Title: Inorganic elemental analysis of decomposition fluids of an *in situ* animal burial

Authors: Henry C. Dick, Jamie K. Pringle

PII: S0379-0738(18)30286-X
DOI: https://doi.org/10.1016/j.forsciint.2018.05.034
Reference: FSI 9329

To appear in: *FSI*

Received date: 11-1-2018
Revised date: 25-4-2018
Accepted date: 22-5-2018

Please cite this article as: Henry C.Dick, Jamie K.Pringle, Inorganic elemental analysis of decomposition fluids of an in situ animal burial, Forensic Science International https://doi.org/10.1016/j.forsciint.2018.05.034

Inorganic elemental analysis of decomposition fluids of an *in situ* animal burial

Henry C. Dick and Jamie K. Pringle*

School of Geography, Geology and Environment, Keele University, Keele, Staffs, ST5 5BG, U.K.

*Corresponding author: J.K. Pringle [j.k.pringle@keele.ac.uk]

Highlights

- Simulated clandestine burial emplaced using pig cadaver
- In situ extraction of decompositional fluids for 18 months
- Inorganic element analysis shows K+, SO42- and Na+ major influence
- Mirrors temporal conductivity changes in other studies
- Implications to detect and potentially date discovered clandestine burial

Abstract

In homicide investigations, it is critically important that post-mortem (PMI) and post-burial interval (PBI) of buried victims are determined accurately. However, clandestine graves can be difficult to locate; and the detection rates for a variety of search methods can be very low. This paper presents elemental analysis results of 18 months of decomposition fluids from an *in situ* buried animal cadaver used as a human clandestine burial proxy. Study results showed potassium, sulphate and sodium are key detectable elements which mirror observed conductivity temporal changes from this and other studies. Seasonal rainfall has a strong influence on both fluid generation and subsequent concentration which needs to be accounted for. Study implications suggest inorganic elements could provide both detection and potential dating of discovered clandestine burials.

Keywords: forensic science; clandestine burial; grave fluid; element concentration; post-mortem interval

1. Introduction

Geoscientific methods are being increasingly utilized by forensic search teams for the detection and location of clandestine burials [1-3]. Clandestine graves of murder victims are usually shallow, less than 3 m and typically 0.5 m below ground level (bgl) [4-5], but current detection rates are low and, without locating the victim's body, obtaining a successful conviction is more difficult [6-7]. Search investigators will typically use a variety of methods, which include scenario-based, feature-focused, intelligence- led, and systematic standard operating procedures [6-7]. Standard operating procedures require investigators to follow sequential workflows, from reviewing case information, sourcing background/intelligence information, and remote data analysis. This process occurs before determining search strategies, undergoing site reconnaissance and phased site investigations, and then intrusively investigating anomalous areas [2, 6, 8]. Geoscientific site investigation methods vary depending upon the specific case, search site, and numerous other factors that are reviewed elsewhere [2-3], but can include scent-trained human remains detection dogs [8-9], forensic geomorphology [10], forensic botany [11-12] and entomology [13-14, near-surface geophysics [15-23], intrusive probing [24-25] and soil geoscience analysis [26-28].

After a body has been found, it is natural for investigators to focus on determining time since death. There has been extensive research on estimating the postmortem interval (PMI) of very recently deceased individuals discovered aboveground that has been reviewed elsewhere [28], commonly using body cadaver temperatures [29-30], entomology [31], entomofauna [32] and thanatochemistry [33]. For longer deceased individuals, other common PMI dating methods include tissue decomposition [34], skeletal remains [35] and tooth odontology [36]. Below ground decomposition rates of discovered individuals have been shown to be highly variable [37], depending on organic content [38], various local environmental factors such as soil type [26,39-41] and organism accessibility [42], among other factors. These factors complicate the estimation of PMI for buried remains. Furthermore, it may be useful to estimate the Post-Burial Interval (PBI) as a guide to the PMI. However, the PMI and PBI may be different: A victim might not be buried immediately after death. In such cases, the PBI can be used as an estimate of the lower limit of the PMI.

The presence of a decomposing cadaver has also been shown to be detectable on the surrounding soil. For example, changes in soil chemistry [38, 43-44], such as changes in the levels of methane [45], phosphates and nitrates [46], ninhydrin-reactive nitrogen [47-48], volatile organic compounds [27,38,43 and pH [46,49] can all be detected. Changes in these soil properties can be used to estimate time since death. The decay of other items such as materials associated with a grave has also been suggested to allow a PBI to be estimated [34,41]. Although relatively poorly understood, grave soil has been shown to be detectable by near-surface geophysical search methods, specifically electrical resistivity [19,21,50,51], and its reciprocal, bulk ground conductivity [16]. Geophysical research using simulated clandestine grave burials can provide critical information, for example, on optimal geophysical detection methods and equipment configurations [15,28,52-54], as well as providing continuous datasets for comparison with real cases [54-60]. Recent research has found that electrical resistivity anomalies over burials are predominantly due to conductive fluids in grave soil that vary temporally that may be due to decomposition [28,54,61]. It has been shown that it is possible to repeatedly extract *in situ* decomposition fluids from both a buried pig cadaver and background soil water, without the need for repeated disturbance or numerous replicate samples as other authors have performed. The resulting fluids can be simply analyzed for conductivity using a handheld meter [28]. However, it is uncertain what elements are causing these observed temporal changes in conductivity, hence the focus of this repeat study.

The aims of this study are to therefore; *firstly* to sample and present results from an 18 month monitoring study of a burial pig carcass, analysing the leachate and background soil water for the major inorganic chemical components and *secondly*, perform a systematic statistical analysis of the resulting element parameters to determine the contributions from individual inorganic elements responsible.

2. Methodology

2.1 Study test site

The selected burial site was located in a restricted area on Keele University campus, approximately 200 m above sea level, close to Newcastle-under-Lyme town in Staffordshire, UK (Fig. 1). Part of the study site was initially used for simulated clandestine graves monitoring and geophysical investigations (see [54,55,61]. Daily climatic records were obtained from a nearby weather observation station within Keele University, with a temperate local climate that is typical for the UK [62]. The study site was a small plot of land approximately 25 m by 20 m, covered with grasses and surrounded by deciduous trees on three sides. The study site scenario is a typical representative of a semi-rural environment.

Information from a nearby borehole records identified the Carboniferous (Westphalian) Butterton Sandstone bedrock geology approximately 2.6 m below ground level (bgl). The local soil is predominantly a made-ground, due to the presence of demolished greenhouses. Initial soil sampling showed a vertical site succession of a shallow (0.01 m) organic-rich, top soil (Munsell colour chart colour (mccc): 5 YR/2/2.5), with underlying ‘A’ Horizon (Mccc: 5

YR/3/3) comprising largely of a natural sandy loam that contained approximately 5% of isolated brick and coal fragments [55]. The natural ground ‘B’ Horizon was located at approximately 0.45 m bgl, consisted mainly sandstone fragments from the underlying bedrock, which suggested a shallower bedrock depth. Also further investigation on soil particle analysis shown that the typical sandy loam soil texture, i.e. approximately 72 % sand, 26 % silt and 2 % clay, in combination with slow lateral water flow led to moderately high moistness of the soil approximately 34 %**.**

2.2 Experimental procedures

The simulated grave was constructed at the eastern part of the site (Fig. 1), which involved removal of the turf and then ground excavation, of a hole up to ~0.6 m deep, ~1.5 m length and ~ 0.5 m wide. The use of human cadavers was prevented due to the ethical issues involved in the use of human cadavers for research in the UK (Human Tissue Act, 2004); therefore a pig cadaver of the species *Sus scrofa* was used as a proxy for a human cadaver, whilst not ideal, they are considered to be similar to humans in weight, fat to muscle ratio, hair coverage, biochemistry and physiology [15,26,63]. A 90 kg pig of length ~1.5m, sourced from a local abattoir, was therefore killed by bolt gun; this prevented excess blood being lost as they usually despatched by electrocution and draining. The pig carcass was interred on 18th March 2014 and a lysimeter model 1900 inserted inside the made-slurry at the base of the hole between the two hind limbs and the grave wall (see Fig. 2). After internment of the pig carcass, the grave was backfilled with the same excavated soil, tamped firmly and leaving a slight mound to account for later settlement, before the turf was replaced to simulate a clandestine grave. A control lysimeter was installed on the same day, approximately 16 m away from the pig grave and uphill to prevent any potential contamination (Fig. 2d). For this control lysimeter, a hole of ~0.3 m by ~0.3 m wide and 0.6 m deep (the same depth as the pig grave) was excavated and refilled. The lysimeters were then left in place throughout the monitoring period. Generating a suction pressure within the lysimeter causes soil water to be drawn through the ceramic cup and into the PVC tube. Leachate and soil samples can then be extracted using a plastic syringe with a narrow tube attachment inserted through the stopper assembly (Fig. 2e).

