Assessment of the distribution of pesticides on soil particle fractions in simulated irrigation run-off using centrifugal SPLITT fractionation and ELISA

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Abstract

This study was designed to measure the distribution of pesticides within the mobile phase of simulated irrigation run-off water, using centrifugal split-flow thin-channel (SPLITT) fractionation, a novel technique providing a gentle separation of natural sediment and suspended particles. Particular attention is paid to the extraction of pesticide residues for enzyme-linked immunosorbent assay (ELISA) analysis; ELISA was used because of the limited sample size.

Centrifugal SPLITT fractionation combined laminar flow hydrodynamics and centrifugal sedimentation to obtain a continuous binary separation of suspended particles. The non-destructive technique allowed an accurate separation of particles into fractions with divisions at 0.5, 2 and 10 μm, with those above 25 μm being performed by wet sieving. ELISA was used to analyse the concentration of endosulfan and diuron for each fraction generated by the SPLITT technique.

This data can be used to determine the role that particulate fines and colloidal fractions play in the transport of bound organic pollutants within the environment and to examine prospects for remediation on farms.
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1. Introduction

The mechanism of colloid-facilitated transport is not included in most current models for predicting contaminant transport because little information is available on the occurrence and nature of mobile colloids [1]. This study is designed to introduce an analytical approach that can provide quantitative information on the colloidal fractions and their role in pesticide transport.

Classical methods to separate, characterise or concentrate naturally occurring aquatic particles include sieving, filtration, settling, centrifugation, cross-flow ultrafiltration, solid phase extraction (XAD or DEAE resins), electron microscopy, electrostimulated dialysis and electron particle counting [2–5].

The limitations of these methods for isolating or characterising colloids are mainly a result of the

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unique nature of colloids. Colloidal dispersions are stabilised by interactions of different interfacial forces, such as electrostatic repulsion, van der Waals attraction, hydration phenomena, hydrodynamic interactions on particle collision and forces due to adsorbed substances [6].

A superior method utilised for the separation and characterisation of colloids would cause the least impacts to the sample. Given the delicate nature of colloidal aggregates in suspension, it is imperative to used gentle conditions to fractionate the samples. This need includes all stages of the analysis of the colloidal material, e.g. in sample collection, transport, storage, processing and analysis.

One approach giving minimal disturbance in the separation and characterisation of colloids and fine particulate material in water stems from the use of field-flow fractionation (FFF) theory and practice. FFF is a separation technique similar to chromatography being an elution technique using a pump, column, detector and fraction collector [7]. An advantage of FFF is that it can separate a wide range of molecules, macromolecules, supramolecular structures, colloidal particles and larger particles, with very good resolution. Due to this, broad range of application FFF can be ideal for environmental samples which are often composed of variable and non-uniform materials. However, a major drawback associated with FFF is that only a small amount of sample can be processed in a single run, which restricts FFF to use primarily as an analytical tool [8,9].

An extension of FFF theory has led to centrifugal split-flow thin-channel (SPLITT) separation, which is able to separate sediments on a more preparative scale. SPLITT fractionation combines laminar flow hydrodynamics and centrifugal sedimentation to obtain a continuous binary separation of suspended particles. The technique is non-destructive and gives an accurate separation of particles into two fractions at each specified cut-off with a "greater than" and "less than" fraction. The most appropriate range for its use is 25–0.2 μm.

A SPLITT channel has two inlets and two outlets, which are separated by the splitters at either end of the channel [10] (Fig. 1). A sample is fed into the channel via inlet (a) and a carrier solution via inlet (b). The sample feed is compressed by the laminar flow. As in FFF a specific force, centrifugal in the SPLITT fractionation described here, is applied perpendicu-
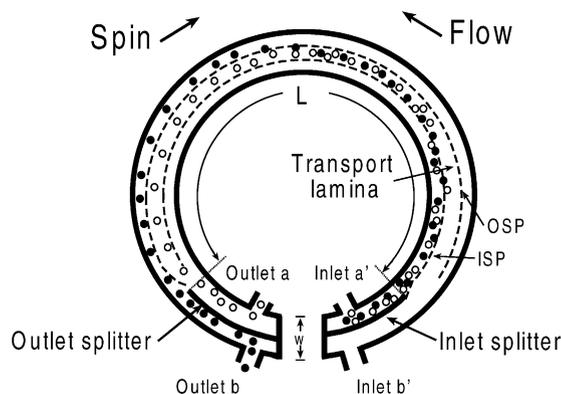

Fig. 1. Schematic diagram (not to scale) of a centrifugal SPLITT cell (after [11]) and the continuous separation of two types of particles according to different sedimentation rates, as a direct result of different interactions with the centrifugal force.

lar to the flow within the channel. Depending on the influence of the field on particles within the sample, movement of particles across the transport laminae occurs. At the outlet-splitter of the channel any material below the outlet splitting plane (OSP), defined by the laminar flow dynamics of the run, will exit from outlet (b). Material not influenced sufficiently by the field will not migrate across the transport region between the inlet-splitting plane (ISP) and the OSP and will exit the channel separately (see Fig. 1). Thus, each run separates the colloidal sample into two fractions, a 'greater than' and 'less than' fraction, with respect to a designated cut-off diameter. The cut-off diameter can be controlled by adjustment of the inlet and outlet flow rates as well as the force applied to the channel [11].

