery of Pb spike was within the range 80 to 120%.

6.4 REFERENCE MATERIAL DATA

Data were accepted if results for the majority of reference materials were within ±25% of the certified value.

6.5 REPLICATE AGREEMENT

Replicate values for a given sample had to have a relative standard deviation of <20% to be acceptable.

7. RESULTS

Pb concentrations measured in each of the samples analysed are shown in Tables 1 – 8. Table 9 summarises the QA data from the study and relevant proficiency test results obtained by Fera during the study are shown in Table 10. Measured concentrations have been corrected for median procedural blank contribution and median spike recovery on a batch by batch basis. Tabulated results are adjusted to 3 significant figures or as appropriate for the LOD. Results are expressed as mg/kg or mg/L as received (i.e. not on a 100% dry matter basis) and values between the LOD and LOQ should be considered semi-quantitative. All replicate analyses showed good agreement except for the herbage samples which showed

greater variability. This is likely to be due to traces of soil contamination, as the herbage was deliberately not washed prior to analysis in order to better reflect dietary intake.

A summary is shown below indicating the minimum, maximum and average Pb levels found in the study samples:

	Pb (mg/kg, as received)		
Sample type	Min	Max	mean
Blood	<0.002	2.16	0.219
Eggs	<0.002	0.382	0.075
Feed	0.044	5.20	1.03
Herbage	0.021	96.8	12.2
Kidney	0.041	4.37	0.967
Liver	<0.002	6.51	0.658
Muscle	<0.002	0.147	0.015
Water	0.00002	0.088	0.006

8. REFERENCES

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2. EFSA, Scientific Opinion of the CONTAM Panel, “Lead in food”. The EFSA Journal 2010, 8, 1570, 147 pp

Table 1. Pb concentrations in Blood

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003299	001-B	0.023
S12-003302	002-B	0.028
S12-003303	003-B	0.052
S12-003305	004-B	0.017
S12-003308	005-B	0.068
S12-003309	006-B	0.029
S12-003311	007-B	0.008
S12-003313	008-B	0.018
S12-003315	009-B	0.034
S12-003317	010-B	0.010
S12-003319	011-B	0.047
S12-003321	012-B	0.032
S12-003323	013-B	0.130
S12-003326	014-B	0.111
S12-003328	015-B	0.140
S12-003329	016-B	0.153
S12-003331	017-B	0.145
S12-003333	018-B	0.135
S12-003335	019-B	0.003
S12-003337	020-B	0.003
S12-003339	021-B	0.003
S12-003341	022-B	0.005
S12-003343	023-B	0.003
S12-003345	024-B	0.007
S12-003347	025-B	1.04
S12-003349	026-B	1.54
S13-025255	027a-B	0.413
S12-003351	027-B	1.52
S13-025257	028a-B	1.13
S12-003353	028-B	1.40
S13-025259	029a-B	0.018
S12-003355	029-B	1.13
S13-025261	030a-B	0.032
S12-003357	030-B	2.16
S13-025263	031a-B	0.016
S12-003359	031-B	0.251
S13-025265	032a-B	0.051
S12-003361	032-B	0.535
S13-025267	033a-B	0.029
S12-003363	033-B	0.227
S12-003365	034-B	0.296
S12-003367	035-B	0.300
S12-003369	036-B	0.232
S12-003371	037-B	0.055
S12-003373	038-B	0.027
S12-003375	039-B	0.026
S12-003377	040-B	0.018
S12-003379	041-B	0.068
S12-003381	042-B	0.030
S12-003383	043-B	0.251
S12-003385	044-B	0.149
S12-003387	045-B	0.005
S12-003389	046-B	0.016
S12-003391	047-B	0.015
S12-003393	048-B	0.111
S12-003395	049-B	0.062
S12-003397	050-B	0.174
S12-003399	051-B	0.168
S12-003401	052-B	0.064
S12-003403	053-B	0.094
S12-003405	054-B	0.013
S12-003407	055-B	0.012

Table 1 continued. Pb concentrations in Blood

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003409	056-B	0.018
S12-003411	057-B	0.254
S12-003413	058-B	0.276
S12-003415	059-B	0.563
S12-003417	060-B	1.50
S12-003419	061-B	0.375
S12-003421	062-B	0.404
S12-003423	063-B	0.110
S12-003425	064-B	0.010
S12-003427	065-B	0.006
S12-003429	066-B	0.007
S12-003431	067-B	0.105
S12-003433	068-B	0.080
S12-003435	069-B	0.081
S12-003437	070-B	0.091
S12-003439	071-B	0.100
S12-003447	075-B	0.024
S12-003449	076-B	0.012
S12-003451	077-B	0.029
S12-003453	078-B	0.088
S12-003455	079-B	0.080
S12-003457	080-B	0.117
S12-003459	081-B	0.059
S12-003461	082-B	0.339
S12-003463	083-B	0.207
S12-003465	084-B	0.239
S12-003467	085-B	0.018
S12-003469	086-B	0.027
S12-003471	087-B	0.052
S12-003473	088-B	0.045
S12-003475	089-B	0.037

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003477	090-B	0.183
S12-003479	091-B	0.261
S12-003481	092-B	0.316
S12-003483	093-B	0.231
S12-003485	094-B	0.170
S12-003487	095-B	0.013
S12-003489	096-B	0.187
S12-003491	097-B	0.380
S12-003493	098-B	0.356
S12-003495	099-B	1.21
S12-003497	100-B	0.379
S12-016541	FSA1-B	0.028
S12-016543	FSA2-B	0.060
S12-016545	FSA3-B	0.036
S12-016547	FSA4-B	0.009
S12-016549	FSA5-B	0.039
S12-016551	FSA6-B	0.043

Table 2. Pb concentrations in Eggs

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003559	001-E	<0.002
S12-003560	002-E	<0.002
S12-003561	003-E	0.002
S12-003562	004-E	<0.002
S12-003563	005-E	<0.002
S12-003564	006-E	<0.002
S12-003565	007-E	0.030
S12-003566	008-E	0.028
S12-003567	009-E	0.022
S12-003568	010-E	0.021
S12-003569	011-E	0.018
S12-003570	012-E	0.023
S12-003571	013-E	0.065
S12-003572	014-E	0.023
S12-003573	015-E	0.060
S12-003574	016-E	0.080
S12-003575	017-E	0.090
S12-003576	018-E	0.088
S12-003577	019-E	0.100
S12-003578	020-E	0.015
S12-003579	021-E	0.294
S12-003580	022-E	0.034
S12-003581	023-E	0.057
S12-003582	024-E	0.382

Table 3. Pb concentrations in Feed

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S13-026990	001-F	0.46
S13-026991	002-F	0.05
S13-026992	003-F	0.04
S13-026993	005-F	0.20
S13-026994	006-F	0.18
S13-026995	007-F	4.55
S13-026996	008-F	5.20
S13-026997	009-F	0.41
S13-026998	010-F	1.22
S13-026999	011-F	0.07
S13-027000	014-F	0.86
S13-027001	015-F	0.77
S13-027002	016-F	0.11
S13-027003	017-F	0.14
S13-027004	018-F	1.10
S13-027005	019-F	0.38
S13-027006	021-F	0.15
S13-027007	022-F	0.07
S13-027008	023-F	1.50
S13-027009	024-F	0.19
S13-027010	025-F	4.08

Table 4. Pb concentrations in Herbage

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S13-026959	001-H	1.48
S13-026960	002-H	1.01
S13-026961	004-H	0.44
S13-026962	005-H	0.60
S13-026963	007-H	19.1
S13-026964	008-H	4.28
S13-026965	009-H	0.02
S13-026966	010-H	0.21
S13-026967	011-H	0.55
S13-026968	012-H	7.17
S13-026969	013-H	0.46
S13-026970	014-H	0.33
S13-026971	018-H	4.86
S13-026972	019-H	3.56
S13-026973	021-H	1.39
S13-026974	022-H	5.50
S13-026975	023-H	1.25
S13-026976	024-H	0.55
S13-026977	025-H	33.5
S13-026978	026-H	18.9
S13-026979	027-H	16.7
S13-026980	028-H	96.8
S13-026981	029-H	54.7
S13-026982	031-H	8.51
S13-026983	033-H	0.75
S13-026984	037-H	3.42
S13-026985	038-H	18.2
S13-026986	039-H	10.6
S13-026987	040-H	1.83
S13-026988	041-H	3.82
S13-026989	043-H	57.3

Table 5. Pb concentrations in Kidney

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003499	001-K	0.145
S12-003500	002-K	0.072
S12-003501	003-K	0.922
S12-003502	004-K	0.050
S12-003503	005-K	0.569
S12-003504	006-K	0.857
S12-003505	007-K	1.60
S12-003506	008-K	2.06
S12-003507	009-K	3.45
S12-003508	010-K	1.60
S12-003509	011-K	1.57
S12-003510	012-K	1.80
S12-003511	013-K	0.995
S12-003512	014-K	0.593
S12-003513	015-K	0.428
S12-003514	016-K	1.25
S12-003515	017-K	0.453
S12-003516	018-K	1.25
S12-003517	019-K	0.399
S12-003518	020-K	4.25
S12-003520	022-K	0.253
S12-003521	023-K	0.206
S12-003522	024-K	0.148
S12-003523	025-K	1.90
S12-003524	026-K	2.15
S12-003525	027-K	2.38
S12-003526	028-K	4.37
S12-003527	029-K	1.70
S12-003528	030-K	1.92
S12-003529	031-K	0.078
S12-003530	032-K	0.273

Table 5 continued. Pb concentrations in Kidney

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003531	033-K	0.127
S12-003532	034-K	0.047
S12-003533	035-K	0.041
S12-003534	036-K	0.091
S12-003535	037-K	0.930
S12-003536	038-K	0.963
S12-003537	039-K	0.900
S12-003538	040-K	0.888
S12-003519	044-K	1.66
S12-005600	101-K	0.931
S13-005602	103-K	0.430
S13-005603	104-K	0.241
S13-005604	105-K	0.128
S13-005605	106-K	0.117
S13-005606	107-K	0.238
S13-005607	108-K	0.744
S13-005608	109-K	0.745
S13-005609	110-K	0.742
S13-005610	111-K	0.387
S13-005611	112-K	2.17
S13-025225	113-K	2.24
S13-025226	114-K	2.25
S13-025227	115-K	0.707
S13-025228	116-K	0.280
S13-025229	117-K	0.217
S13-025230	118-K	0.463
S13-025231	119-K	0.300
S12-016593	FSA1-K	0.597
S12-016594	FSA2-K	0.977
S12-016595	FSA3-K	0.434
S12-016596	FSA4-K	0.089
S12-016597	FSA5-K	0.571
S12-016598	FSA6-K	0.560

Table 6. Pb concentrations in Liver

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003093	001-L	0.008
S12-003100	002-L	0.012
S12-003101	003-L	0.023
S12-003102	004-L	0.007
S12-003103	005-L	0.032
S12-003104	006-L	0.012
S12-003105	007-L	0.129
S12-003106	008-L	0.138
S12-003107	009-L	0.211
S12-003108	010-L	0.088
S12-003109	011-L	0.442
S12-003110	012-L	0.282
S12-003111	013-L	3.03
S12-003112	014-L	4.76
S12-003113	015-L	4.10
S12-003114	016-L	1.33
S12-003115	017-L	1.48
S12-003116	018-L	1.22
S12-003117	019-L	<0.002
S12-003118	020-L	<0.002
S12-003119	021-L	<0.002
S12-003120	022-L	<0.002
S12-003121	023-L	<0.002
S12-003122	024-L	<0.002
S12-003123	025-L	0.695
S12-003124	026-L	0.945
S13-025215	027a-L	0.270
S12-003125	027-L	0.953
S13-025216	028a-L	1.09
S12-003126	028-L	0.878
S13-025217	029a-L	0.252