2.3 Sample collection and on-site measurements

Initial sample extraction was conducted two days before the sampling day, to enable a fresh accumulation of leachate fluid in the grave which should be representative during the sampling period. The lysimeter clamp ring used to fold the neoprene tubing was removed, giving access to extract any fluid present in the grave, before a vacuum hand pump was employed to generate a vacuum pressure of approximately 65 centibars (kPa) (Fig. 2e). This pressure is capable of causing moisture to move from the soil through the porous ceramic cup, and into the vacuum sampler [64]. The same extraction procedure was repeated on each sampling day. Samples were extracted from both the pig grave and the control once a month for a period of 18 months, except for the first month that was sampled fortnightly, to enhance and validate the initial soil and leachate conditions. The samples were transferred to 100 ml labelled plastic sample bottles (Fig. 2e) after a portable WTW^{TM} Instrument Multi-line P4 conductivity meter was used on-site to measure conductivity and temperature values (Fig. 2e). These were automatically corrected by the conductivity meter to a reference temperature (25 ^{0}C) and are 0.1 ^{0}C accurate, thus avoiding any potential temperature variation effects when collecting samples. This procedure was repeated to check reading repeatability and reliability. Samples pH was also measured onsite in the laboratory with standards at pH 4, 7 and 10 before each use. Approximately 10 ml of the collected samples were used for the ICP-OES analysis, which was conducted within 1 hour after sampling and the remaining portion kept frozen until further Dionex laboratory analysis was conducted.

2.4 Climatological data collection

Climatological information was obtained from the closest weather station, ~ 0.2 km from the test site managed by the U.K. Meteorological Office. The recorded data include average daily rainfall and air temperature reading over the corresponding monitoring periods (see Table 1). It measured monthly minimum, maximum, and average total rainfall of 15 mm, 113 mm, and 66 mm, respectively, over the 549 day monitoring period. Calculated monthly total rainfall data of the site were used to correct measured soil water measurements for local rainfall variation, in which conductivity values were multiplied by a rainfall correction factor, generated by dividing the average monthly rainfall for England in a given year by the average monthly rainfall for the local area in the same year (see [28] for background). The reason for the correction is to adjust the rainfall value in case of relatively high rainfall rates, which could potentially dilute grave soil water and hence reduce the measured values for physicochemical parameters, and in case of relatively low rainfall rates would increase the concentration of grave soil water and hence increase values for the measured parameters.

2.5 Ion Chromatography (IC) Dionex system

Ion Chromatography (IC) is a standard analytical technique used to detect inorganic anions and cations in liquid samples down to parts per million (ppm) – see [65] for background. Forensic applications include characterisation of gunshot [66] and explosive [67] residues, elemental profiling of illegal tobacco [68] and fingerprint analysis [69]. It is also a well established regulatory method for this purpose for environmental forensic studies, quantifying inorganic contaminants in, for water, wastewater, leachate, soil extracts, surface and groundwater, and other environmental sample matrixes [65].

Analysis of the inorganic anion concentrations was performed using a standard Dionex™ ICS-1000 ion chromatograph with improved suppressed conductivity detection. This machine was initially calibrated by running three standards of Fluoride, Nitrate, Phosphate and Sulphate of varying concentrations (1ppm – 10ppm) through the equipment and comparing linearity of measured results following standard procedures (see [70]). 0.5 ml of fluid from both the control and grave soil water samples were prepared and put onto the sample loader, before the machine measured and analysed the results. The standards were also re-run between each set of samples to avoid cross-contamination and monitor any instrument measurement drift. Blanks of deionised water were also used after each sample measurement for quality control.

2.6 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is similarly a standard analytical tool used to measure analyte concentrations in an aqueous sample, then comparing them to known element concentrations in a standard sample and can detect metallic elements down to 1 part per billion (ppb). Forensic applications include analysis of evidentiary materials [71], discriminating glass analysis [72] and determining fatal mercury poisoning [73].

Elemental ICP-OES metal analysis used a Varian™ Vista MPX ICP-OES instrument (Agilent Technologies, Santa Clara, CA). Samples were centrifuged at 5000 rpm for 10 mins and then 7 mL of each sample was transferred into 10 mL auto sampler tubes following standard methodologies [74]. The commercial cyclonic spray chamber (#98301) was used with a Seaspray concentric nebuliser (#86779). Operating parameters of this instrument are listed in the appendix. Sample calibration against approved standard reference materials (P, S, V and Si) was undertaken and serial dilutions were prepared, dissolved in 5% (v/v) HNO_3 and used to produce calibration curves from which respective sample amounts were calculated. Between each measurement, blank samples were analysed to reduce/eliminate measurement drift. The known standards were also periodically run between samples to check for measurement accuracy.

3. Results

The observed sinusoidal seasonal temperature variation was typical for northern latitudes, with broadly typical wetter winter and dryer summer months. Monthly local temperature variations greatly affect decomposition rates [4]; these could be corrected for by converting post-burial (day) interval (PBI) to Accumulated Degree Days (ADD), as detailed in the methodology.

However, there were notable variations between the first and second year monthly rainfall, which could result in a significant variation in the concentration of grave soil water if not properly accounted for, as relative higher rainfall rates will reduce measured conductivities and concentrations of other element parameters.

The control soil water measurements from the Keele test site (Fig. 4) evidenced consistent background conductivity values over the 549-day study period, averaging 367 (± 0.1) mS/cm (Table 2). This compared well with the 411 (± 0.)1 mS/cm averages of previous studies [28]. The grave soil water (leachate) evidenced a steady increase in conductivity values to 2,850 ± 0.1 mS/cm (58 days) before a consistent increase up to 34,400 ± 0.1 mS/cm (272 days). After this time, conductivity values then steadily decreased until the end of the monitoring period to 13,830 ± 0.1 mS/cm (549 days)- see Fig. 4a.

Climatic variations were able to be adjusted for by converting Post-Burial Days (PBI) to calculate Accumulated Degree Days (ADD) to correct for temperature, these results compared favourably with previous studies [28]– see Fig. 4b. Monthly rainfall variations were also able to be taken into account by using a correction factor for each conductivity, using the percentage difference of individual months compared to the total study average rainfall – see Table 2 and Fig. 4b.

The grave soil water element analysis averages showed Calcium element concentrations to rapidly increase from 171 ± 0.1 mg/l at 14 PBD up to a maximum of 1,620 ± 0.1 mg/l at 150 PBD before declining back to ~303 ± 0.1 mg/l at the end of the monitoring period (Table 3). The control soil water values average 82 ± 0.1 mg/l. Grave soil water Potassium and Sodium element concentrations showed similar trends to Calcium, increasing from 11 ± 0.1 mg/l and 58 ± 0.1 mg/l at 14 PBD up to 1,230 ± 0.1 mg/l and 536 ± 0.1 mg/l at 212 PBD before declining back to 338 ± 0.1 mg/l and 119 ± 0.1 mg/l respectively (Table 3). Their respective control soil water element concentrations were not detectable. Grave soil water chloride, sulphate, nitrate and phosphate element concentrations also showed similar trends, albeit at lower concentrations (Table 3) with their control soil water element concentrations mostly not detectable (Table 3).

After the respective control element concentration values were subtracted from the 'grave' soilwater element concentrations, the resulting concentration element variations Pig decomposition leachate and corresponding background soil water chemistry results are shown in Tables 3 and 4 respectively. A combined graph of elemental concentration against the post burial days (PBD) is presented in Figure 5. The removal of soil water effect from leachate water was conducted by subtracting the corresponding concentration of elements in soil water from the values obtained in leachate water in order to obtain the concentration of a pure leachate sample used for further analysis (see Figure 4c).

Generally, there was a notably high correlation between the concentrations of inorganic elements and the electrical conductivity temporal variations in the leachate sample (Fig. 4). The degree of linear association between any two of the parameters were measured by simple correlation coefficient (R) values for the leachate and background soil water samples respectively (Tables 4-6).

