A method for the simultaneous extraction of seven pesticides from soil and sediment

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Organic pesticides are difficult compounds to extract from soils and sediments due to their strong affinity for soil particulates. Different pesticide compounds migrate to varying extents in the environment depending on their chemical structures, with effects ranging from strong adsorption to soil particles to rapid dissolution in water. Published methodologies report procedures for extracting and analysing discrete compounds or chemical classes but there is a lack of previous work concerned with methodologies for the simultaneous extraction and analysis of organic pesticides from different chemical classes. The soil environment is a complex matrix and farmers use a variety of compounds for pest control; with regular crop rotation a diverse mixture of chemicals is likely to be present in arable fields. Mindful of a legacy of pesticide contamination in water and the requirement for management of water resources at catchment scales there is a need to quantitatively assess the storage and transport of a variety of organic pesticides in different phases; soil, sediment and water. This paper describes a methodology for analysing seven different organic pesticides representative of five different classes of pesticide using a single methodology. Prior to use all glassware was deactivated using dimethyldichlorosilane to prevent adsorption effects. Soils then underwent a triple ultrasonication in acetone before being methylated with trimethylsilyldiazomethane. Final extracts were dissolved in hexane and analysed by GC/MS. Recoveries from the soil were determined to range between 70% and 114%.

**Introduction**

It has been estimated that 34% of global crop production can be lost on an annual basis to pests including insects, plant pathogens and weeds.1,2 To prevent crop losses large quantities of pesticides are applied annually to arable land pre- and post-crop emergence. With more than nine hundred active ingredients available for pest control, there is a significant and rising concern about soil and water quality, especially where extraneous compounds persist in the environment for very long periods of time.1,4

The distribution of a pesticide between the particulate and water phase depends upon the soil characteristics, particularly soil type, soil structure, moisture, temperature and availability of oxygen,5 and the chemical structure of the compound along with its half-life, toxicity, aqueous solubility and soil–water partition coefficient.6 These chemical characteristics are also influenced by local environmental conditions.7 At the field or catchment scale the fate of pesticides is directly influenced by land-use management such as crop rotation, tillage and irrigation. Differences in soil type and the potential for pesticides to travel longer distances over time can create a greater spatial distribution of chemicals. Crop rotation is practised to mitigate against persistent pests and to improve soil structure and fertility thereby maximising benefits from the soil. Different pesticides are applied on the varying crops which can lead to a mixture of chemicals in catchment soils. Approximately 500 different pesticide products are available worldwide; comprising a mix of some of the 908 active compounds.8 The seven pesticides selected for this study are frequently used by UK arable farmers and have been reported as causing a contamination threat to raw water supplies.8 This is due to chemical persistence, ability to adsorb to soil particulates and their potential for leaching.9 The pesticides represent five different chemical groups which have not been previously reported as being extracted and analysed simultaneously.

Establishing the links between hydrological processes, soil characteristics, erosion dynamics and pesticide applications, is key to understanding the fate of pesticides, including their chemical alteration, transport mechanisms and storage timescales in soils and sediments. However, there is currently limited understanding of the role of soil- and sediment-bound pesticides in surface and subsurface water contamination over different timescales. Sediments on slopes, floodplains, in streams, lakes or reservoirs can act as a long-term sink for
pesticides\textsuperscript{11,13} which can be released episodically to water courses long after the original application took place. Contaminated soils or sediments have the potential for remobilisation and release of pesticides to water bodies during storm events. Compounds which have a particularly high affinity for solids are found preferentially bound to sediment particles and tend to follow slower and more convoluted pathways from source to sink.\textsuperscript{13,14}

Previous communications describe how to extract and analyse different organic pesticides from water\textsuperscript{15,16} and soil where several reviews are available.\textsuperscript{13,17–19} Techniques of extracting pesticides from soil range from traditional Soxhlet\textsuperscript{20–22} which use large quantities of solvent and are very time consuming to more environmentally friendly methods of ultrasonication,\textsuperscript{22–29} accelerated solvent extraction (ASE),\textsuperscript{29–32} supercritical fluid extraction (SFE),\textsuperscript{30} microwave assisted extraction (MAE)\textsuperscript{20,23,33–35} and subcritical water extraction.\textsuperscript{36} These latter procedures are less time-consuming and require lower volumes of solvent, thereby reducing cost.\textsuperscript{18} The techniques of MAE, ASE and SFE reduce solvent volume and increase efficiency of extraction through enhanced solubility\textsuperscript{19,22} however, specialist instrumentation is required for the extraction and a clean-up step is often required using solid-phase extraction (SPE) or solid-phase microextraction (SPME). An ultrasonic probe has been tested for sonication of soil phase extraction (SPE) or solid-phase microextraction (SPME). Analysis of extracts is for single compounds or a single chemical class. There is a general lack of understanding of the storage and transport of pesticides bound to sediment particles, primarily due to the cost and complexity of simultaneous extraction and analysis of multiple compounds. Analysis of compounds with different functional groups is complex as they may undergo different chemical reactions within the soil/ sediment and during extraction and subsequent analysis. Furthermore, compounds possessing different functional groups may also express diverse physical properties during the extraction process, such as adsorption or evaporation, which may obfuscate the results of any analysis of multiple compounds.