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003127	029-L	0.929
S13-025218	030a-L	0.186
S12-003128	030-L	1.38
S13-025219	031a-L	0.133
S12-003129	031-L	0.119
S13-025220	032a-L	0.155
S12-003130	032-L	0.777
S13-025221	033a-L	0.208
S12-003131	033-L	0.122
S12-003132	034-L	0.181
S12-003133	035-L	0.085
S12-003134	036-L	0.116
S12-003135	037-L	0.167
S12-003136	038-L	0.189
S12-003137	039-L	0.513
S12-003138	040-L	0.304
S12-003139	041-L	2.17
S12-003140	042-L	0.682
S12-003141	043-L	3.21
S12-003142	044-L	0.587
S12-003143	045-L	0.050
S12-003144	046-L	0.046
S12-003145	047-L	0.044
S12-003146	048-L	1.58
S12-003147	049-L	1.59
S12-003148	050-L	1.62
S12-003149	051-L	6.51
S12-003150	052-L	2.03
S12-003151	053-L	2.11
S12-003152	054-L	0.141
S12-003153	055-L	0.293

Table 6 continued. Pb concentrations in Liver

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003154	056-L	0.195
S12-003155	057-L	0.581
S12-003156	058-L	0.194
S12-003157	059-L	0.422
S12-003158	060-L	0.536
S12-003159	061-L	0.296
S12-003160	062-L	0.271
S12-003161	063-L	0.521
S12-003162	064-L	0.016
S12-003163	065-L	0.014
S12-003164	066-L	0.027
S12-003165	067-L	0.351
S12-003166	068-L	0.412
S12-003167	069-L	0.290
S12-003168	070-L	0.679
S12-003169	071-L	0.468
S12-003171	073-L	0.323
S12-003172	074-L	0.099
S12-003173	075-L	0.107
S12-003174	076-L	0.127
S12-003175	077-L	0.121
S12-003176	078-L	0.278
S12-003177	079-L	0.345
S12-003178	080-L	0.307
S12-003179	081-L	0.097
S12-003180	082-L	2.64
S12-003181	083-L	3.29
S12-003182	084-L	2.23
S12-003183	085-L	0.009
S12-003184	086-L	0.010
S12-003185	087-L	0.017
S12-003186	088-L	0.016
S12-003187	089-L	0.009
S12-003188	090-L	0.147
S12-003189	091-L	0.134
S12-003190	092-L	0.261
S12-003191	093-L	0.186
S12-003192	094-L	0.111
S12-003193	095-L	0.004
S12-003194	096-L	0.111
S12-003195	097-L	0.332
S12-003196	098-L	0.182
S12-003197	099-L	1.09
S12-003198	100-L	0.114
S12-016489	FSA1-L	0.240
S12-016490	FSA2-L	0.377
S12-016491	FSA3-L	0.161
S12-016492	FSA4-L	0.035
S12-016493	FSA5-L	0.177
S12-016494	FSA6-L	0.186

Table 7. Pb concentrations in Muscle

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003199	001-M	0.003
S12-003200	002-M	0.006
S12-003201	003-M	<0.002
S12-003202	004-M	<0.002
S12-003203	005-M	0.015
S12-003204	006-M	<0.002
S12-003205	007-M	0.005
S12-003206	008-M	0.003
S12-003207	009-M	0.004
S12-003208	010-M	<0.002
S12-003209	011-M	0.006
S12-003210	012-M	0.005
S12-003211	013-M	0.027
S12-003212	014-M	0.147
S12-003213	015-M	0.055
S12-003214	016-M	0.020
S12-003215	017-M	0.024
S12-003216	018-M	0.018
S12-003217	019-M	0.003
S12-003218	020-M	<0.002
S12-003219	021-M	<0.002
S12-003220	022-M	<0.002
S12-003221	023-M	<0.002
S12-003222	024-M	<0.002
S12-003223	025-M	0.020
S12-003224	026-M	0.047
S13-025235	027a-M	0.013
S12-003225	027-M	0.030
S13-025236	028a-M	0.029
S12-003226	028-M	0.041
S13-025237	029a-M	0.008

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003227	029-M	0.031
S13-025238	030a-M	0.007
S12-003228	030-M	0.029
S13-025239	031a-M	<0.002
S12-003229	031-M	0.012
S13-025240	032a-M	0.003
S12-003230	032-M	0.016
S13-025241	033a-M	0.003
S12-003231	033-M	0.011
S12-003232	034-M	0.006
S12-003233	035-M	0.033
S12-003234	036-M	0.009
S12-003235	037-M	0.011
S12-003236	038-M	0.008
S12-003237	039-M	0.011
S12-003238	040-M	0.003
S12-003239	041-M	0.011
S12-003240	042-M	0.005
S12-003241	043-M	0.046
S12-003242	044-M	0.017
S12-003243	045-M	<0.002
S12-003244	046-M	<0.002
S12-003245	047-M	<0.002
S12-003246	048-M	0.014
S12-003247	049-M	0.019
S12-003248	050-M	0.031
S12-003249	051-M	0.076
S12-003250	052-M	0.022
S12-003251	053-M	0.031
S12-003252	054-M	<0.002
S12-003253	055-M	0.005

Table 7 continued. Pb concentrations in Muscle

[LOD = 0.002 mg/kg, LOQ = 0.007]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003254	056-M	0.003
S12-003255	057-M	0.006
S12-003256	058-M	0.010
S12-003257	059-M	0.009
S12-003258	060-M	0.006
S12-003259	061-M	0.009
S12-003260	062-M	0.006
S12-003261	063-M	0.008
S12-003262	064-M	<0.002
S12-003263	065-M	<0.002
S12-003264	066-M	0.002
S12-003265	067-M	0.008
S12-003266	068-M	0.005
S12-003267	069-M	0.003
S12-003268	070-M	0.011
S12-003269	071-M	0.005
S12-003271	073-M	0.008
S12-003272	074-M	0.003
S12-003273	075-M	0.003
S12-003274	076-M	0.004
S12-003275	077-M	0.003
S12-003276	078-M	0.003
S12-003277	079-M	0.003
S12-003278	080-M	0.003
S12-003279	081-M	<0.002
S12-003280	082-M	0.037
S12-003281	083-M	0.033
S12-003282	084-M	0.037
S12-003283	085-M	<0.002
S12-003284	086-M	<0.002
S12-003285	087-M	<0.002
S12-003286	088-M	<0.002
S12-003287	089-M	<0.002
S12-003288	090-M	0.005
S12-003289	091-M	0.005
S12-003290	092-M	0.013
S12-003291	093-M	0.005
S12-003292	094-M	0.004
S12-003293	095-M	<0.002
S12-003294	096-M	0.010
S12-003295	097-M	0.017
S12-003296	098-M	0.009
S12-003297	099-M	0.020
S12-003298	100-M	0.006
S12-016515	FSA1-M	0.010
S12-016516	FSA2-M	0.011
S12-016517	FSA3-M	0.004
S12-016518	FSA4-M	<0.002
S12-016519	FSA5-M	0.003
S12-016520	FSA6-M	0.005

Table 8. Pb concentrations in Water

[LOD = 0.01 mg/kg, LOQ = 0.03 mg/kg]

Fera LIMS code	Sample ID	Pb (mg/kg)
S12-003539	001-W	0.04
S12-003540	002-W	0.10
S12-003541	003-W	7.89
S12-003542	004-W	0.12
S12-003543	005-W	0.33
S12-003544	006-W	0.28
S12-003545	007-W	2.16
S12-003546	008-W	0.14
S12-003547	009-W	0.15
S12-003548	010-W	0.02
S12-003549	011-W	0.27
S12-003550	012-W	0.78
S12-003551	013-W	0.33
S12-003552	014-W	1.77
S12-003553	015-W	3.22
S12-003554	016-W	7.97
S12-003555	017-W	1.96
S12-003556	018-W	20.9
S12-003557	019-W	12.3
S12-003558	020-W	14.0
S13-000900	021-W	2.82
S13-000901	022-W	0.85
S13-001194	023-W	0.10
S13-001195	024-W	7.21
S13-001196	025-W	0.90
S13-001197	026-W	1.19
S13-001198	027-W	0.09
S13-001199	028-W	0.53
S13-001200	029-W	87.6
S13-001201	030-W	0.54

Table 9. Quality Assurance

Reference materials		Pb (mg/kg)	
BCR185r Bovine liver	Measured Certified	0.163 ± 0.01 0.172	n= 30
In- House Ref freeze-dried liver	Measured Assigned	0.459 ± 0.03 0.459	n= 30
IAEA A-13 Animal blood	Measured Certified	0.147 ± 0.01 0.180	n= 7
ERM CA010a Hard drinking water	Measured Certified	0.100 ± 0.002 ~0.095	n= 3
Spike recovery:		98% ± 5%	n= 30
Measurement Uncertainty: (Coverage factor of 2)		14%	

Table 10. Fera participation in FAPAS Series 7 (Pb) over the duration of the study

Date	Matrix	Round	Pb (z-score)
Dec.11	Soya flour	07166	-0.1
Jan-Feb 12	Cocoa Powder	07167	-0.8
Feb-Mar 12	Fruit juice	07168	-0.1
Mar-Apr 12	Vegetable puree	07170	-0.1
Apr-May 12	Milk powder	07172	-0.4
Jun-Aug 12	Tomato paste	07175	0.1
Oct-Nov 12	Wine	07181	0.2
Jan.13	Soya flour	07184	0.2
Feb-Mar 13	Fruit juice	07186	0.4
Mar-Apr 13	Vegetable puree	07188	-0.1
Apr-May 13	Milk powder	07190	0.0
May-Jun 13	Canned crab meat	07192	0.0
Jun-Jul 13	Tomato paste	07193	1.2
Sep-Oct 13	Edible oil	07198	0.0
Oct-Dec 13	Milk Powder	07201	-0.1

Appendix 3 - Isotope Ratio Analysis Report Provided by the British Geological Survey

Pb isotope analysis of animal products provided as solutions by the Food and Environment Research Agency, on behalf of Prof Toby Knowles, Bristol.

NERC Isotope Geosciences Laboratory (NIGL) report by J.A. Evans and V. Pashley

November 2013.

Introduction

The sample solutions were prepared at the Food and Environment Research Agency (FERA), York. They comprised 0.7grams of material digested in 1.4ml of HNO_3 and 0.4 ml of HCl. This was then made up to 10ml total volume using Milli-Q water to give a 20% (v/v) acid solution. Approximately 5ml of each of these solutions was received by NIGL.