Significance tests were undertaken of the correlation coefficients of fluid conductivity against measured element concentrations in both the grave (Table 5) and control (Table 6) soil water samples. Results showed potassium, sulphate, sodium and phosphate elements to have significant correlation coefficients in the grave soil water samples (Table 4), although phosphate was also significant in the control soil water samples (*cf.* Tables 4-5).

Finally two variable least-square approaches were used to investigate the relationship between fluid conductivity as an independent variable and the main measured elements as dependent variables. Linear regression analysis were carried out and evidenced that electrical fluid conductivity was the controlling variable (Table 6). Regression results for potassium, sulphate, sodium and phosphate were significant at 1% (≤ 0.001), whilst the equation of nitrate is significant at 10% level of confidence (Table 6). A F test (ratio of regression mean square : error mean square) also showed high values. Phosphate was the only significant measured element at 1% level of confidence in control soil water (Table 7).

Multiple R2 values (0.88) indicated that 88% of the variability could be associated to the combined effect of potassium (24%), sulphate (25%), sodium (20%) and phosphate (31%) respectively (Table 7). However, the variability of fluid conductivity values in background soil water was mainly caused by the combined effect of phosphate and chlorate (Table 6). The combined multiple R2 value (0.73) indicates that 73% of the variability in EC of soil water could be linked to the presence of phosphate (56%) and chlorate (44%) respectively (Table 7).

4. Discussion

Although each search for buried murder victim will be unique, the burial will always lead to changes in soil composition and the accumulation of substances and elements [75]. This section will discuss the paper aims and objectives.

Study aims were firstly; *to sample and present results from an 18 month monitoring study of a burial pig carcass, analysing the leachate and background soil water for the major inorganic chemical components*. This study has demonstrated that, using an animal pig cadaver as a human proxy, that over time, grave soil water could be differentiated from background soil water by measuring element parameters, in particular, by potassium, sulphate and sodium elements. The observed electrical conductivity temporal changes corresponded with these elements, with the measured conductivity changes having a good similarity with previous studies (see [28,38]) and thus giving confidence that results were replicable. Temporal variations of these elements post-burial, after correcting for temperature and rainfall, all increased up to 220 PBI before declining, with a small increase and decrease again until the end of the monitoring period which mirrors [38], which may also be able to date a discovered clandestine burial of a murder victim, as others have shown with fluid conductivity studies [76]. An interesting observation was the trend of element values immediately after values peaked (Fig. 5), which other authors have also reported (e.g. [47,77]).

The second study aim was *to perform a systematic statistical analysis of the resulting element parameters to determine the contributions from individual inorganic ions responsible*. In this study, analysis of correlation coefficients (Table 4) showed statistically significant positive correlation between fluid conductivity and potassium, sulphate, sodium and phosphate, although phosphate was also statistically significant with the control soil water (Table 5). This suggests that fluid conductivity depends on dissolved salts [78]. The choice of dependent and independent variables in a regression model was crucial. The dependent variable is a variable to be explained, while the independent variable is a moving force [79]. Two variable least squares approach were used here to quantify the relationship between fluid conductivity as an independent variable and different inorganic elements (Table 6). Different dependent characteristics of grave soil water were calculated using a regression equation and by substituting the values for the independent parameter in the equations. Results for potassium, sulphate, sodium and phosphate evidenced that electrical conductivity was significant at 1% level of confidence, while nitrate was significant at 10% level, although phosphate was also significant in the control soil water. The significance of the relationship was also validated by an F-test (Table 7) evidencing that independent variables were significant in predicting changes in fluid conductivity values.

5. Conclusions

This study has importantly shown why observed grave soil water conductivity changes over time as others have observed (see [28,76]); this is primarily due to inorganic ion element changes, specifically potassium, sulphate and sodium, when compared to control values. Forensic soilwater surveys could therefore be undertaken by initial soil sampling of suspected burial sites, careful sample storage, and then centrifuging to extract soil water [47], before measuring their respective inorganic element concentrations. This would not be recommended as an initial search method; rather, it should be undertaken when a search area has been narrowed down to an appropriate size. This does, however, have promise as other studies have shown decomposition fluids to be retained in the local soil environment and to be electrically detectable, even when physical remains have decayed [80].

Further work should *firstly* test this potential post-burial interval method in a real forensic case of a discovered clandestine grave to determine its usefulness for forensic investigation. *Secondly* this study should be repeated with different burial patterns and contrasting soil types, possibly, in a mass burial scenario. *Thirdly* the repeat studies should look at organic element temporal changes to determine if these affect conductivity results. *Finally* it should be investigated if multi-elemental concentrations in soil could be detected, either using hand-held X-ray or laser-induced breakdown spectrometers onsite for a quick reconnaissance survey to potentially locate a potential clandestine burial site.

6. Acknowledgements

I. Wilshaw and A. Cowles (both Keele University) are acknowledged for assistance with the Dionex and ICP-OES PerkinElmer™ equipment training and sample preparation respectively. D. Thompson is thanked for ICP-OES expert advice. V. Heaton is acknowledged for PMI advice. H. Dick is financially supported by the Nigerian Tertiary Education Fund. An anonymous reviewer is thanked for improving the manuscript.

References

[1] A. Ruffell, J. McKinley, Geoforensics. Chichester, U.K.: Wiley Pubs, 2008.

[2] J.K. Pringle, A. Ruffell, J.R. Jervis, L. Donnelly, J. McKinley, J. Hansen, et al., The use of geoscience methods for terrestrial forensic searches, Earth Sci. Rev. 114 (2012a) 108-123.

[3] A. Ruffell, J.K. Pringle, J.P. Cassella, R. Morgan, M. Ferguson, V.G. Heaton, C. Hope, The use of geoscience methods for aquatic forensic searches, Earth Sci. Rev. 171 (2017) 323-337.

[4] M.H. Manhein, Decomposition rates of deliberate burials: a case study of preservation. in: W.D. Haglund, M.H. Sorg. (Eds), Forensic Taphonomy: the post-mortem fate of human remains, CRC Press, 1996, pp. 469-482.

[5] J. Hunter, M. Cox, Forensic archaeology: advances in theory and practice, Routledge Pubs, 2005.

[6] M. Harrison, L.J. Donnelly, Locating concealed homicide victims: developing the role of geoforensics, in: K. Ritz, L. Dawson (Eds.), Criminal and environmental soil forensics, Springer Pubs, (2009), pp. 197–219.

[7] D.O. Larson, A.A. Vass, M. Wise, Advanced scientific methods and procedures in the forensic investigation of clandestine graves, J Contemp. Crim. Just. 27 (2011) 149–182.

[8] T.L. Dupras, J.J. Schultz, S.M. Wheeler, L.J. Williams, Forensic recovery of human remains, CRC Press, 2006.

[9] A. Lasseter, K.P. Jacobi, R. Farley, L. Hensel, Cadaver dog and handler team capabilities in the recovery of buried human remains in the southeastern United States. J Forensic Sci. 48 (2003) 1–5.

[10] A. Ruffell, J. McKinley, Forensic geomorphology. Geomorphology 206 (2014) 14–22.

[11] H.M. Coyle, Forensic botany: principles and applications to criminal casework, CRC Press, 2005.

[12] I. Aquila, F. Ausania, C. Di Nunzio, A. Serra, S. Boca, A. Capelli, et al., The role of forensic botany in crime scene investigation: case report and review of literature, J Forensic Sci. 59 (2014) 820–824.

[13] J. Amendt, C.P. Campobasso, E. Gaudry, C. Reiter, H.N. LeBlanc, M.J.R. Hall, Best practice in forensic entomology: standards and guidelines, Int. J Legal Med. 121 (2007) 90–104.

[14] D. Gennard, Forensic entomology: an introduction, 2nd ed., Wiley-Blackwell, 2012.

[15] D.L. France, Griffin, T.J., Swanburg, J.G., Lindemann, J.W., Davenport, G.C., Trammell, V., et al., A multi-disciplinary approach to the detection of clandestine graves, J Forensic Sci. 37 (1992) 1445–1458.

[16] D.C. Nobes, The search for Yvonne: a case example of the delineation of a grave using near-surface geophysical methods, J Forensic Sci. 45 (2000) 715–721.

[17] A. Witten, R. Brooks, T. Fenner, The Tulsa Race Riot of 1921: a geophysical study to locate a mass grave, Leading Edge 20 (2001) 655–660.

[18] K. Powell, Detecting human remains using near-surface geophysical instruments, Expl. Geophys. 35 (2004) 88–92.