Gravitational SPLITT fractionation (SF) has been used successfully in a number of environmental studies. Water samples (both terrestrial and oceanographic) have been separated using SPLITT and analysed by other methods [12,13]. These studies provided validation of the SPLITT system. The continuing application of centrifugal SPLITT to environmental samples involves the further development of the process and technique as well as combining the technique with other detection and characterisation techniques. The experiment presented here is believed to be the first that involves the use of centrifugal SPLITT to fractionate sediment samples for the analysis of pesticide content by immunoassay.

2. Methods and Materials

This study involved the flow of water over prepared beds of soil with pesticides applied at field rates to produce contaminated run-off. The aim of the experiment was to fractionate this run-off and to analyse each fraction for the concentration endosulfan and diuron, to determine the distribution.

Erosion trays were made of stainless steel for ease of manufacture, durability and resistance to pesticide sorption. The basic design features include: a solid rectangular body (dimensions $L \times W \times H$ being 1 m $\times$ 0.25 m $\times$ 0.25 m) with drainage holes at the 'flume end' to enable collection of any water that drains downwards through the soil profile; a flume at one end protruding from mesh ($\sim$1 cm^2, to help support a furrow structure within the soil) for the collection of irrigation water; and, a mesh support ($\sim$1 mm^2) raised 3 cm from the base of the box. This support is designed to bear the weigh of the soil, estimated roughly at 40 kg (based on a bulk density of 1.1 g cm^{-1}).

Four stainless steel erosion trays were packed with two soil types collected from a field site of Wee Waa, in northern New South Wales, Australia. Two trays contained a 'red dermosol' soil and two trays contained a 'grey vertisol' soil [14]. The soil was packed at field density (1.4 and 1.2 g cm^{-1} for red and grey soil, respectively) and a series of wetting and drying cycles were used to ensure the soil structure mimicked the field soil structure [15].

Diuron (0.033 g) and endosulfan (0.0185 g) were applied to the erosion trays at a concentration similar to the field application rate [16]. A mixture of the endosulfan isomers, alpha and beta, was used in a 3:2 ratio similar to the commercially available mixture. The chemical was dissolved in acetone ($\sim$10 ml) and then water ($\sim$70 ml) and applied directly to the soil, 30 min before the start of the irrigation, using a pump-pressurised garden pesticide spray bottle.

Watering of the erosion trays was achieved by using a system similar to the siphon water delivery system used on most irrigated cotton farms. A container with adjustable height was used to represent the head ditch, which creates the head of water required for siphoning. This container was continually replenished with water from two 225 l drums, via a float valve (0.5 in.), filled with water (pH 7.7).

Samples were collected in 1 l amber bottles and sealed with Teflon lined lids at timed intervals after the first "elution" of irrigation water from the end of the erosion tray. The intervals were at 1, 5, 10 and 30 min after the first sample (T0) was collected. One other sample (G0) completed the set and this sample was taken from the lower drainage tubes.

2.1. SPLITT fractionation

All samples were fractionated within 24 h of collecting the sample. The fractionation process started with two wet-sieving processes, one with a pore-size of 62 μm and a second with a pore size of 25 μm. A sub-sample (120 ml) was taken before and after each sieving process and the residue was collected, dried and weighed.

The remaining liquid, containing a suspension of particles of less than 25 μm, was then delivered to the SPLITT apparatus. Three size cut-off were obtained from this suspension, 0.5, 2 and 10 μm. For each cut-off a "greater than" and "less than" fraction was obtained. Samples bottles were sealed with foil-lined lids and frozen for transport and storage until analysis.

2.2. Specifications of the SPLITT

Channel dimensions of the SPLITT channel and the operational rotation velocities are listed in Table 1.

All residue collected from the sieve was transferred to a clean dry weighed glass container. Samples were dried for 48 h at 120 °C and the mass of the residue was determined by mass difference.

The masses of fractions collected from the SPLITT instrument were estimated by turbidity. A sub-sample (20 ml) of the <25 μm suspension for each sample was diluted sequentially and a turbidity reading taken for each dilution. These samples were then

Table 1
Centrifugal SPLITT run conditions shown together with the flow rates used for all fractionations

Cut-off, d_c (μm)	Rotation of channel (rpm)	Flow rates (ml min^{-1})	
		a$'$	3.01
10	91	b$'$	26.99
2	454	a	26.99
0.5	1816	b	3.01

dried and weighed and a standard curve (mass versus turbidity) plotted. The linear relationship between mass and turbidity was used to determine the mass of each fraction collected based upon a measured turbidity.

2.3. Analysis of endosulfan and diuron by ELISA

Samples collected and generated during the study were analysed for diuron and endosulfan using enzyme-linked immunosorbent assay (ELISA). Immunoassay was deemed to be the method of choice as the samples collected were generally expected to be too low in volume and concentration to be analysed by GC or HPLC. The latter methods usually require half a litre or more of sample, versus a minimum limit of 100 μl for ELISA depending upon the sample concentration.