A pilot study, conducted at NIGL, attempted to analyse the Pb isotope ratios of a sub-set of samples directly i.e. as supplied by FERA. However, the results of this pilot showed that these solutions could not be analysed in their original form because the excessive sample matrix had a detrimental impact on the sample introduction system of the instrument used (MC-ICP-MS; multicollector-inductively coupled plasma-mass spectrometer) e.g. nebuliser capillary blockages and sooting to the sample and skimmer cones. Consequently, it was deemed necessary to isolate the Pb from each sample using ion exchange techniques, as follows:

Sample Preparation

Each solution was decanted into a clean Savillex beaker. The source container was rinsed with c.1ml Milli-Q water and this too was added to the Savillex beaker. c. 1ml of quartz distilled 8M HNO_3 was also added to help digest organic material. The samples were evaporated to dryness overnight on a hot plate. 2-3 ml of 0.5HBr was added to the residue to convert it to its bromide form, and this was again dried down. The sample was then taken up in c. 1ml of 0.5HBr in preparation for ion exchange separation of the Pb. Procedural blanks, produced by FERA, were received as part of the sample set. These were processed in the same manner as the samples.

Five drops of cation exchange resin, (AG 1X8), were added to pre-cleaned polypropylene columns, each fitted with a 35µm polyethylene frit. The resin was cleaned by eluting first with one column volume (CV) of 6M HCl, followed by 1 CV Milli-Q water. The HCl efficiently removes any Pb contamination present on the resin, whilst the water serves to re-swell the resin thus allowing more effective cleaning by a subsequent elution of HCl. The resin was then pre-conditioned by addition of 0.5 CV 0.5M HBr. The sample was then added to the column. Any Pb present in the sample forms stable bromide complexes with the pre-conditioned

column surface. Other elements present in the sample matrix are eluted off the column by washing with 1.5 CV of 0.5M HBr. The isolated Pb fraction is then eluted off the column by washing with 1 CV 6M HCl. This fraction was collected into a pre-cleaned Savillex beaker. 1ml 8M HNO_3 was added to each individual sample. This breaks down any resin which may have co-eluted off the column with the sample. The samples were again evaporated to dryness and finally taken up in 2% HNO_3 ready for isotope analysis by MC-ICP-MS.

Isotope analysis

Pb isotope analysis of the samples was conducted using a Nu Instuments Nu Plasma, MC-ICP-MS. Prior to analysis, each sample was filtered (Millipore 0.25µm PFA) and spiked with a thallium (Tl) solution, which was added to allow for the correction of instrument induced mass bias. Samples were then introduced into the instrument via an ESI 50µl/min PFA micro-concentric nebuliser attached to a de-solvating unit, (Nu Instruments DSN 100). For each sample, five ratios were simultaneously measured ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$). Each individual acquisition consisted of 60 sets of ratios, collected at 5-second integrations, following a 60 second de-focused baseline.

The precision and accuracy of the method was assessed through repeat analysis of an NBS 981 Pb reference solution, (also spiked with Tl). The average values obtained for each of the measured NBS 981 ratios were then compared to the known values for this reference, (Thirlwell, 2002). All sample data were subsequently normalised, according to the relative daily deviation of the measured reference value from the true. Normalisation to an international standard in this way effectively cancels out the effects of slight daily variations in instrumental accuracy, and allows the direct comparison of the data obtained during different analytical sessions. Internal uncertainties (the reproducibility of the measured ratio) were propagated relative to the external uncertainty (i.e. the excess variance associated with the reproducibility of the reference material analysed during the session).

Results

Blanks: The total Pb signal (V) obtained for each of the blanks supplied by FERA is listed in Table 1. The contribution of this blank has been estimated as a percentage

for each sample, based on a comparison of the appropriate blank signal (batch specific) with the total Pb signal achieved for each undiluted sample within the same batch. The estimated blank contribution varies between 0.1 and 11.9%. A blank contribution of >2% was considered excessive, and these data have been excluded from the data plots. Also, samples that produced a beam size of below 1V were deemed unsuitable for analysis, due to limits of analytical capability.

The average blank composition is given below. It falls within typical UK airborne Pb isotope range (Noble et al 2008).

	206Pb/204Pb	207Pb/204Pb	208Pb/204Pb	207Pb/206Pb	208Pb/206Pb
Average ±1SD	17.702 ± 0.23	15.493 ± 0.11	37.453±0.43	0.875±007	2.117 ±009

Analytical reproducibility was monitored by measuring eight samples in duplicate, during different analytical sessions. In each instance, the duplicates lie within error of one another.

Discussion of data (excluding those samples with excessive blank contribution and those containing insufficient Pb),

The data are presented in Table 2 and plotted in Figures 1-3.

In the absence of any information about these samples a few observations are made below about the data, but these should be understood to be made through observation of the dataset alone. Knowledge of the nature of the samples and their geographic origin would provide important constraints on the interpretation.

Figure 1 shows the relationship between each samples $^{206}Pb/^{207}Pb$ ratio and Pb concentrations (provided by FERA). The diagram shows two clear peaks of isotope compositions at approximately $^{206}Pb/^{207}Pb$ = 1.176 and 1.161. Two reference fields are displayed on the diagram. The first is taken from 17th-18th century Pb isotope data obtained from human teeth, which represent the average biosphere uptake of lead that predates the modern introduction of Australian Pb in petrol (Millard et al 2012). The second field represents the range of recent Pb isotope measurements of air pollution in London showing the residual effect of the Australian petrol Pb ie modern anthropogenic Pb (Noble et al 2008). The samples show two major peaks in Pb

isotope composition. The first peak ($^{206}Pb/^{207}Pb = 1.176$) coincides with the typical pre-modern pollution composition seen frequently in British archaeology studies (Montgomery et al 2011). The second peak ($^{206}Pb/^{207}Pb = 1.161$) plots between conventional English values and the field of modern pollution. In the absence of any other evidence, there are two likely explanations for the data cluster of $^{206}Pb/^{207}Pb$ at c. 1.161. The first is that the samples come from an areas partially contaminated by modern anthropogenic Pb. The other option is that these samples come from areas of Britain, such as Scotland or Wales, where geogenic Pb has a lower $^{206}Pb/^{207}Pb$ ratio than English Pennine ores.

Figures 2 and 3 present the data on a diagram commonly used in environmental studies ($^{206}Pb/^{207}Pb$ vs $^{208}Pb/^{207}Pb$) and archaeological/geological studies ($^{207}Pb/^{206}Pb$ vs $^{208}Pb/^{206}Pb$) respectively. Both of these diagrams highlight the range of values and subgroups of data within the overall study.

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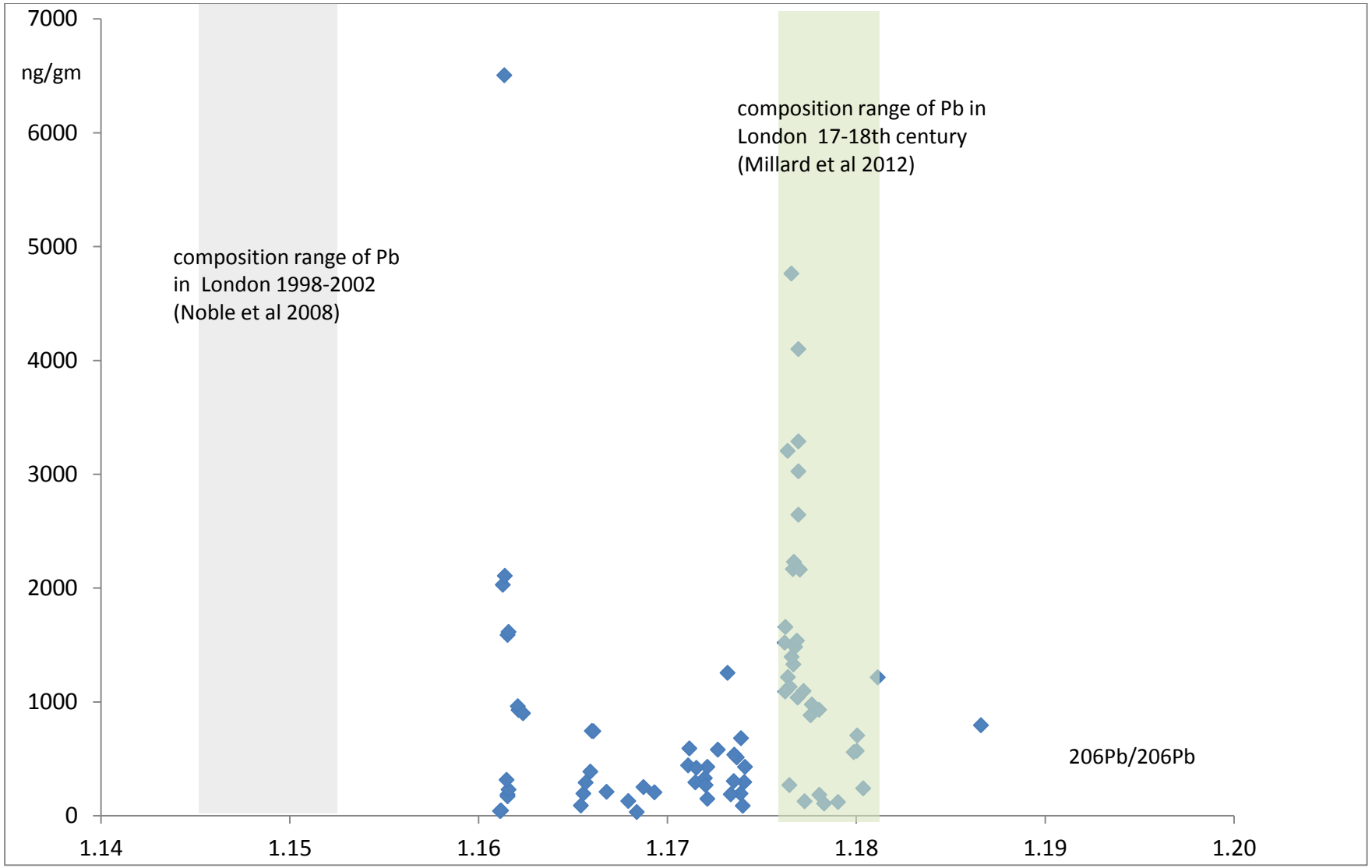


Figure 1. $^{206}Pb/^{207}Pb$ vs ng/gm of Pb for samples. Purple shading is London air Pb isotope composition (Noble et al 2008) and the green shade area is typical biosphere Pb isotope composition from historical and archaeological samples (Millard et al 2012, Montgomery et al 2011) .