[19] P. Cheetham, Forensic geophysical survey, in: J. Hunter, M. Cox (Eds.), Forensic Archaeology: Advances in Theory and Practice, Routledge, 2005, pp. 62–95.

[20] J.J. Schultz, Using ground-penetrating radar to locate clandestine graves of homicide victims: forming forensic archaeology partnerships with law enforcement, Homicide Studies 11 (2007) 15–29.

[21] J.K. Pringle, J.R. Jervis, Electrical resistivity survey to search for a recent clandestine burial of a homicide victim, UK, Forensic Sci. Int. 202 (2010) e1–e7.

[22] A. Novo, H. Lorenzo, F. Ria, M. Solla, 3D GPR in forensics: finding a clandestine grave in a mountainous environment, Forensic Sci. Int. 204 (2011) 134–138.

[23] J-P Fernandez-Alvarez, D. Rubio-Melendi, A. Martinez-Velasco, J.K. Pringle, D. Aguilera, Discovery of a mass grave from the Spanish Civil War using GPR and forensic archaeology, Forensic Sci. Int. 267 (2016) e10-e17.

[24] D.W. Owsley, Techniques for locating burials, with emphasis on the probe, J Forensic Sci. 40 (1995) 735–740.

[25] E.W. Killam, The detection of human remains, Charles C. Thomas Pubs., Springfield, USA, 2004.

[26] D.O. Carter, M. Tibbett, Cadaver decomposition and soil: processes, in: M. Tibbett, D.O. Carter, (Eds.), Soil analysis in forensic taphonomy: chemical and biological effects of burial of human remains, CRC Press, 2009, pp. 29-52.

[27] J. Dekeirsschieter, F.J. Verheggen, M. Gohy, F. Hubrecht, L. Bourguignon, G. Lognay, et al., Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus*) in different biotopes, Forensic Sci. Int. 189 (2009) 46–53.

[28]M. Megyesi, S. Nawrocki, N. Haskell, Using Accumulated Degree-Days to estimate the post-mortem interval from decomposed human remains, J. Forensic Sci. 50 (2005) 1-9..

[29]C. Moffatt, T. Simmons, J. Lynch-Aird, An Improved Equation for TBS and ADD: establishing a reliable postmortem interval framework for casework and experimental studies, J. Forensic Sci. 61 (2016) S201-S207..

[30] C. Henssge, Death time estimation in case work: the rectal temperature time of death nomogram, Forensic Sci. Int. 38 (1988) 209–236.

[31] M. Arnaldos, I. Garcia, E. Romera, J.J. Presa, A. Luna, Estimation of post-mortem interval in real cases based on experimentally obtained entomological evidence, Forensic Sci. Int. 149 (2005) 57–65.

[32] M.I. Marchenko, Medicolegal relevance of cadaver entomofauna for the determination of the time of death, Forensic Sci. Int. 120 (2001) 89–109.

[33] B. Madea, Is there recent progress in the estimation of the post-mortem interval by means of thanatochemistry? Forensic Sci. Int. 151 (2005) 139–149.

[34] R.C. Janaway, Decomposition of materials associated with buried cadavers, in: M. Tibbett, D.O. Carter (Eds.), Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains, CRC Press, 2009, pp. 153–201.

[35] F. Ramsthaler, K. Kreutz, K. Zipp, M.A. Verhoff, Dating skeletal remains with luminol-chemiluminescence: validity, intra- and inter-observer error, Forensic Sci. Int. 187 (2009) 47–50.

[36] A.H. McKeown, J.L. Bennett, A preliminary investigation of post-mortem tooth loss, J Forensic Sci. 40 (1995) 755–757.

[37] W.C. Rodriguez, Decomposition of buried and submerged bodies, in: W.D. Haglund, M.H. Sorg (Eds.), Forensic taphonomy: the post-mortem fate of human remains, CRC Press, 1997, pp. 459–468.

[38] A.A. Vass, W.M. Bass, J.D. Wolt, J.E. Foss, J.T. Ammons, Time since death determinations of human cadavers using soil solution, J Forensic Sci. 37 (1992) 1236–1253.

[39] B. Turner, P. Wiltshire, Experimental validation of forensic evidence: a study of the decomposition of buried pigs in heavy clay soil, Forensic Sci. Int. 101 (1999) 113–122.

[40] S.L. Forbes, B.B. Dent, B.H. Stuart, The effect of the burial environment on adipocere formation, Forensic Sci. Int. 154 (2005) 24–34.

[41] A.S. Wilson, R.C. Janaway, A.D. Holland, H.I. Dodson, E. Barran, A.M. Pollard, et al., Modelling the buried human body environment in upland climes using three contrasting field sites, Forensic Sci. Int. 169 (2007) 6–18.

[42] D.W. Hopkins, The role of soil organisms in terrestrial decomposition, in: M. Tibbett D.O. Carter (Eds.), Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains, CRC Press, 2009, pp. 53–66.

[43] A.A. Vass, R.R. Smith, C.V. Thompson, M.N. Burnett, N. Dulgerian, B.A. Eckenrode, Odor analysis of decomposing human remains, J Forensic Sci. 53 (2008) 384–391.

[44] D.O. Carter, D. Yellowlees, M. Tibbett, Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soil, Appl. Soil Ecol. 40 (2008a) 129-137.

[45] M. Davla, M. Kalacska, T.R. Moore, A. Costopopoulos, Detecting graves with methane, Geoderma 189–190 (2012) 18–27.

[46] M.J. Matias, M. Marques da Silva, L. Goncalves, C. Peralta, C. Grangeia, E. Martinho, An investigation into the use of geophysical methods in the study of aquifer contamination by graveyards, Near Surf. Geophys. 2 (2004) 131–136.

[47] D.O. Carter, D. Yellowlees, M. Tibbett, Using ninhydrin to detect gravesoil, J Forensic Sci. 53 (2008b) 397–400.

[48] L.E. Van Belle, D.O. Carter, S.L. Forbes, Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (*Sus domesticus*) decomposition, Forensic Sci. Int. 193 (2009) 37–41.

[49] L.A. Benninger, D.O. Carter, S.L. Forbes, The biochemical alteration of soil beneath a decomposing carcass, Forensic Sci. Int. 180 (2008) 70–75.

[50] J.D. Hansen, J.K. Pringle, J. Goodwin, GPR and bulk ground resistivity surveys in graveyards: locating unmarked burials in contrasting soil types, Forensic Sci. Int. 237 (2014) e14–29.

[51] H.C. Dick, J.K. Pringle, K.D. Wisniewski, R. van der Putten, G. Evans, J. Goodwin, et al., Determining geophysical responses from burials in graveyards and cemeteries, Geophysics, 82 (2017) B245-255.

[52] J.K. Pringle, M. Giubertoni, N.J. Cassidy, K. Wisniewski, J.D. Hansen, N. Linford, R. Daniels, The use of magnetic susceptibility as a forensic search tool, Forensic Sci. Int. 246 (2015) 31–42.

[53] J.J. Schultz, M.M. Martin, Controlled GPR grave research: comparisons of reflection profiles between 500 and 250 MHz antennae, Forensic Sci. Int. 209 (2011) 64–69.

[54] J.K. Pringle, J.R. Jervis, D. Roberts, H.C. Dick, K.D. Wisniewski, N.J. Cassidy, J.P. Cassella, Geophysical monitoring of simulated clandestine graves using electrical and ground penetrating radar methods: 4-6 years, J Forensic Sci. 61 (2016) 309-321.

[55] J.K. Pringle, J.R. Jervis, J.D. Hansen, N.J. Cassidy, G.M. Jones, J.P. Cassella, Geophysical Monitoring of Simulated Clandestine Graves Using Electrical and Ground-Penetrating Radar Methods: 0-3 Years after Burial, J Forensic Sci. 57 (2012b) 1467-1486.

[56] J.J. Schultz, M.E. Collins, A.B. Falsetti, Sequential monitoring of burials containing large pig cadavers using ground-penetrating radar, J Forensic Sci. 51 (2006) 607–616.

[57] J.J. Schultz, Sequential monitoring of burials containing small pig cadavers using ground-penetrating radar, J Forensic Sci. 53 (2008) 279–287.

[58] J.J. Schultz, M.M. Martin, Monitoring controlled graves representing common burial scenarios with ground penetrating radar, J Appl. Geophys. 83 (2012) 74–89.

[59] C.M. Molina, J.K. Pringle, M. Saumett, G.T. Evans, Geophysical and botanical monitoring of simulated graves in a tropical rainforest, Colombia, South America, J Appl. Geophys. 135 (2016a) 232-242.