The majority of previous pesticide studies concentrate on pesticides solely in the soluble phase in the water environment, primarily due to the lack of a single technique which has hindered the systematic analysis of sediment-bound pesticides. Herein, a new method developed for the concurrent extraction and analysis of seven different organic pesticides, representative of five distinct chemical classes, from soils and sediments is reported. The method optimizes the use of solvents and reduces the time taken for extraction to ensure an efficient, accurate and precise quantitative analysis. These protocols are unique to previously published methodologies as they incorporate simultaneous extraction followed by analysis using GC/MS for seven key pesticides which have not been previously analysed together. The described protocol is cheap and cost effective, enabling the analysis for large numbers of soil samples where multiple compounds are present.

**Chemicals**

Pesticide standards were purchased as solids, from Sigma-Aldrich with a purity of >98%. Compounds used in the analysis were: 2,4,6,8-tetramethyl-1,3,5,7-tetraoxacenemacetaldehyde \textsuperscript{metaldehyde}, 3-(3-chloro-4-methylphenyl)-1,1-dimethylurea \textsuperscript{chlorotoluron}, 3-(4-isopropylphenyl)-1,1-dimethylurea \textsuperscript{isoproturon}, \textit{(R,S)}-2-(4-chloro-2-methylphenoxy)propanoic acid \textsuperscript{mecoprop}, 4-(4-chloro-2-methylphenox)butanoic acid \textsuperscript{MCPB}, 1-chloro-3-ethylamino-5-isopropylamine-2,4,6-triazine \textsuperscript{atrazine} and 2-chloro-N-(2,6-dimethylphenyl)-N-(1H-pyrazol-1-yl)methacetamide \textsuperscript{metazachlor}. Internal standards \textit{d}_\textsubscript{5}-atrazine and methyl palmitate were purchased from LGC Standards and Sigma-Aldrich, respectively. Stock pesticide standard solutions were prepared at a concentration of 1 mg ml\textsuperscript{−1} using methanol and refrigerated at $<5\degree C$ for no more than one year. All pesticide standards in powder form and trimethylsilyldiazomethane (TMSDAM) were stored at $-20\degree C$. Solvents (methanol, acetone, hexane and toluene) used in the process were HPLC grade and purchased from Rathburn Chemicals Ltd. TMSDAM and dimethylchlorosilane (DMDCS) at 5% in toluene were purchased from Sigma-Aldrich.

**Methodology**

**Experimental preparation**

Prior to extraction the soils were prepared for analysis as follows: wet soil was frozen and dried using a Thermo Scientific Heto PowerDry LL3000 freeze dryer for up to 5 days depending upon soil moisture content. Soil was then ground using a pestle and mortar and passed through a 2 mm sieve to remove stones, vegetation and other extraneous objects. The resulting soil or sediment sample was then stored, frozen, in sealed glass vials prior to extraction. Soil and sediment samples can be stored frozen for up 450 days without major degradation.\textsuperscript{38}

A silylation procedure was undertaken for all glassware used in the experiments to prevent adsorption of compounds during the extraction and methylation processes thereby maximising recovery of all compounds. All glassware was furnace for 4 hours at 450 \degree C and left to cool before being treated. DMDCS in 5% toluene was applied to the glassware (including pipette tips, vials and flasks) for 2 minutes. The glassware was then double rinsed in toluene and then methanol before being left in a fume cupboard to air dry overnight. Tests showed a consistently higher average recovery for all compounds from silylated (81% ± 22) rather than non-silylated (45% ± 3) glassware used in the experiments. The results indicate that a considerable quantity of pesticide is adsorbed to glassware if it is not deactivated before being used.

**Extraction and methylation**

A solvent extraction was undertaken which enables the separation of the bound constituents from the soil particles without chemically altering the target compounds. Previous methods have used a range of individual or mixed solvent systems for extraction including methanol, acetonitrile, acetone and sulfuric acid.\textsuperscript{37,39,40} In the method reported herein, pesticide extraction was carried out by ultrasonication with methanol or
acetone as these solvents have been previously proven to efficiently extract pesticides from soil particles. Approximately 2 g of soil was weighed into a 28 ml silylated vial and 8 ml of HPLC grade solvent added. The vial was then sonicated for 20 minutes. Extracts were separated from soil by centrifugation for 5 minutes at 3000 rpm and the solution removed using a silylated glass pipette and stored in another silylated vial. The ultrasonication step was repeated twice more resulting in a final volume of solution of 24 ml. This was then dried under a gentle stream of nitrogen for a minimum time period and stored ready for analysis.