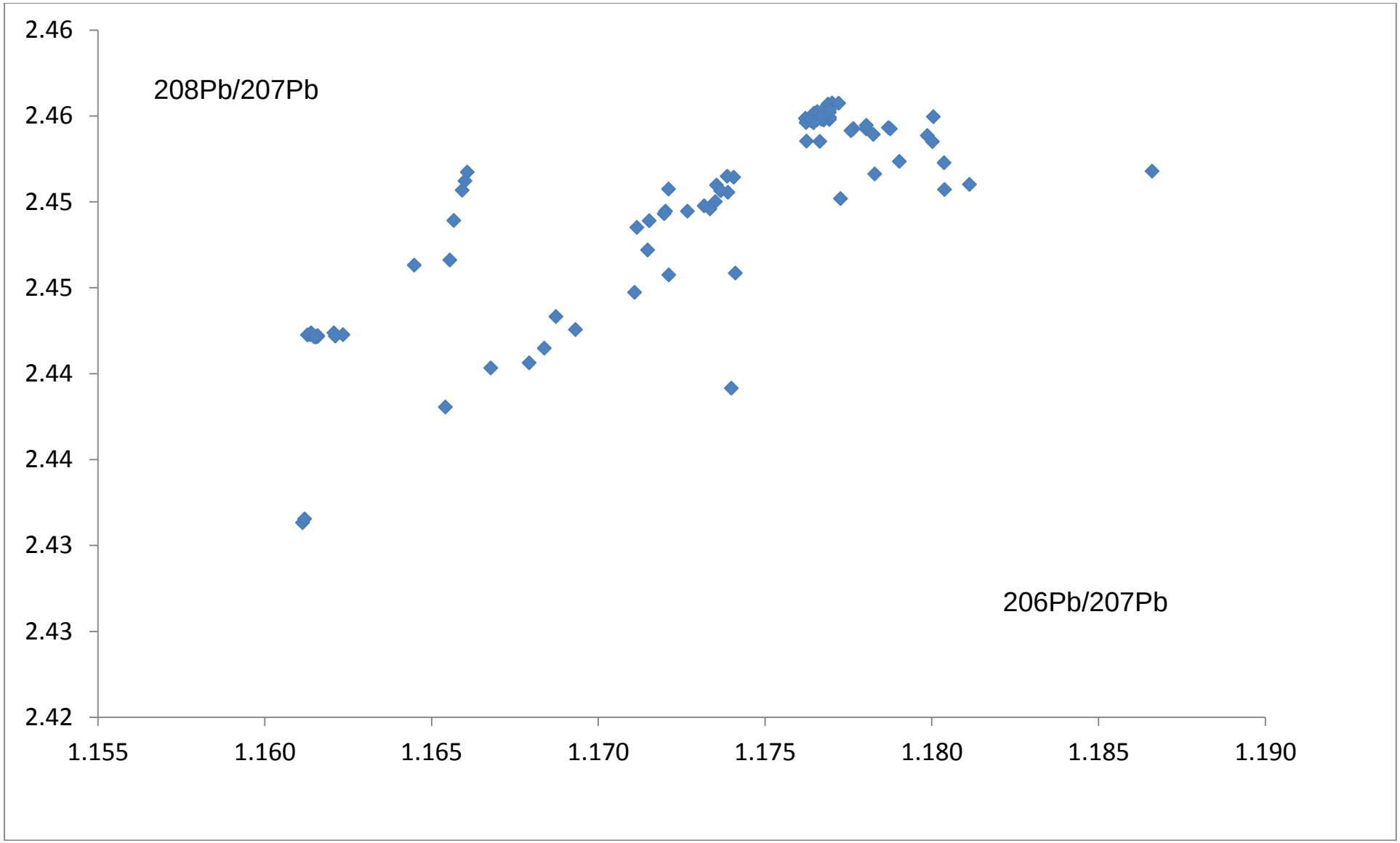


Figure 2. Data displayed using a diagram axes typically used in environmental studies.

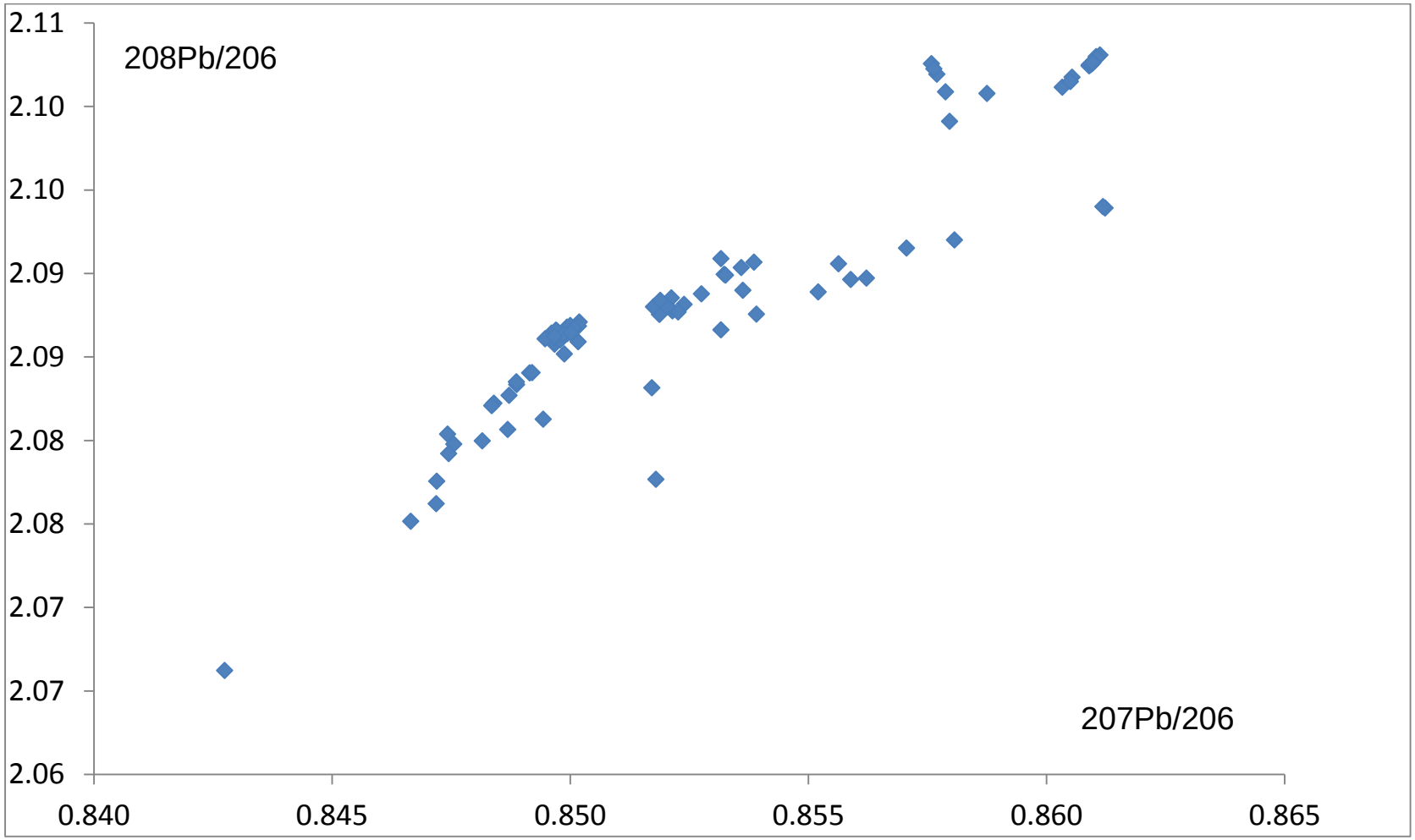


Figure 3. Data displayed on axes typically used in archaeological and geological studies.

Table 1. Voltage intensities for the blank solutions provided by FERA

Sample Name	Total Pb/(V)
YCC_23_Blank	0.025
YDB_22_Blank	0.057
YCR_22_Blank	0.23
XBR_3_Blank	0.16
XDD_27_Blank	0.15
XJG_10_Blank	0.09
XBM_15_Blank	0.13
XAU_21_Blank	0.22
YDF_15_Blank	0.90
XDK_6_Blank	0.23
YBC_4_Blank	0.17
XIN_11_Blank	0.10
XDH_11_Blank	0.36
XFS_21_Blank	0.11
YAQ_16_Blank	0.03
XJB_11_Blank	0.02

Table 2. Running voltage, Pb isotope ratios, blank contributions and ng/gm (supplied by FERA) for the samples submitted to NIGL for Pb isotope analysis.

Sample Name	**Total Pb/(V)**	**206Pb/ 204Pb**	**2σ abs**	**207Pb/ 204Pb**	**2σ abs**	**208Pb/ 204Pb**	**2σ abs**	**207Pb/ 206Pb**	**2σ abs**	**208Pb/ 206Pb**	**2σ abs**	**208Pb/ 207Pb**	**206Pb/ 207Pb**	**Dilution Factor**	**% blank**	**ng/gm (FERA)**
XAU_01	<500mv total Pb													Neat		7
XAU_03	8.1	18.296	0.002	15.623	0.002	38.193	0.007	0.85391	0.00003	2.0876	0.00017	2.4447	1.1711	1:2	1.4	442
XAU_05	4.6	18.541	0.003	15.627	0.002	38.203	0.005	0.84280	0.00005	2.0601	0.00006	2.4443	1.1865	Neat	4.8	282
XAU_13	6.5	18.249	0.002	15.619	0.002	38.134	0.004	0.85589	0.00005	2.0896	0.00005	2.4415	1.1684	1:4	0.8	32
XAU_16	1.9	18.548	0.004	15.632	0.003	38.163	0.008	0.84279	0.00004	2.0576	0.00011	2.4414	1.1865	Neat	11.8	12
XAU_27	<1V total Pb													Neat		8
XAU_30	10.6	18.026	0.003	15.601	0.003	37.948	0.007	0.86551	0.00002	2.1052	0.00010	2.4323	1.1554	Neat	2.1	138
XAU_31	5.7	18.216	0.002	15.612	0.002	38.102	0.004	0.85706	0.00005	2.0915	0.00005	2.4403	1.1668	1:4	1.0	211
XBM_02	4.3	18.598	0.002	15.637	0.002	38.261	0.005	0.84080	0.00005	2.0573	0.00005	2.4468	1.1893	Neat	2.9	23
XBM_12	10.8	18.394	0.002	15.636	0.001	38.382	0.004	0.85007	0.00005	2.0867	0.00006	2.4548	1.1764	Neat	1.2	1218
XBM_21	3.2	18.398	0.003	15.632	0.002	38.382	0.005	0.84966	0.00005	2.0862	0.00005	2.4554	1.1769	1:2	2.0	4100
XBM_25	15.3	18.400	0.002	15.636	0.001	38.384	0.004	0.84979	0.00005	2.0860	0.00005	2.4548	1.1768	1:4	0.2	1484
XBM_30	12.5	18.398	0.002	15.637	0.001	38.386	0.004	0.84994	0.00005	2.0864	0.00006	2.4548	1.1766	1:2	0.2	4764
XBM_31	9.1	18.399	0.002	15.636	0.002	38.387	0.004	0.84986	0.00005	2.0864	0.00005	2.4550	1.1767	1:2	0.3	1331
XBM_36	2.1	18.632	0.004	15.632	0.003	38.233	0.008	0.83890	0.00006	2.0518	0.00010	2.4458	1.1920	Neat	6.1	12
XBM_37	10.4	18.401	0.002	15.634	0.001	38.387	0.004	0.84967	0.00005	2.0861	0.00005	2.4552	1.1769	1:4	0.3	3026
XBR_01	<500mv total Pb													Neat		7
XBR_04	<500mv total Pb													Neat		3
XBR_05	<500mv total Pb													Neat		3
XBR_06	<500mv total Pb													Neat		3
XBR_11	<500mv total Pb													Neat		3
XBR_12	<500mv total Pb													Neat		5