[60] C.M. Molina, J.K. Pringle, M. Saumett, O. Hernandez, G.T. Evans, Geophysical monitoring of simulated graves with resistivity, magnetic susceptibility, conductivity and GPR in Colombia, South America, Forensic Sci. Int. 261 (2016b), 106-115.

[61] J.R. Jervis, J.K. Pringle, G. Tuckwell, Time-lapse resistivity surveys over simulated clandestine graves, Forensic Sci. Int. 192 (2009) 7–13.

[62] M.C. Peel, B.L. Finlayson, T.A. McMahon, Updated world map of the Koppen-Geiger climate classification, Hydro. Earth System Sci. 11 (2007) 1633-1644.

[63] L. Swann, S. Forbes, S.W. Lewis, S.W. Observations of the temporal variation in chemical content of decomposition fluid: A preliminary study using pigs as a model system, Aust. J Forensic Sci. 42 (2010) 199-210.

[64] J. Grossmann, P. Udluft, The extraction of soil water by the suction-cup method: a review, J Soil Sci. 42 (1991) 83-93.

[65] P.E. Jackson, 2001. Determination of inorganic ions in drinking water by ion chromatography, Trends Anal. Chem. 20 (2001) 320-329.

[66] E. Gilchrist, E. Jongekrijg, L. Harvey, N. Smith L. Barron, Characterisation of gunshot residue from three ammunition types using suppressed anion exchange chromatography, Forensic Sci. Int. 221 (2012) 50-56.

[67] K. Szomborg, F. Jongekrijg, E. Gilchrist, T. Webb, D. Wood, L. Barron, Residues from low-order energetic materials: The comparative performance of a range of sampling approaches prior to analysis by ion chromatography, Forensic Sci. Int. 233 (2013) 55-62.

[68] K. Quayle, G. Clemens, T.G. Sorribes, H.M. Kinvig, P.G. Stevenson, X.A. Conlan, M.J. Baker, Elemental and molecular profiling of licit, illicit, and niche tobacco, Forensic Sci. Int. 266 (2016) 549-554.

[69] C. Love, E. Gilchrist, N. Smith, L. Barron, Detection of anionic energetic material residues in enhanced fingermarks on porous and non-porous surfaces using ion chromatography, Forensic Sci. Int. 23 (2013) 150-156.

[70] R. Michalski, Ion chromatography as a reference method for determination of inorganic ions in water and wastewater, Critical Rev. Anal. Chem. 36 (2006) 107-127.

[71] R.C. Carpenter, The analysis of some evidential materials by inductively coupled plasma-optical emission spectrometry, Forensic Sci. Int. 27 (1985) 157-163.

[72] E.R. Schenk, J.R. Almirall, Elemental analysis of glass by laser ablation inductively coupled plasma optical emission spectrometry (LA-ICP-OES), Forensic Sci. Int. 217 (2012) 222-228.

[73] T. Lech, ICP OES and CV AAS in determination of mercury in an unusual fatal case of long-term exposure to elemental mercury in a teenager, Forensic Science Int. 237 (2014) e1-e5.

[74] D. Thompson, Rapid production of cyclonic spray chambers for inductively coupled plasma applications using low cost 3D printer technology, J Anal. At. Spectrom. 29 (2014) 2262.

[75] J.A. Aitkenhead-Peterson, C.S. Owings, M.B. Alexander, N. Larison, J.A. Bytheway, J.A. Mapping the lateral extent of human cadaver decomposition with soil chemistry, Forensic Sci. Int. 216 (2012) 127-134.

[76] J.K. Pringle, J.P. Cassella, J.R. Jervis, Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims, Forensic Sci. Int. 198 (2010) 126–133.

[77] S.L. VanlearHoven, G.S. Anderson, Insect succession on buried carrion in two biogeoclimatic zones of British Columbia, J Forensic Sci. 44 (1999) 32-43.

[78] M.C. Shah, P. Shilpkar, S. Sharma, Correlation, regression study on physico-chemical parameters and water quality assessment of ground water of Mansa Taluka in Gujarat, Asian J Chem. 19 (2007) 3449-3454.

[79] R.S. Pindyk, D.L. Rubinfeld, Econometric models and economic forecasts, 4th ed., Irwin McGraw-Hill Coy USA, 1998.

[80] A. Juerges, J.K. Pringle, J.R. Jervis, P. Masters, Comparisons of magnetic and electrical resistivity surveys over simulated clandestine graves in contrasting burial environments, Near Surf. Geophys. 8 (2010) 529-539.

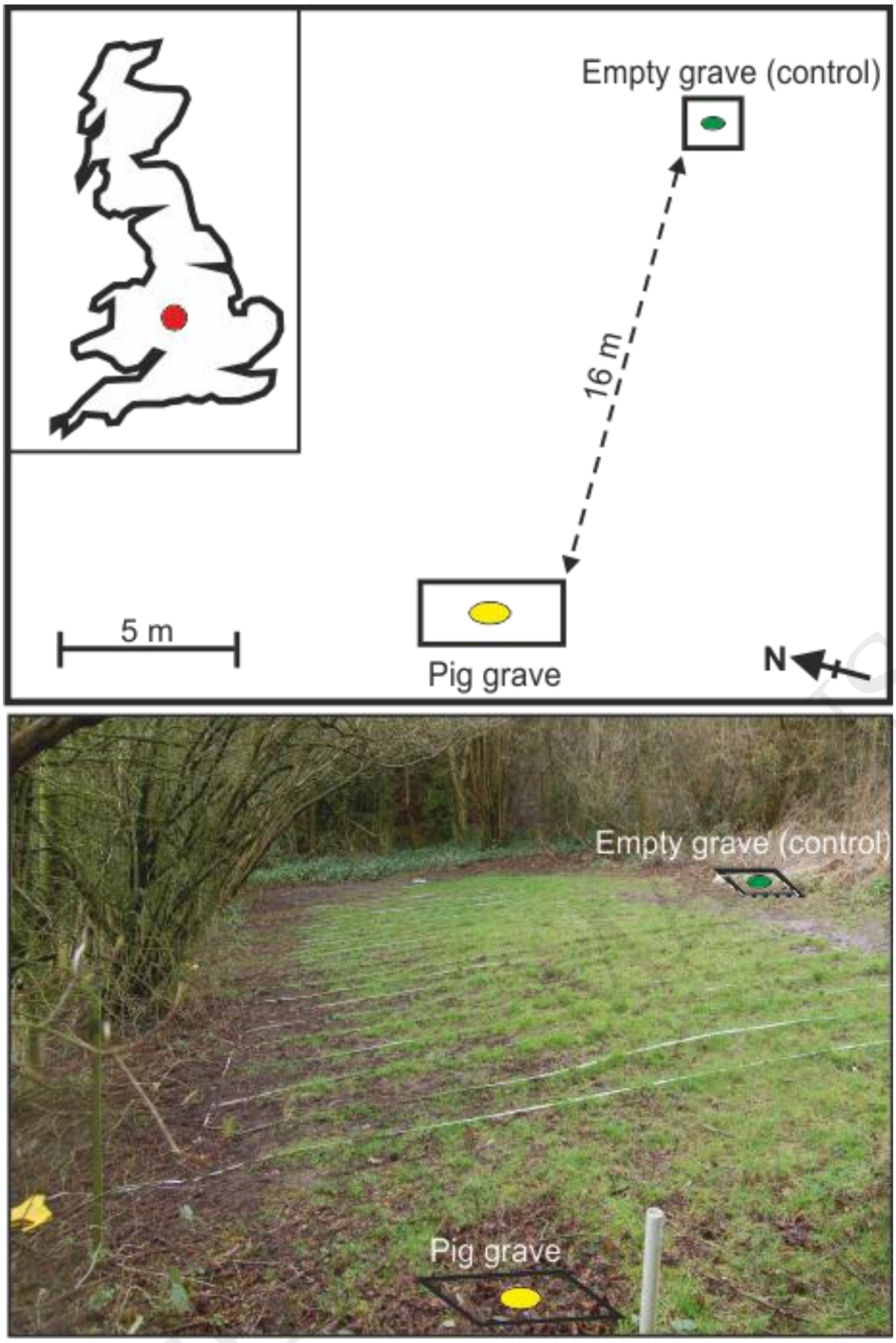

Fig. 1. Schematic diagram (above) and photograph (below) of the study site, showing the surveyed area with pig grave (yellow dot) and control (green dot) positions approximately indicated and location map (inset). Survey tapes indicate area used for the [54-55] Pringle et al., (2012b/2016) long-term geophysical monitoring studies.