Ultrasonic extraction uses a lower volume of solvent than Soxhlet extractions and, although it is not as exhaustive a process, it is substantially less costly. Pesticides are also less likely to degrade due to the lower temperatures used. Extraction tests using Soxhlet apparatus and rotary evaporation showed high losses of the more volatile compounds (average loss: metaldehyde 33%, chlorotoluron 30%, mecoprop 18%, atrazine 43%, MCPB 35%, isoproturon 21% and metazachlor 3%). These losses could have occurred due to the high temperatures and long time period of extraction using Soxhlet extractors and, subsequently, rotary evaporation at the end of the procedure to remove large volumes (~250 ml) of solvent. Tests between drying using rotary evaporation and nitrogen blow-down apparatus showed losses were on average 5% less using the nitrogen blow-down. After extraction a test was conducted whereby the samples were subjected to a clean-up procedure using Supelclean™ ENVI-Carb™ SPE tubes. These were used to increase pesticide recovery by avoiding losses using drying techniques alone. The tests showed percentage recovery to range between 76% and 106% which was similar to those not subjected to this procedure (70% to 114% using acetone). In addition, the chlorotoluron was not detected on the GC-MS after the SPE phase. Therefore this was deemed an unnecessary step and abandoned, thereby reducing overall cost and time for analysis.

To quantify compounds and account for changes in the instrument over time and between sample runs an internal standard was used. Initial tests were conducted using a deuterated version of one of the compounds of interest, (d5-atrazine) as this will exhibit almost identical chemical and physical properties to the target analogue. However, GC/MS atrazine) as this will exhibit almost identical chemical and physical properties.

produce a more chromatographically resolved distribution. Previous studies have used diazomethane for methylating acid herbicides prior to analysis but this is explosive. Due to the hazardous nature of diazomethane the safer alternative, TMSDAM was used for this procedure. Addition of the TMSDAM converts the chemical compounds to their methyl esters. 10 µl of TMSDAM was added to a 1 ml mixture of toluene and methanol at a ratio of 4:1. Approximately, 200 µl of the TMSDAM and solvent solution was added to the dry sample and left in a fume hood to react. 1 ml aliquots of standard solution at a concentration of 50 ng µl⁻¹ were left in contact with TMSDAM for periods between 10 minutes and 24 hours. Analysis showed there to be no significant difference (ANOVA F = 7.08, P < 0.05) in percentage of compound recovery and therefore the actual time period for methylation proved unimportant. One hour was used in this study for consistency between experiments. The reaction with the TMSDAM was quenched using a few drops of acetic acid. Solvent and excess TMSDAM were then removed by evaporation under a gentle stream of nitrogen just to the point of complete solvent evaporation then re-dissolved in 50 µl hexane prior to analysis by GC/MS.

Recovery tests were carried out using spiked samples with ultrasonic extraction to validate the method. Reference soil was purchased for recovery testing from LGC Standards certified free from most pesticides (below limit of detection). The reference soil used for recovery testing was classified as a clay loam and has the properties described in Table 1. This soil was selected based on the soil characteristics for a small agricultural catchment in south west England where field based monitoring of pesticides in soil and sediments is taking place. 500 µl of the working solution of the pesticide mixture was applied to the soil being tested to make a concentration of 25 µg g⁻¹ soil (equivalent to 50 ng µl⁻¹ in solution) and left for 24 hours. The extraction, methylation and analysis procedures were then conducted and recoveries established.

**Table 1 Physical properties of the reference soil used in the recovery test**

<table>
<thead>
<tr>
<th>Property</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Clay</td>
<td>10%</td>
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<tr>
<td>Sand</td>
<td>50%</td>
</tr>
<tr>
<td>Silt</td>
<td>40%</td>
</tr>
<tr>
<td>pH</td>
<td>8.4</td>
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<tr>
<td>Organic matter</td>
<td>1.85%</td>
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operated in Selected Ion Monitoring (SIM) mode using the target m/z values as specified in Table 2 and shown in the example chromatogram in Fig. 1. For calibration a working solution of a mixture of all seven pesticides was prepared from the 1 mg ml⁻¹ standard solutions to make a range of different concentrations from 0.5 ng ml⁻¹ to 200 ng ml⁻¹. Analytes were quantified using a linear calibration on an eight point calibration graph. The Limit of Detection (LOD) and Limit of Quantification* varied slightly for each of the compounds (Table 2).