XBR_17	11.0	18.2397	0.0032	15.6169	0.0030	38.1165	0.0075	0.8562	0.0000	2.0897	0.0001	2.4406	1.1679	Neat	1.5	129
XBR_25	6.0	18.0015	0.0032	15.5970	0.0030	37.8993	0.0076	0.8665	0.0000	2.1054	0.0001	2.4299	1.1541	Neat	2.7	88
XDD_02	12.5	18.3327	0.0023	15.6264	0.0017	38.2823	0.0063	0.8524	0.0000	2.0882	0.0001	2.4498	1.1732	1:5	0.2	1255
XDD_03	5.7	18.2563	0.0025	15.6193	0.0024	38.1974	0.0074	0.8556	0.0000	2.0923	0.0002	2.4455	1.1688	Neat	2.6	514
XDD_06	5.8	18.2805	0.0024	15.6207	0.0024	38.2153	0.0073	0.8545	0.0000	2.0905	0.0002	2.4464	1.1702	Neat	2.5	996
XDD_08	9.5	18.3152	0.0023	15.6260	0.0023	38.2962	0.0072	0.8532	0.0000	2.0909	0.0002	2.4507	1.1721	Neat	1.5	429
XDD_19	8.1	18.3046	0.0019	15.6255	0.0013	38.2399	0.0052	0.8536	0.0000	2.0890	0.0001	2.4472	1.1715	Neat	1.8	294
XDD_25	6.8	18.2691	0.0023	15.6196	0.0023	38.2071	0.0072	0.8550	0.0000	2.0913	0.0002	2.4460	1.1696	Neat	2.1	1014
XDH_01	8.4	18.5496	0.0021	15.6316	0.0016	38.3271	0.0050	0.8427	0.0000	2.0662	0.0001	2.4518	1.1866	1:4	1.1	796
XDH_19	7.5	18.4472	0.0022	15.6282	0.0016	38.3164	0.0050	0.8472	0.0000	2.0770	0.0001	2.4516	1.1804	1:2	2.4	1204
XDH_28	3.8	18.4599	0.0023	15.6287	0.0014	38.3078	0.0055	0.8467	0.0000	2.0752	0.0001	2.4510	1.1811	1:5	1.9	1216
XDK_03	13.9	18.4036	0.0022	15.6372	0.0022	38.3999	0.0043	0.8497	0.0000	2.0866	0.0014	2.4557	1.1769	1:8	0.2	1038
XDK_04	17.0	18.3972	0.0021	15.6360	0.0021	38.3904	0.0039	0.8499	0.0000	2.0868	0.0014	2.4553	1.1766	1:8	0.2	1395
XDK_08	8.7	18.3897	0.0023	15.6344	0.0022	38.3803	0.0045	0.8502	0.0000	2.0871	0.0014	2.4549	1.1762	1:16	0.2	1521
XDK_09	11.2	18.3952	0.0024	15.6361	0.0020	38.3878	0.0065	0.8500	0.0000	2.0869	0.0001	2.4552	1.1765	1:8	0.3	1133
XDK_18	9.0	18.4005	0.0022	15.6354	0.0022	38.3932	0.0044	0.8497	0.0000	2.0866	0.0014	2.4556	1.1769	1:16	0.2	1539
XDK_25	14.6	18.4033	0.0022	15.6355	0.0022	38.3969	0.0044	0.8496	0.0000	2.0864	0.0014	2.4558	1.1770	1:16	0.1	2164
XFS_03	13.7	18.3471	0.0019	15.6290	0.0014	38.3005	0.0047	0.8519	0.0000	2.0876	0.0001	2.4506	1.1739	Neat	0.8	682
XFS_07	9.0	18.3001	0.0021	15.6252	0.0016	38.2592	0.0049	0.8539	0.0000	2.0907	0.0001	2.4485	1.1712	1:2	0.6	593
XFS_11	9.7	18.3948	0.0022	15.6366	0.0022	38.3844	0.0068	0.8501	0.0000	2.0867	0.0002	2.4547	1.1764	Neat	1.1	3206
XFS_15	12.7	18.3418	0.0020	15.6273	0.0014	38.2979	0.0046	0.8520	0.0000	2.0880	0.0001	2.4506	1.1737	Neat	0.9	513
XFS_16	6.4	18.3915	0.0024	15.6303	0.0024	38.3506	0.0074	0.8499	0.0000	2.0852	0.0002	2.4535	1.1766	Neat	1.7	2167
XFS_18	11.2	18.3862	0.0022	15.6307	0.0022	38.3497	0.0070	0.8502	0.0000	2.0859	0.0002	2.4535	1.1762	Neat	1.0	1659
XFS_26	15.1	18.3382	0.0020	15.6266	0.0015	38.2866	0.0046	0.8521	0.0000	2.0878	0.0001	2.4500	1.1735	Neat	0.7	304
XFS_34	14.7	18.3331	0.0020	15.6241	0.0014	38.2740	0.0046	0.8523	0.0000	2.0877	0.0001	2.4496	1.1733	Neat	0.7	189
XIN_02	10.9	18.4406	0.0024	15.6292	0.0019	38.3522	0.0057	0.8476	0.0000	2.0798	0.0002	2.4539	1.1799	1:2	0.5	560
XIN_03	8.8	18.4420	0.0027	15.6283	0.0019	38.3431	0.0058	0.8474	0.0001	2.0792	0.0002	2.4535	1.1800	1:2	0.6	571
XIN_17	11.3	18.3389	0.0021	15.6205	0.0016	38.1024	0.0049	0.8518	0.0000	2.0777	0.0001	2.4392	1.1740	Neat	0.9	89
XIN_21	10.7	18.3347	0.0022	15.6229	0.0018	38.2925	0.0055	0.8521	0.0000	2.0885	0.0002	2.4510	1.1735	1:2	0.5	536

XIN_26	11.9	18.3014	0.0023	15.6216	0.0019	38.2565	0.0055	0.8536	0.0000	2.0904	0.0002	2.4489	1.1715	Neat	0.8	422
XIN_29	6.2	18.3433	0.0024	15.6263	0.0020	38.3080	0.0057	0.8519	0.0000	2.0884	0.0002	2.4515	1.1739	Neat	1.6	194
XIN_30	10.5	18.3453	0.0023	15.6252	0.0019	38.3054	0.0056	0.8517	0.0000	2.0880	0.0002	2.4514	1.1741	Neat	1.0	296
XIN_32	8.9	18.3097	0.0023	15.6221	0.0020	38.2665	0.0055	0.8532	0.0000	2.0899	0.0002	2.4495	1.1720	Neat	1.1	271
XIN_35	8.7	18.3237	0.0023	15.6253	0.0020	38.2739	0.0057	0.8528	0.0000	2.0888	0.0002	2.4495	1.1727	1:2	0.6	581
XJB_05	2.7	18.2026	0.0031	15.6311	0.0024	38.2386	0.0072	0.8588	0.0000	2.1008	0.0001	2.4463	1.1645	Neat	0.7	141
XJB_06	5.4	18.1635	0.0021	15.6406	0.0016	38.2000	0.0058	0.8611	0.0000	2.1031	0.0001	2.4423	1.1613	1:5	0.1	2029
XJB_12	11.2	18.2185	0.0017	15.6306	0.0011	38.2433	0.0048	0.8580	0.0000	2.0991	0.0001	2.4466	1.1655	Neat	0.2	195
XJB_13	7.9	18.1648	0.0020	15.6399	0.0015	38.1998	0.0056	0.8610	0.0000	2.1030	0.0001	2.4424	1.1614	1:5	0.1	2109
XJB_16	4.3	18.1643	0.0024	15.6405	0.0018	38.1981	0.0061	0.8611	0.0000	2.1029	0.0001	2.4423	1.1614	1:5	0.1	6505
XJB_18	13.7	18.2242	0.0017	15.6337	0.0010	38.2873	0.0048	0.8579	0.0000	2.1009	0.0001	2.4489	1.1657	Neat	0.1	293
XJB_22	12.2	18.1638	0.0023	15.6370	0.0018	38.1887	0.0062	0.8609	0.0000	2.1025	0.0001	2.4422	1.1616	1:5	0.0	1616
XJB_25	11.5	18.1644	0.0023	15.6386	0.0018	38.1927	0.0060	0.8609	0.0000	2.1025	0.0001	2.4422	1.1615	1:5	0.0	1589
XJB_28	insufficient sample (not 5mls)															1576
XJG_07	10.2	18.408	0.002	15.631	0.002	38.362	0.006	0.84915	0.00003	2.0840	0.00010	2.4543	1.1776	1:10	0.1	978
XJG_09	8.4	18.408	0.002	15.632	0.002	38.364	0.006	0.84920	0.00003	2.0841	0.00009	2.4541	1.1776	1:10	0.1	882
XJG_18	9.6	18.194	0.002	15.612	0.002	38.063	0.005	0.85807	0.00004	2.0920	0.00008	2.4381	1.1654	Neat	0.9	91
XJG_23	4.4	18.113	0.002	15.599	0.002	37.931	0.006	0.86118	0.00003	2.0940	0.00010	2.4315	1.1612	Neat	1.9	47
XJG_27	4.5	18.120	0.002	15.605	0.002	37.942	0.005	0.86123	0.00004	2.0939	0.00008	2.4313	1.1611	Neat	1.9	41
YAQ_09	12.0	18.418	0.002	15.634	0.002	38.371	0.006	0.84887	0.00004	2.0834	0.00016	2.4543	1.1780	1:2	0.1	931
YAQ_14	12.6	18.172	0.002	15.638	0.002	38.194	0.005	0.86053	0.00004	2.1018	0.00015	2.4424	1.1621	1:2	0.1	963
YAQ_23	14.0	18.173	0.002	15.635	0.002	38.186	0.005	0.86033	0.00004	2.1012	0.00016	2.4423	1.1623	1:2	0.1	900
YAQ_28	4.4	18.1675	0.0026	15.6327	0.0022	38.1793	0.0060	0.8605	0.0000	2.1015	0.0002	2.4422	1.1621	1:4	0.2	930
YBC_01	9.6	18.4248	0.0022	15.6266	0.0022	38.3223	0.0043	0.8482	0.0000	2.0800	0.0014	2.4524	1.1790	Neat	1.8	121
YBC_02	10.8	18.3945	0.0023	15.6246	0.0023	38.2832	0.0047	0.8494	0.0000	2.0813	0.0014	2.4502	1.1773	Neat	1.6	127
YBC_06	12.8	18.2576	0.0022	15.6136	0.0022	38.1381	0.0044	0.8552	0.0000	2.0889	0.0014	2.4426	1.1693	Neat	1.3	206
YBC_08	9.5	18.4104	0.0020	15.6244	0.0015	38.3059	0.0048	0.8487	0.0000	2.0807	0.0001	2.4516	1.1783	Neat	1.8	107
YBC_35	6.9	18.3041	0.0020	15.6166	0.0022	38.1940	0.0062	0.8532	0.0000	2.0866	0.0001	2.4458	1.1721	1:2	1.2	148
YBC_36	6.4	18.3330	0.0020	15.6140	0.0022	38.1901	0.0062	0.8517	0.0000	2.0832	0.0001	2.4459	1.1741	1:4	0.7	430