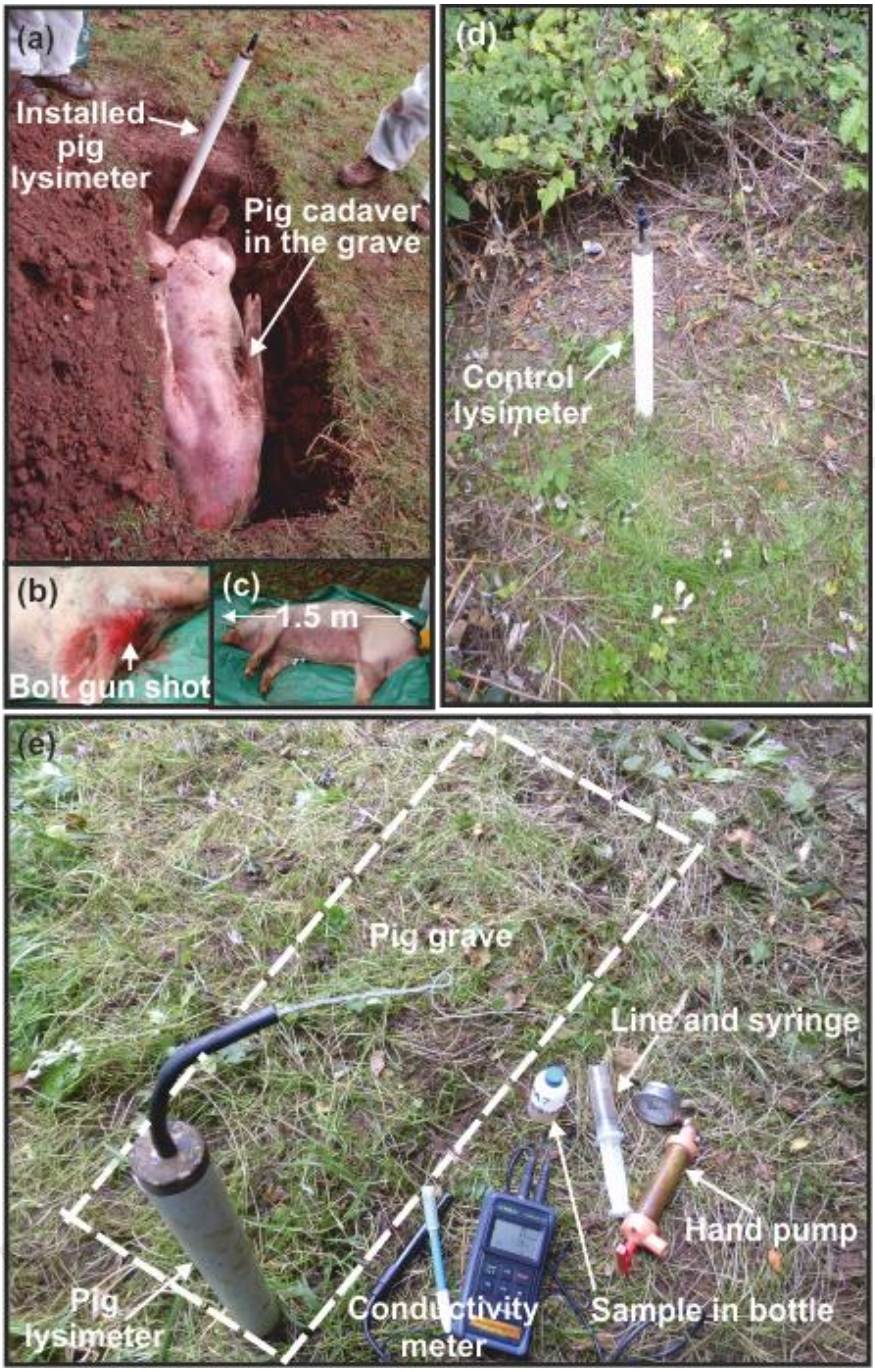

Fig. 2. Study site. **a.** pig lysimeter grave, **b/c.** pig carcass, **d.** control site with lysimeter only and **e.** control soil water measurements with instruments.

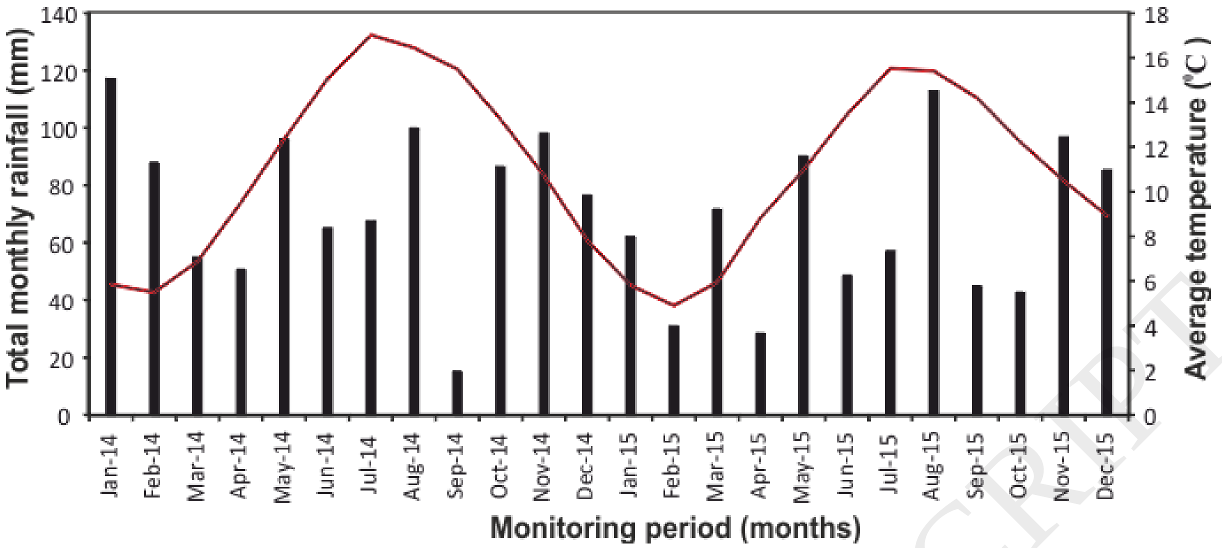

Fig. 3. Graphical climate summary of rainfall (bars) and temperature (line) data from Keele University weather station over the monitoring period.