### Results and discussion

Loss tests for the entire chemical extraction process with no soil were conducted using both methanol and acetone to determine whether compounds are lost, chemically altered or degraded during the extraction. The working solution was added to a vial and three extractions were undertaken using the same method as for soil. Some losses for metaldehyde, chlorotoluron and metazachlor were observed during the extraction process, however, the remaining four chemicals isoproturon, mecoprop, MCPB and atrazine showed no losses during the extraction phase (Table 3).

The experiments were replicated eight times using methanol or acetone as the solvent. Atalay and Hwang* showed methanol to be an efficient solvent to extract a high proportion of pesticides, however, it has a low volatility and evaporation of solvent with nitrogen can take more than 8 hours in this case. Acetone exhibits a similar efficiency but is more volatile (boiling point of 56 °C vs. 65 °C for methanol) and evaporates more rapidly when left under nitrogen (~2 hours). Recovery tests indicate acetone extractions to be more consistent with lower variability between replicate samples compared with using methanol (Table 3). Due to previously reported losses and degradation of chlorophenoxy acid compounds during extraction* both single and triple

<table>
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<tr>
<th>Pesticide compound</th>
<th>Methanol extraction recovery (%)</th>
<th>Acetone extraction recovery (%)</th>
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<tr>
<td></td>
<td>Loss tests</td>
<td>Soil recovery</td>
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<tr>
<td>Metaldehyde</td>
<td>79</td>
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<tr>
<td>Chlorotoluron</td>
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<tr>
<td>Isoproturon</td>
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<td>89</td>
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<tr>
<td>Mecoprop</td>
<td>102</td>
<td>96</td>
</tr>
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<td>MCPB</td>
<td>104</td>
<td>95</td>
</tr>
<tr>
<td>Atrazine</td>
<td>105</td>
<td>96</td>
</tr>
<tr>
<td>Metazachlor</td>
<td>70</td>
<td>61</td>
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ultrasonic extractions were compared. Results showed a triple extraction to be approximately 20% more efficient than a single extraction without large pesticide loss. Experimental results are summarised in Table 3. Results showed recovery from soil between 70% and 114%.

This procedure provides a potential means of analysing multiple pesticides from soils and sediments for catchment scale studies where a high throughput of samples is essential. The technique is efficient as it optimises the use of solvent and extraction time increasing the number of samples which can be potentially analysed for any given study. Using these protocols, soil and sediment samples can be extracted, analysed and used to assess storage and transport of pesticide chemicals either laterally or vertically in the soil profile and in sediment bound particles in water through runoff, stream transport and deposition in water bodies.

This technique has been tested for a range of soil samples taken from a small catchment in south west England between 0 and 10 cm depth in arable fields with similar soil properties to those in the reference soil. Chlorotoluron, mecoprop, and metazachlor were found in the soil at concentrations between 400 ng g⁻¹ and 3200 ng g⁻¹ soil. This was consistent with expected results where known arable activity is taking place and pesticides are used on a regular basis. The analysis did not detect any pesticides in the areas of alternative land use, as expected.

Conclusions

The combined use of ultrasonic extraction and GC/MS analysis is a well-established analytical protocol for this type of study. Within the context of this study the additional processes of silylation of glassware and the use of TMSDAM for methylation are new procedures. Tests have shown silylation increases the consistency of recovery from soil and helps reduce chemical loss during each stage of the process. TMSDAM is an effective methylation agent which is easy to use and results in derivatives that yield diagnostic ions of high abundance under electron ionisation conditions thereby aiding the application of SIM-GC/MS for detection and quantification of the targets.

Using the described procedures for preparation, extraction and analysis of pesticide chemicals from soil or sediment will enhance research on commonly used pesticides. The processes enable different compounds from five chemical classes to be extracted and analysed simultaneously. Recoveries from soil ranged between 64% and 114%. A triple sonication was shown to be 20% more efficient than a single extraction at removing pesticides from the soil. The combined use of acetone as a solvent, ultrasonic extraction with silylated glassware and methylation using TMSDAM produces a viable method for chemical extraction of seven different but key pesticide species from soil, maximising efficiency of time and resources.

This paper has described a rapid and accurate analytical technique for use in assessing and monitoring the movement and storage of pesticides in soil and sediments. This technique can be used in combination with established analytical techniques for the soluble phase to gain a more complete understanding of the behaviour and impact of pesticides in the environment. The method reported here enables simultaneous analysis of seven distinct pesticides from particulates throughout a catchment which are applied to a diverse range of crops in different seasons. Utilising this rapid and efficient extraction procedure maximises the number of samples which can be analysed throughout a catchment using a single extraction.

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References