YBC_37	11.9	18.2462	0.0021	15.6120	0.0016	38.1459	0.0050	0.8556	0.0000	2.0906	0.0001	2.4433	1.1687	1:2	0.7	253
YBC_38	11.4	18.4466	0.0020	15.6273	0.0015	38.3242	0.0047	0.8472	0.0000	2.0776	0.0001	2.4523	1.1804	1:2	0.7	241
YCC_01	2.4	18.4235	0.0035	15.6313	0.0029	38.3635	0.0080	0.8484	0.0000	2.0822	0.0001	2.4543	1.1787	Neat	1.0	45
YCC_05	10.5	18.1657	0.0017	15.6395	0.0010	38.1951	0.0046	0.8609	0.0000	2.1025	0.0001	2.4421	1.1615	Neat	0.2	187
YCC_07	2.9	18.4173	0.0030	15.6309	0.0024	38.3573	0.0074	0.8487	0.0000	2.0827	0.0001	2.4539	1.1782	Neat	0.9	52
YCC_10	3.7	18.1612	0.0024	15.6362	0.0019	38.1868	0.0050	0.8610	0.0000	2.1026	0.0001	2.4422	1.1615	1:4	0.2	316
YCC_17	1.8	18.4578	0.0036	15.6368	0.0032	38.3226	0.0074	0.8472	0.0001	2.0762	0.0001	2.4507	1.1804	Neat	1.4	18
YCC_18	14.0	18.1658	0.0017	15.6388	0.0010	38.1932	0.0045	0.8609	0.0000	2.1024	0.0001	2.4422	1.1616	Neat	0.2	231
YCC_19	<1V total Pb													Neat		27
YCC_20	2.1	18.427	0.004	15.632	0.003	38.366	0.009	0.84835	0.00003	2.0821	0.00009	2.4543	1.1788	Neat	1.2	37
YCC_25	<500mv total Pb													Neat		13
YCC_27	4.8	18.165	0.002	15.639	0.002	38.195	0.005	0.86097	0.00003	2.1026	0.00008	2.4422	1.1615	1:2	0.3	261
YCC_30	11.0	18.168	0.002	15.641	0.001	38.197	0.005	0.86093	0.00003	2.1025	0.00008	2.4421	1.1615	Neat	0.2	170
YCC_34	10.7	18.423	0.002	15.638	0.001	38.384	0.005	0.84887	0.00003	2.0835	0.00008	2.4545	1.1780	Neat	0.2	183
YCR_03	4.7	18.404	0.004	15.634	0.003	38.379	0.008	0.84947	0.00003	2.0853	0.00011	2.4548	1.1772	Neat	4.8	114
YCR_05	13.1	18.407	0.003	15.636	0.003	38.399	0.007	0.849	0.000	2.086	0.000	2.456	1.177	1:2	0.864	1095
YCR_17	9.1	18.318	0.003	15.630	0.003	38.283	0.008	0.853	0.000	2.090	0.000	2.449	1.172	1:2	1.250	332
YCR_18	14.6	18.397	0.002	15.637	0.002	38.385	0.005	0.850	0.000	2.086	0.000	2.455	1.176	Neat	1.551	270
YCR_21	10.2	18.403	0.003	15.631	0.003	38.368	0.007	0.849	0.000	2.085	0.000	2.454	1.177	Neat	2.224	182
YCR_23	13.3	18.391	0.003	15.635	0.003	38.380	0.007	0.850	0.000	2.087	0.000	2.455	1.176	1:10	0.171	1092
YCR_25	11.4	18.236	0.003	15.640	0.003	38.338	0.007	0.858	0.000	2.102	0.000	2.451	1.166	Neat	1.986	745
YCR_26	5.0	18.238	0.002	15.642	0.001	38.349	0.004	0.85766	0.00003	2.1027	0.00013	2.4517	1.1660	1:2	2.3	744
YCR_27	10.8	18.438	0.003	15.626	0.003	38.345	0.008	0.84752	0.00002	2.0797	0.00010	2.4539	1.1799	Neat	2.1	280
YCR_28	11.1	18.233	0.003	15.639	0.003	38.325	0.008	0.85770	0.00002	2.1019	0.00010	2.4507	1.1659	1:2	1.0	387
YCR_29	7.2	18.239	0.003	15.640	0.003	38.347	0.008	0.85758	0.00002	2.1026	0.00009	2.4517	1.1661	1:2	1.6	742
YCR_31	13.8	18.446	0.003	15.631	0.003	38.373	0.008	0.84742	0.00002	2.0804	0.00010	2.4550	1.1800	1:2	0.8	707
YDB_16	17.5	18.400	0.002	15.634	0.002	38.380	0.005	0.84967	0.00004	2.0858	0.00016	2.4548	1.1769	1:2	0.2	3289
YDB_26	12.2	18.394	0.002	15.632	0.002	38.373	0.005	0.84984	0.00004	2.0862	0.00016	2.4548	1.1767	1:2	0.2	2232
YDB_29	16.8	18.395	0.002	15.629	0.002	38.370	0.006	0.84966	0.00004	2.0859	0.00016	2.4549	1.1769	1:2	0.2	2644

YDF_11	5.8	18.420	0.002	15.628	0.002	38.270	0.005	0.84845	0.00003	2.0778	0.00140	2.4489	1.1786	1:2	7.8	217
YDF_12	14.4	18.425	0.002	15.633	0.002	38.304	0.004	0.84850	0.00003	2.0789	0.00140	2.4501	1.1785	1:2	3.1	463
YDF_17	15.0	18.424	0.002	15.635	0.002	38.268	0.004	0.84862	0.00003	2.0770	0.00140	2.4476	1.1784	Neat	6.0	300
YDF_17	15.0	18.424	0.002	15.635	0.002	38.268	0.004	0.84862	0.00003	2.0770	0.00140	2.4476	1.1784	Neat	6.0	300

Appendix 4 – Investigation into Within-Field Pb Variation

To investigate the variability of spot Pb soil samples within fields in areas of known high geochemical Pb a simple survey was carried out on one of the farms participating in the survey. A convenience sample was used. A farm belonging to one of the more helpful farmers on an area already identified as being in the 'high' geochemical Pb category, and not too distant from Aberystwyth University, where the portable X-Ray fluorescence spectrometer (pXRF) was based, was used. Spot readings were taken by an experienced operator using the pXRF at intervals during a number of traverses of three different fields. At each spot a small patch of the turf, with roots, was removed to expose the soil beneath. The pXRF used was the instrument described in Appendix 5, and as in Appendix 5 a plastic sample bag was placed between the exposed soil and the instrument and a sampling time of approximately 70 seconds used per sample. The sample positions and the spot Pb measured are shown on the page below. Histograms of the results for each field are shown in Figure 1.

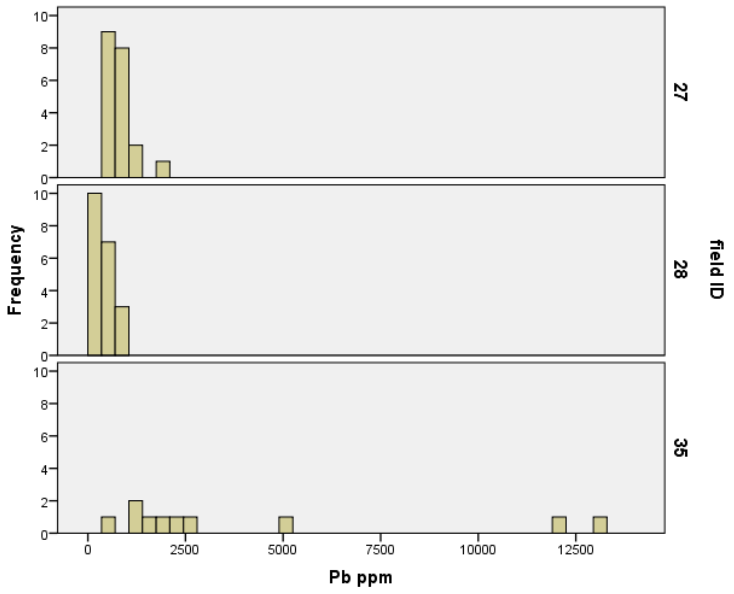
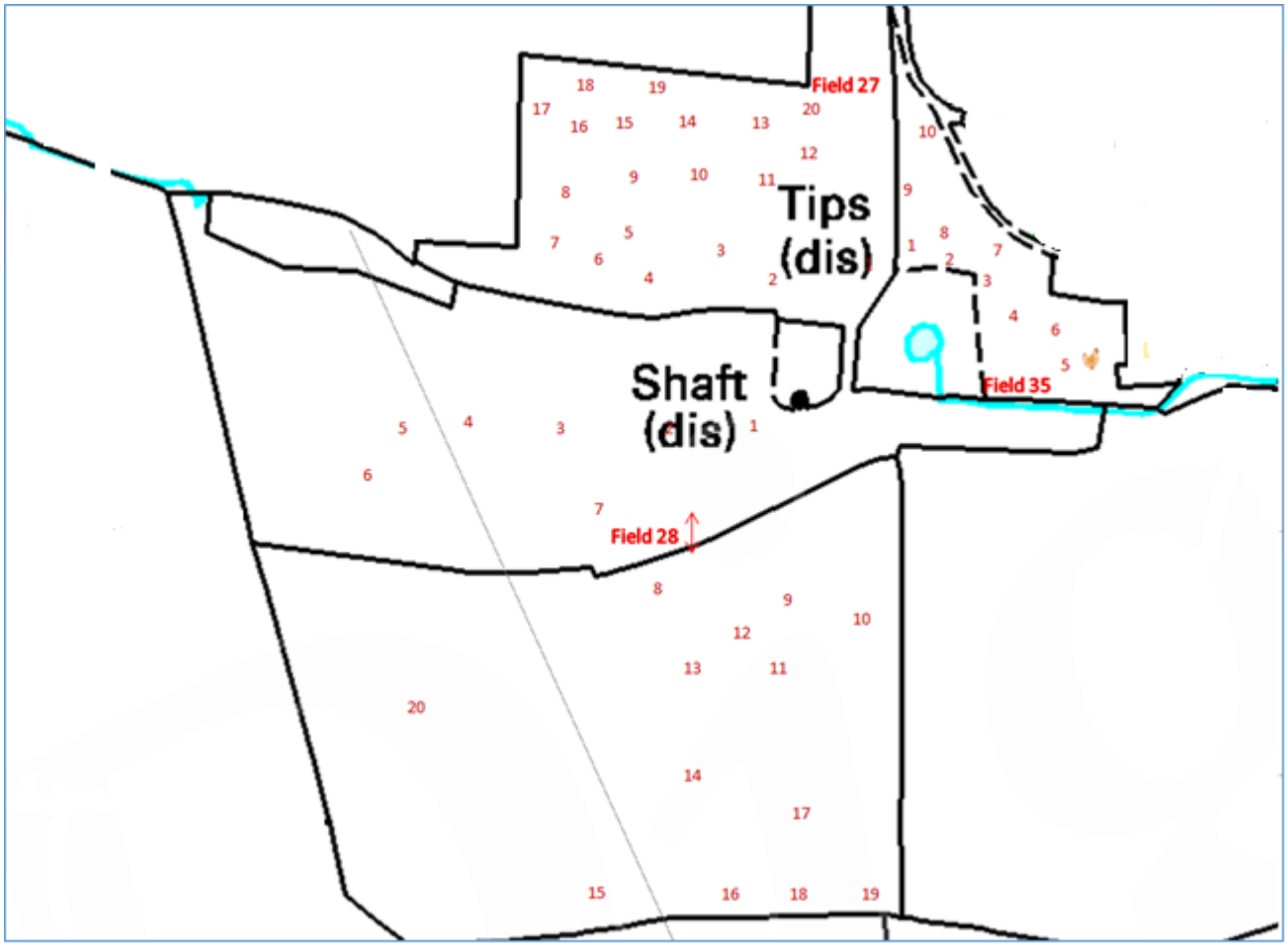


Figure 1. Histograms showing the sampling distribution in levels of geochemical Pb measured by pXRF across the three fields on soil in situ. Note that units of ppm and mg/kg are interchangeable.