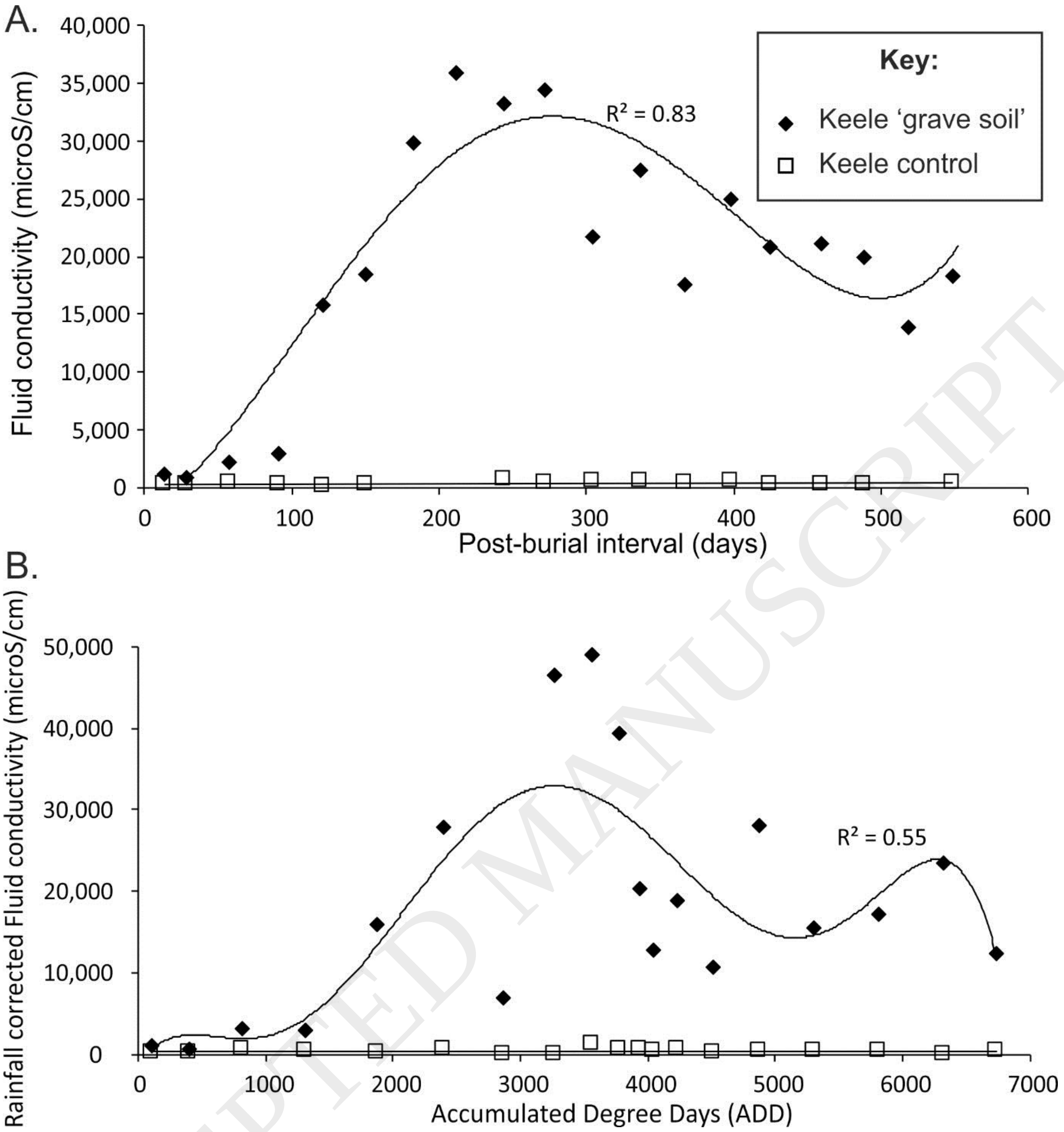

Fig. 4. (A) Measured soil water fluid conductivities over the 18 month (shown as post-burial days) study period, with (B) corrections for temperature (Accumulated Degree Days) and varying monthly rainfall (see text for details and data shown in Table 2).

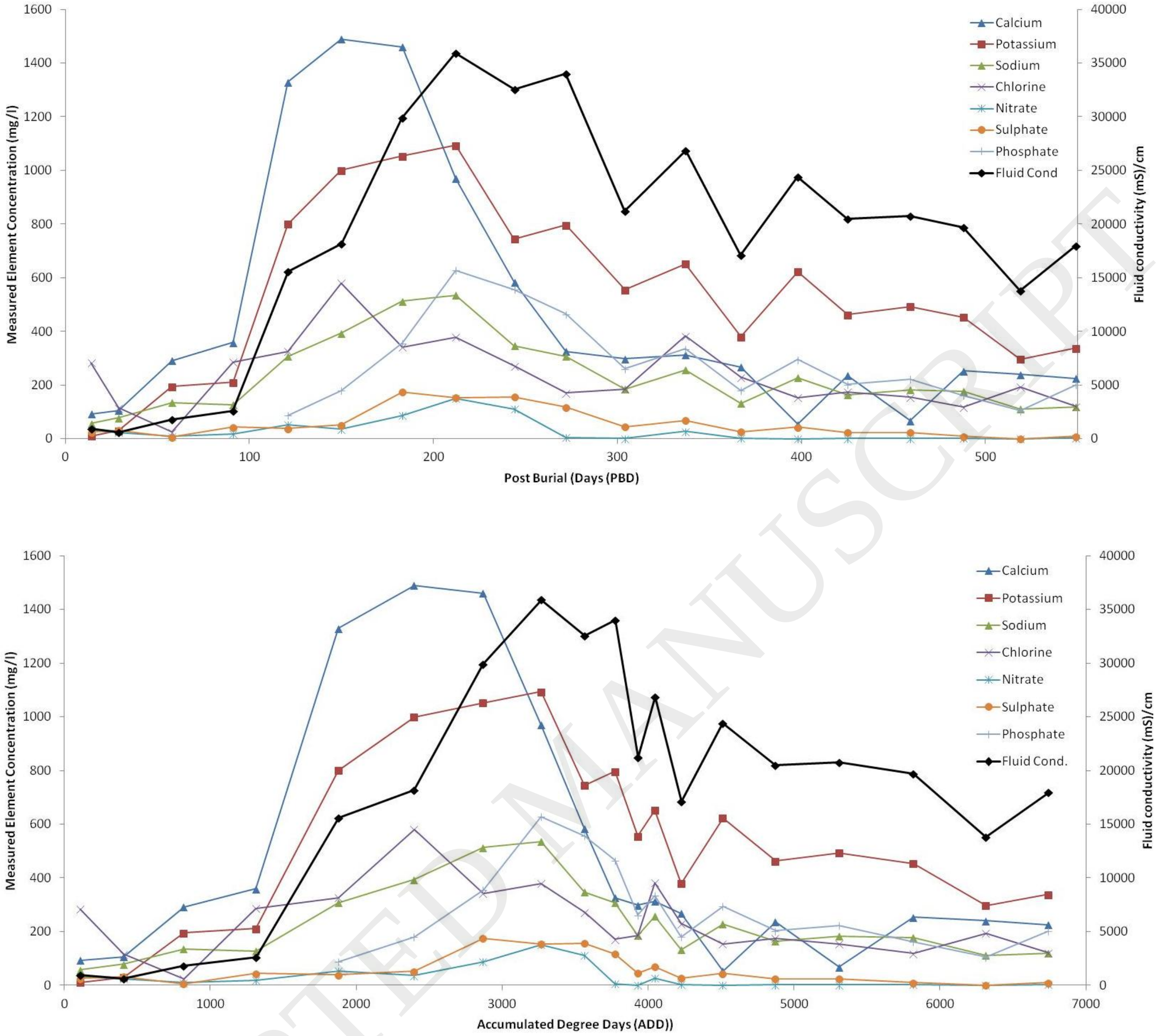

Fig. 5. Combined plots of measured element concentrations elements against: a. Post-Burial Days (PBD) and b. Accumulated Degree Days (ADD), with the control element concentrations subtracted from respective grave element concentrations (see text). Fluid conductivity also plotted (black) on right y axis for comparison.

Table 1. Summary of measured monthly local average temperature and total rainfall data from the study site over the 18 months monitoring period. Stated measurements are averages with ± 0.1 ^{0}C and 0.1 mm accuracy. ADD – Accumulated Degree Days, see [28] for more information. Bgl – below ground level.

Sampling Date	Sample Day After Burial	Monthly ADD 0.3 m bgl	Monthly Average Temperature (^{0}C)			Monthly Total Rainfall (mm)
			0.3 m bgl	1.0 m bgl	Average	
18/03/14	0	0	0	0	0	0
01/04/14	14	111	7	7	7	54.8
16/04/14	29	406	10	9	9.5	50.6
15/05/14	58	818	13	11	12	96
17/06/14	91	1,314	17	14	15.5	65
17/07/14	121	1,878	18	16	17	67.4
15/08/14	150	2,398	17	16	16.5	99.8
17/09/14	183	2,870	16	15	15.5	15
16/10/14	212	3,269	13	14	13.5	86.4
17/11/14	244	3,566	10	12	11	98
15/12/14	272	3,776	7	9	8	76.4
16/01/15	304	3,930	5	7	6	62
18/02/15	337	4,049	4	6	5	30.8
18/03/15	367	4,228	6	6	6	71.6
18/04/15	398	4,513	10	8	9	28.4
15/05/15	425	4,873	12	10	11	90
18/06/15	459	5,309	15	12	13.5	48.6
17/07/15	488	5,819	16	15	15.5	57
18/08/15	519	6,316	16	15	15.5	112.6
18/09/15	549	6,742	14	14	14	44.8

Table 2. On-site measured (± 0.1) *in situ* grave soilwater (leachate) and control soil water samples over the 18 month monitoring period, with temperature (ADD) and rainfall corrections also undertaken (see text). NS = No Sample.

Sampling Date	Accumulated Degree Days (ADD)	Accumulated Degree Days (ADD)	Field-measured Grave Conductivity (µS/cm)	Rainfall-corrected "Grave" Conductivity (µS/cm)	Field-measured Control Conductivity (µS/cm)
01/04/2014	111	111	1,157	878	195
16/04/2014	406	406	816	619	200
15/05/2014	818	818	2,180	3,138	344
17/06/2014	1,314	1,314	2,850	2,778	228
17/07/2014	1,878	1,878	15,740	15,908	162
15/08/2014	2,398	2,398	18,520	27,715	319
17/09/2014	2,870	2,870	29,900	6,725	
16/10/2014	3,269	3,269	35,900	46,511	
17/11/2014	3,566	3,566	33,300	48,935	750
15/12/2014	3,776	3,776	34,400	39,409	396
16/01/2015	3,930	3,930	21,700	20,174	484
18/02/2015	4,049	4,049	27,400	12,655	573
18/03/2015	4,228	4,228	17,530	18,821	425
18/04/2015	4,513	4,513	24,900	10,604	477
15/05/2015	4,873	4,873	20,800	28,071	310
18/06/2015	5,309	5,309	21,100	15,377	316
17/07/2015	5,819	5,819	20,000	17,094	313
18/08/2015	6,316	6,316	13,830	23,351	
18/09/2015	6,742	6,742	18,330	12,314	384

Table 3. Analysed elements from grave soil (Leachate) and control soil water samples measured during the monitoring period (see text). *ND = Not Detected and NS = No Sample*

Sampling Date	Accumulated Degree Days (ADD)	Grave soil water (Leachate) ± 0.1 (mg/l)							Control Soil water ± 0.1 (mg/l)						
		Ca^{2+}	K$^+$	Na$^+$	Cl^{2-}	SO$_4^{2-}$	NO^{3-}	PO$_4^{3-}$	Ca^{2+}	K$^+$	Na$^+$	Cl^{2-}	SO$_4^{2-}$	NO^{3-}	PO$_4^{3-}$
01/04/14	111	171	10.9	57.7	287.1	29.9	36.7	ND	78.3	ND	ND	3.4	4.7	4.7	2.7
16/04/14	406	176	30.2	78.3	119.1	35.4	26.3	ND	69.6	ND	ND	3.3	3.9	2.4	1.7
15/05/14	818	390	195	135	28.1	9.5	12.7	ND	97.8	ND	ND	3.3	3.3	2.8	2.6
17/06/14	1,314	440	211	127	289.0	47.7	23.4	ND	80.6	ND	ND	2.8	3.7	4.6	3.2
17/07/14	1,878	1,390	800	307	328.4	41.3	55.1	90.8	60.5	ND	ND	2.3	3.0	2.1	2.9
15/08/14	2,398	1,620	1,000	394	591.9	55.2	50.8	184.7	130	ND	ND	11.1	3.7	14.8	4.7
17/09/14	2,870	1,540	1,190	513	342.2	175.0	88.1	356.1	NS	NS	NS	NS	NS	NS	NS
16/10/14	3,269	1,050	1,230	536	379.6	153.5	151.6	627.9	NS	NS	NS	NS	NS	NS	NS
17/11/14	3,566	664	882	347	286.3	158.2	132.4	563.2	80.1	13.7	ND	14.2	2.3	20.7	6.2
15/12/14	3,776	409	797	308	175.5	139.3	9.6	469.3	82.4	ND	ND	4.2	21.6	3.7	4.1
16/01/15	3,930	402	559	185	198.3	70.6	2.2	267.5	104	3.58	ND	13.3	24.9	1.2	6.0
18/02/15	4,049	428	652	257	405.9	92.3	28.7	341.0	115	ND	ND	23.4	22.9	1.2	6.2
18/03/15	4,228	354	380	133	235.1	46.3	3.1	183.5	86.7	ND	ND	5.7	19.3	0.8	3.8
18/04/15	4,513	110	623	228	159.9	56.0	2.1	297.7	56.1	ND	ND	6.1	11.8	1.4	2.7
15/05/15	4,873	305	463	164	176.3	35.2	2.1	206.7	69	ND	ND	1.2	10.7	0.8	3.3
18/06/15	5,309	125	498	183	156.3	33.2	1.9	225.3	57.6	ND	ND	1.6	9.5	0.1	3.2
17/07/15	5,819	327	453	177	118.9	19.7	3.2	165.4	73.2	5.54	ND	1.2	10.0	0.1	3.3
18/08/15	6,316	317	297	111	194.1	0	0	105.4	NS	NS	NS	NS	NS	NS	NS
18/09	6,742	30	33	11	12	14.	4.0	20	77	ND	ND	1.2	5.0	0.5	4.

/15	3	8	9	1.6	9		6.1							1
averages	553.7	558.4	229.5	241.8	63.8	33.4	286	82.4	-	-	6.1	10.0	3.9	3.8

Table 4: Correlation coefficients among the different measured grave soil water parameters with those deemed moderately statistically significant (r > 0.525) shown in bold.

parameters	**EC**(*µS/cm*)	**K^+**	**SO_4^{2-}**	**Na^+**	**PO_4^{3-}**	**NO_3^-**	**Cl^-**	**Ca^{2+}**
Fluid conductivity	1.000							
K^+	**0.833**	1.000						
SO_4^{2-}	**0.710**	**0.731**	1.000					
Na^+	**0.750**	**0.953**	**0.846**	1.000				
PO_4^{3-}	**0.947**	**0.761**	**0.808**	**0.749**	1.000			
NO_3^-	0.452	**0.597**	**0.807**	**0.775**	**0.613**	1.000		
Cl^{2-}	0.286	**0.615**	0.446	**0.623**	0.277	0.525	1.000	
Ca^{2+}	0.321	**0.759**	**0.533**	**0.799**	0.263	**0.616**	**0.740**	1.000

NB: Except for fluid Electrical Conductivity (EC), all other parameters are in mg/l

Table 5: Correlation coefficients among the different measured control soil water parameters with those deemed moderately statistically significant (r > 0.525) shown in bold.

parameters	**EC** (*µS/cm*)	**SO_4^{2-}**	**PO_4^{3-}**	**NO_3^-**	**Cl^-**	**Ca^{2+}**
Fluid conductivity	1.000					
SO_4^{2-}	0.393	1.000				
PO_4^{3-}	**0.790**	0.468	1.000			
NO_3^-	0.423	-0.400	0.436	1.000		
Cl^{2-}	**0.706**	0.447	**0.823**	0.380	1.000	
Ca^{2+}	0.279	0.265	**0.596**	0.349	**0.664**	1.000

NB: Except for fluid Electrical Conductivity (EC), all other parameters are in mg/l

Table 6: Quantified statistical correlation between fluid conductivity as the dependent variable and independent elements in grave soil water samples (see text).

Measured elements	Regression equation	R^2	t Value	P Value	F Value
Potassium	79.8 + 0.024EC	0.69	6.199	0.000**	38.428
Sulphate	-9.7 + 0.003EC	0.71	4.161	0.001**	17.315
Sodium	53.1 + 0.009EC	0.56	4.674	0.000**	21.843
Phosphate	-72.1 + 0.016EC	0.90	12.000	0.000**	146.412
Nitrate	-2.7 +0.002EC	0.20	2.090	0.052*	4.369
Chlorate	174.2 + 0.003EC	0.08	1.230	0.235	1.514
Calcium	215.5 + 0.014EC	0.10	1.396	0.181	1.950

***NB**: * indicates significant at 10% level, ** indicates significant at 1% level of confidence*

Table 7: Quantified statistical correlation between fluid conductivity as the dependent variable and independent elements in control soil water samples (see text).

Measured elements	Regression equation	R^2	t Value	P Value	F Value
Sulphate	2.6 + 0.020EC	0.15	1.599	0.132	2.556
Phosphate	1.2 + 0.007EC	0.62	4.817	0.000**	23.207
Nitrate	-1.9 +0.016EC	0.18	1.744	0.103	3.043
Chlorate	-4.5 + 0.029EC	0.50	3.726	0.002**	13.882
Calcium	68.6 + 0.037EC	0.08	1.085	0.296	1.177

***NB**: ** indicates significant at 1% level of confidence*

Appendix1. Operating conditions of the Varian Vista MPX ICP-OEX

Instrument conditions	
Plasma power (kW)	1.0
Plasma gas flow (L min^{-1})	15.0
Aux gas fow (L min^{-1})	1.5
Nebuliser gas flow (L min^{-1})	0.75
Viewing height (mm)	10
Replicates	3
Replicate read time (s)	15
Monitored wavelengths (nm)	
Al	396.152
Ca	422.673
Cu	327.395
Fe	238.204
K	766.491
Mg	279.553
Mn	257.610
Na	589.592
P	213.618
S	181.972
Si	251.611
Sr	215.283

V	389.310