It is apparent from the data that although all three fields can be considered to contain elevated and extreme concentrations of lead, and although the fields are adjacent to each other, the manner in which lead was distributed both within and between fields differed considerably. Minimum and maximum values for fields 27, 28 and 35, respectively were: 442 to 2061, 177 to 986 and 486 to 13100 mg/kg.



field ID	sample no	Pb ppm	+/-value
27	1	2061	43
27	2	1075	39
27	3	1034	35
27	4	1120	41
27	5	781	29
27	6	546	27
27	7	590	26
27	8	721	29
27	9	712	27
27	10	783	31
27	11	610	37
27	12	616	31
27	13	835	36
27	14	839	29
27	15	711	27
27	16	442	34
27	17	682	27
27	18	604	28
27	19	556	31
27	20	674	27
28	1	986	35
28	2	781	38
28	3	521	26
28	4	545	20
28	5	419	20
28	6	683	25
28	7	701	44
28	8	329	20
28	9	369	22
28	10	563	55
28	11	357	18
28	12	242	15
28	13	276	16
28	14	277	16
28	15	253	17
28	16	215	14
28	17	259	17
28	18	177	14
28	19	277	27
28	20	235	14
35	1	2663	42
35	2	2049	49
35	3	2241	46
35	4	13100	100
35	5	4948	72
35	6	1407	28
35	7	1321	51
35	8	12000	100
35	9	1397	30
35	10	486	53

Appendix 5 - A comparison of ICP-MS and pXRF Pb Determinations

A measure of the agreement between the determinations of soil Pb by inductively coupled plasma mass spectrometry (ICP-MS) instrumentation following acid extraction, and a portable X-ray fluorescent (pXRF) analyzer.

Material and Methods

Twenty soil samples chosen at random from the project's retained soil sample fractions were measured for concentrations of Pb using the hand held Thermo Scientific Niton XLt 700 Analyser belonging to Aberystwyth University. The XLt 700 is capable of 3-sigma precision whilst maintaining the ability for fast data collection. This pXRF is equipped with a low power (1.0W) X-ray tube with Ag anode target (Thermo Electron Corporation). Internal calibration was carried out as per the instructions in the user guide (version 5.0 P/N500/905). The sampling mode was set to 'soils' and a sampling time of approximately 70s per sample was used. Measurements were compared with ICP-MS acid extraction results provided by Eurofins UK from another portion of the same sample. Eurofins is UKAS accredited for this analysis. Details of soil sampling, sample handling and the Eurofins ICP-MS method are given within the main project report.

For the pXRF analysis, samples were of weights varying from approximately 0.2 to 0.6 kg, held in transparent polythene sample bags. Samples had been mixed and broken by hand in the field and later subjected to some drying. Thus they still contained soil peds of varying size and given the amount of drying could be classified as either air-dry or moist. Samples were shaken to move the finer particles to one side, and one corner of the bag was then placed on a bench on top of three layers of corrugated cardboard. With the finer particles placed uppermost, the pXRF window was pressed against this corner of the bag for analysis.

To test repeatability two further measurements were made of each sample; for the first, the bags were returned to their storage box and then withdrawn in random order and a second measurement taken, following the same procedure as for the first measurement. For the third measurement on a sample, the bag was not moved from its position following the second measurement but the pXRF removed and then immediately replaced in the same position. Thus the repeatability on a relatively unprepared sample could be judged by comparison of measurements one and two, and the repeatability on a matched, spot measurement judged by comparison of measurements two and three.

The comparisons reported here are based upon the methodology described in Bland and Altman (1999). Given the nature of Pb contamination and the range of concentrations across control and 'high' samples combined (18.9 to 23800 mg/kg), the raw data were natural log (Ln) transformed before the measurements were compared. A plot of the raw values and a plot of the Ln values of one method against the other were produced. A Bland – Altman plot of the Ln values was constructed. A regression model was fitted to the mean v difference Ln data to test for a linear relationship. From the Bland – Altman plot a similar diagram, but of untransformed values was constructed to allow the reader to

immediately appreciate the difference between the methods at any level within the range. Moisture content is thought to affect the pXRF measurement and so a general linear model was constructed to test the effect of dry or moist classification on the predicted ICP-MS value. The repeatability of the pXRF method on the Ln scale is also reported for the comparison between repeated measurements one and two, and between two and three.

Results

Figure 1 shows a plot of the raw data from one measurement method against the other. It can be seen from this that the pXRF almost consistently gave a lower value than ICP-MS instrumentation and that the errors increase the greater the levels of Pb in the soil.

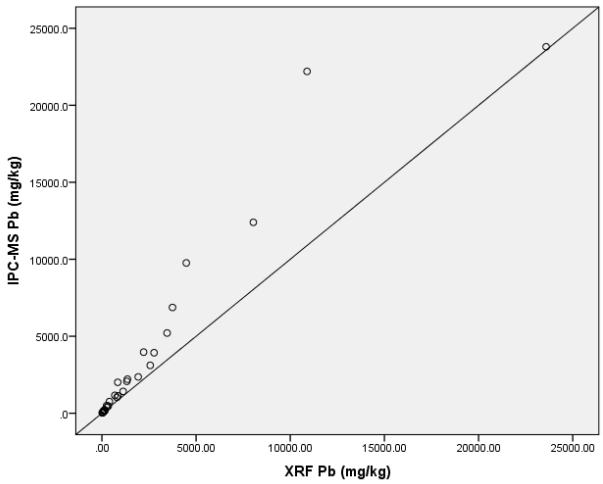


Figure 1. A plot of the pXRF determinations against the IPC-MS measurements. The line of perfect agreement is shown.

Figure 2 shows a similar plot to that of Figure 1, but with the Ln transformed data. It can now be seen from this diagram that the lower values from the pXRF are lower in proportion to the Pb content of the soil and that the variability is now uniform throughout the range, indicating that the errors are also in proportion to the magnitude of the soil Pb content.

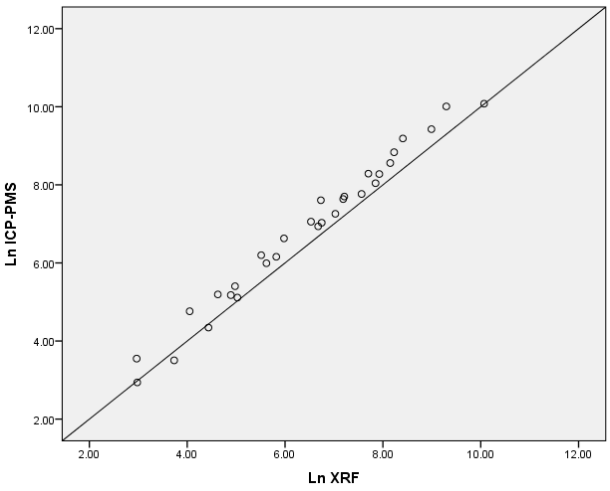


Figure 2. A plot of the Ln pXRF determinations against the Ln IPC-MS measurements. The line of perfect agreement is shown.

The Bland – Altman plot of the difference against the mean of the natural log of the measurements is given as Figure 3 and shows that the mean difference pXRF determination is 0.39118 Ln value lower than those from the ICP-MS procedure and that the error variability between the methods appears to be evenly spread about the mean difference, with an even distribution of error throughout the range of Ln soil Pb values. The upper and lower limits of agreement show the limits that 95% of paired measurements at a given mean value of soil Pb would be expected to fall within. A linear regression model was fitted to the data shown in Figure 3 to test for a linear relationship between the 'errors' and the mean soil Pb but no significant fit was found (p = 0.122).

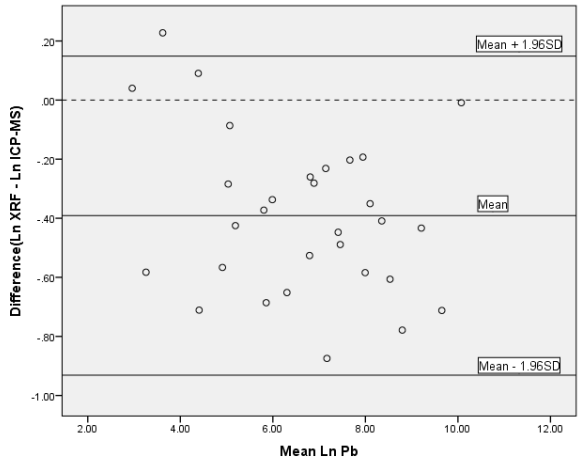


Figure 3. A Bland - Altman plot showing the mean Ln Pb levels by the two methods against the difference between their Ln values. The mean difference (Ln pXRF – Ln IPC-MS) was -0.39118, and the upper and lower limits of agreement were 0.14887 to -0.93112, respectively. The line of perfect agreement is shown as the broken horizontal line at a difference = 0.

In Figure 4 the line of perfect agreement, mean difference and upper and lower limits of agreement from Figure 3 are plotted using untransformed axes so that it can be seen directly how the two measurement methods would be expected to perform across the range of soil Pb values.

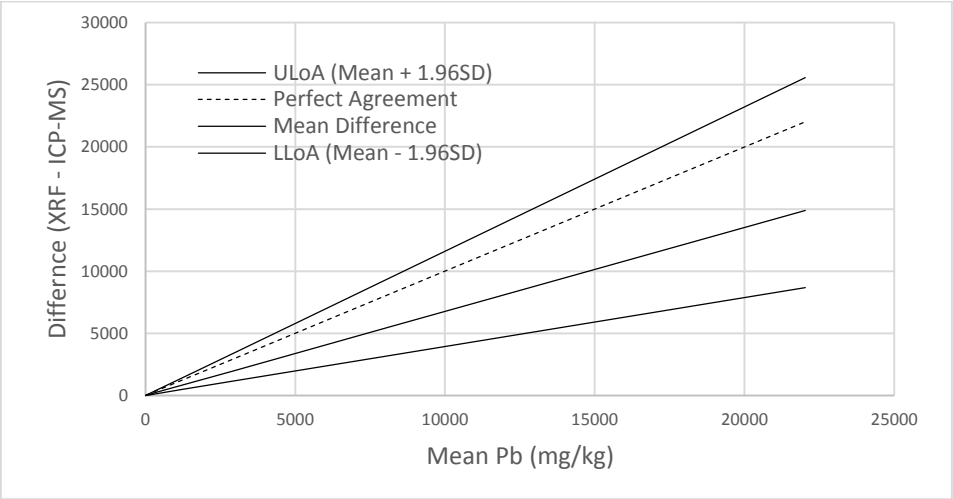


Figure 4. The Bland – Altman plot in Figure 3 plotted with untransformed data. ULoA and LLoA = upper and lower limits of agreement, respectively.

This demonstrates directly how the pXRF measurements are a constant proportion of the measurement from the ICP-MS and shows how the variability in the errors also increases in proportion to the magnitude of the soil Pb values.

It is reported that the values given by the pXRF depend to some extent upon the moisture content of the soil. To test this a general linear model was fitted with Ln ICP-MS values as the predicted variable and pXRF and soil moist or dry (a binary variable) as predictors. A significant effect of moist soil ($p = 0.041$) was found. The parameter estimates of the model were:-

Ln ICP-MS = 0.04 (0.172) + 0.21 (0.098) (if soil was moist) + (1.033 x Ln pXRF)

where the se of the estimates are given in brackets following the parameter estimate.

As a measure of repeatability, the within-sample standard deviation for the natural log measurements one and two was 0.18390, and for measurements two and three, 0.10625.

Discussion

As would be expected from the nature of the concentrations of Pb found in soil, and similarly to the distribution of values from other contaminants, a log scale was required to investigate the agreement between the ICP-MS determination and that of the pXRF. The analyses above shows that the pXRF consistently reported approximately 67% of the mean value of the pXRF and ICP-MS combined and that

the variability in the 'disagreement' between the two measures was, again, proportional to the magnitude of the soil Pb. This type of measurement error is very common when measurements are taken across a very great range. For example, the error in height measurements made on an ant compared with those of the height of a human would show a similar relationship.

From the practical point of view, relevant to this survey, these measures of agreement show that the pXRF would be a perfectly adequate tool for immediate, spot verification in the field of soils containing higher concentrations of Pb where identification and collection of potentially interesting animal and food samples is required.

The assessment above was made with the practical application of how the pXRF would likely be used in the field and made no attempt to refine or improve agreement. The general linear model reported above suggests that the correspondence between the two measures can be more precisely predicted if the moisture content of the soil is factored into the pXRF measurement.

The measurements of repeatability, within use, for the pXRF are reported above, and as would be anticipated the fresh measurement on each soil sample after the bag has been moved and the pXRF reoriented shows less repeatability than when the pXRF is simply removed and replaced in as close as possible to its original position. Of greater interest would be a comparison of the repeatability of the pXRF measurements for a set of samples with the repeatability of the ICP-MS method. However, duplicate analyses were not carried out for the ICP-MS procedure.

Reference

Bland, J.M. and Altman, D.G. (1999) Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* **8** 135-160.

Appendix 6 – Comparison of Soil Pb Levels Using ICP-MS with either EDTA or Acid Extraction

Twenty five of the bulked soil samples were analysed for levels of Pb using both acid and EDTA extraction. A plot of the natural log values for acid extraction are shown against those for EDTA extraction in the Figure below. It can be seen that there is a very strong linear relationship between the two measures and one outlying value. The outlier was most probably due to a mistake in the chemical analysis and has been deleted from further analyses in this Appendix.

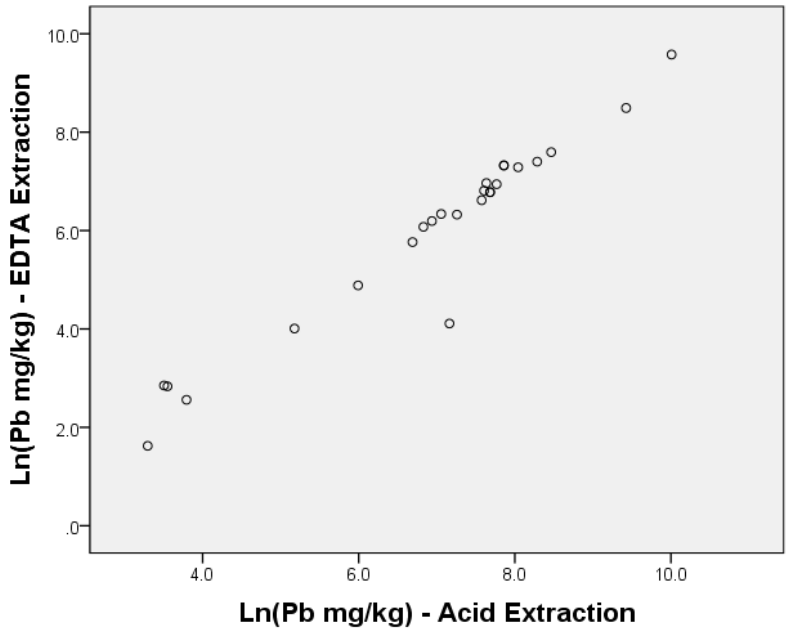


Figure. A scatter plot of the natural log Pb values from the soil analysis using acid extraction plotted against those from EDTA extraction.

A linear regression equation derived from the natural logged values was highly statistically significant:-

$$\text{Ln (Pb EDTA)} = -1.406\ (0.411) + 1.066\ (0.058) \times \text{Ln(Pb acid)}$$

However, the relationship is more simply described on the normal (not log) scale. The level of Pb by EDTA extraction was approximately 50% (actual value 50.47%) that obtained by acid extraction.

Appendix 7 – Snapshot of Pb Isotope Ratio Visual Analysis

The graphics package ggobi allows the seven dimensional isotope ratio data to be reduced to a two dimensional projection which is then dynamically rotated, arbitrarily about the seven dimensions. This allows underlying patterns in the data to be seen more clearly. The electronic file Supplement 3 with this report shows a screen capture of part of the data visualisation process using ggobi.

The snapshot shown in the Figure, below, summarises aspects of the data. Samples from the four regions are labelled: Aberystwyth in red, Derbyshire in green, Mendips in orange and control samples in blue. The clustering of samples by isotope ratios within region is very apparent, with Aberystwyth completely distinct and some minor overlap between Derbyshire and Mendip samples. The control samples were obtained from a diversity of areas and this is reflected in their distribution in space, but nonetheless they do not appear to overlap the three 'high' regions to any great extent. Four possible outliers are labelled, three from the Mendips and one from Derbyshire. They were not extreme outliers and were not associated with unusual level of Pb and so none were excluded from the survey data.

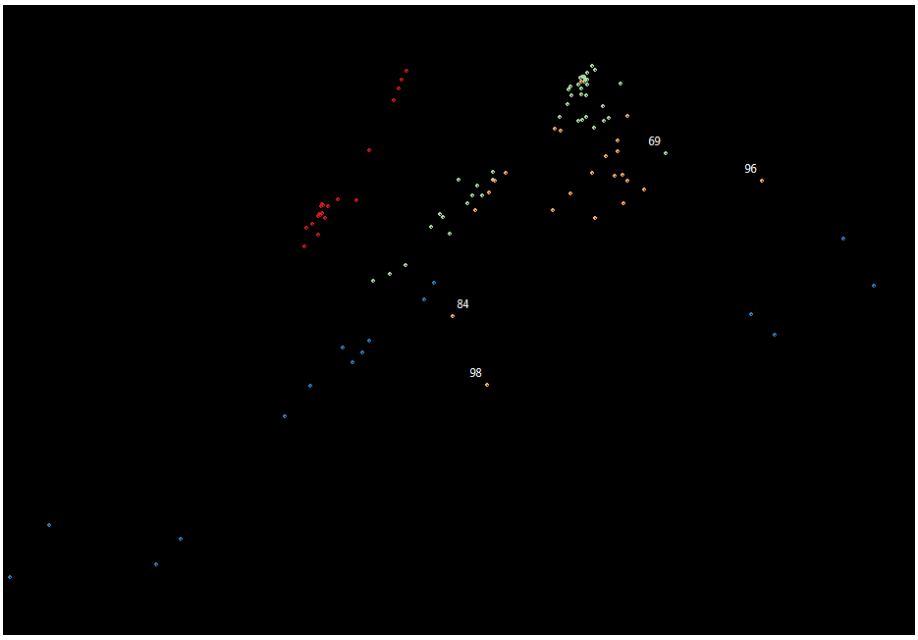


Figure A snapshot from the dynamic, visual exploration of the isotope ratio analysis, highlighting the clustering between geographic areas (Aberystwyth = Red, Derbyshire = Green, Mendips = Orange, Control samples = Blue). Four potential outliers are numbered.

Appendix 8 – The Use of the Parameter Estimates to Construct a Predictive Equation

Table 3 from the report is reproduced below. A predictive equation can be constructed for each type of animal from the parameter estimates given in the Table. The estimates for cattle are used here to demonstrate. It should be remembered that the equations were derived using the natural log transformed Pb data (measured in mg/kg) and so should only be used with natural log transformed data. The predicted value on the log scale can be back-transformed to give the actual Pb level predicted, on a normal scale.

Predictor Variable		Broiler			Cattle			Layer			Sheep	
	β	se	p	β	se	p	β	se	p	β	se	p
Constant (Liver)	-0.465	0.178	0.009 **	1.3	0.388	<0.001 ***	-0.289	0.171	0.091	2.537	0.309	<0.001 ***
Muscle	-3.554	0.155	<0.001 ***	-5.107	0.285	<0.001 ***	-3.867	0.219	<0.001 ***	-4.173	0.096	<0.001 ***
Kidney	-	-	-	0.592	0.285	0.038 *	-	-	-	0.039	0.092	0.672
Egg	-	-	-	-	-	-	-1.528	0.219	<0.001 ***	-	-	-
Blood	1.07	0.05	<0.001 ***	0.943	0.112	<0.001 ***	1.162	0.088	<0.001 ***	0.983	0.092	<0.001 ***
Muscle x Blood	-0.559	0.045	<0.001 ***	-0.421	0.083	<0.001 ***	-0.732	0.112	<0.001 ***	-	-	-
Kidney x Blood	-	-	-	-0.113	0.083	0.173	-	-	-	-	-	-
Egg x Blood	-	-	-	-	-	-	0.264	0.112	0.018 *	-	-	-

When constructing the equation the treatment of continuous variables (eg blood, age, Pb in feed) is different from that of categorical variables (eg tissue type). The predictive equation for Ln Pb levels in the different cattle tissues from the parameter estimates (β) in the Table can be constructed thus:

Ln(Pb) = 1.3 – 5.107[if muscle] + 0.592[if kidney] + (0.943 x (Ln(blood Pb)))

+ (-0.421 x (Ln(Blood Pb)) [if muscle]) + (-0.113 x (Ln(Blood Pb))[if kidney])

The estimate for kidney has been included in the equation, even though not statistically significant, for the reason given in the report. The constant in the equation (and all equations in this report) has been arbitrarily tied to Liver.

The equation can now be used to predict Ln(Pb) levels in any of the consumed tissues for a given level of Ln(Pb) in the blood, for example, suppose blood Pb had been measured as 0.05 mg/kg. Taking the natural log of 0.05 gives a value of -2.996, which would lead to a prediction equation for each tissue as follows:-

Ln(Liver Pb) = 1.3 + (0.943 x -2.996)

Ln(Muscle Pb) = 1.3 – 5.107 + (0.943 x -2.996) + (-0.421 x -2.996)

Ln(Kidney Pb) = 1.3 + 0.592 + (0.943 x -2.996) + (-0.113 x -2.996)

These give predicted values of:-

Ln(Liver Pb) = -1.5252

Ln(Muscle Pb) = -5.371

Ln(Kidney Pb) = -0.5947

These values can then be back transformed by exponentiation to give predicted values for each of the tissues of:-

Liver Pb = 0.2176 mg/kg

Muscle Pb = 0.0047 mg/kg

Kidney Pb = 0.5517 mg/kg

These are the expected values in cattle found with a blood Pb of 0.05 mg/kg.