Tests showed it was advantageous to extract the samples with organic solvent to ensure that all pesticide was removed from the particulate material fractionated by the SPLITT instrument, also eliminating any matrix effects.

Fractionated samples were mixed thoroughly to ensure even dispersion of sediment and a 30 ml aliquot transferred to a glass centrifuge tube. A volume of 1.6 ml nano-grade methanol was added and sample shaken (orbital shaker) for 45 min. Samples were then centrifuged at $1076 \times g$ for 15 min and then 100 μl of the supernatant loaded into immunoassay well for analysis.

2.4. Endosulfan immunoassay

All samples were analysed using an ELISA technique validated for environmental samples in [17]. Optimisation was achieved by determining the best dilution of the enzyme conjugate. The cross reactivity of the endosulfan immunoassay is specifically limited to endosulfan and endosulfan sulphate, as well as other cyclodiene pesticides. As it is uncommon for other cyclodiene pesticides to be applied with endosulfan, the immunoassay is considered as specific for the most toxicologically harmful forms of endosulfan.

Immunoassays were performed as described in [18]. Fig. 2 shows the average of eight standard curve plots for endosulfan analyses. It can be seen that the IC_{50} is around 2.0 ppb, giving a range of 0.5–10 ppb with a limit of detection of 0.2 ppb.
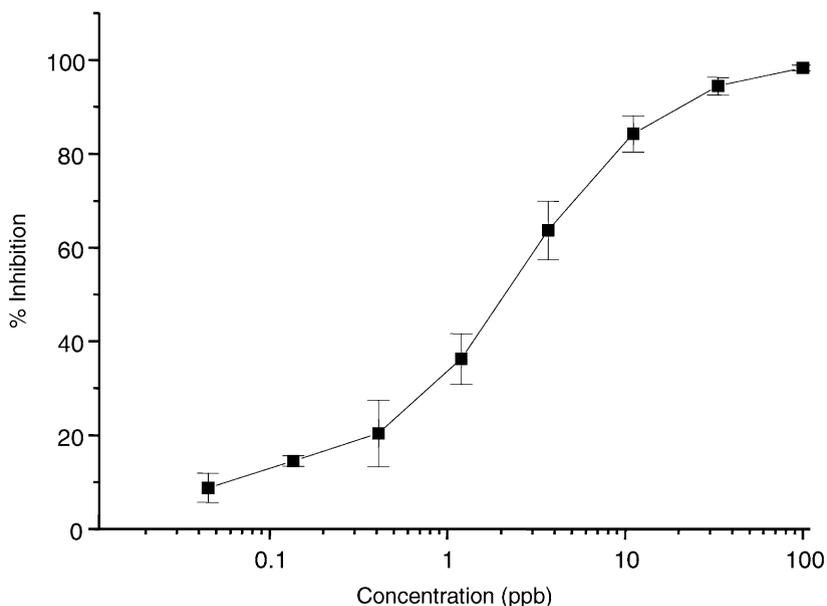

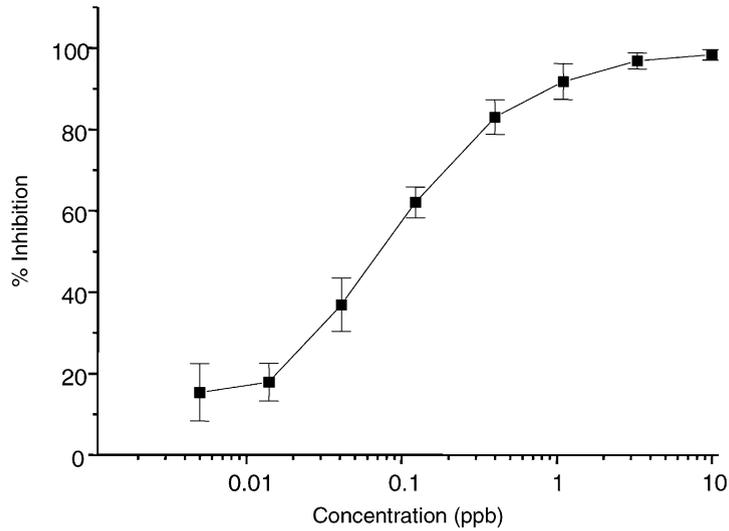Fig. 2. Average endosulfan standard curve of all analyses; standard deviation shown by error bars.

Fig. 3. Average diuron standard curve of all analyses; standard deviation shown by error bars.

2.5. Diuron immunoassay

All samples were analysed using an immunoassay technique described in [19]. Adjusting the concentration of the enzyme conjugate and performing a double layer coating method gave optimal results. Assay specificity is restricted to other phenylurea compounds with neburon and monuron giving similar IC_{50} and IC_{10} to diuron [19]. Fig. 3 shows a plot of the averaged standard curves of all the assays performed in

the experiment. Based on the plot the IC_{50} is 0.08 ppb and the limit of detection is 0.02 ppb.

3. Results and discussion

3.1. Total sediment analysis

In Fig. 4, a plot of the total endosulfan and diuron associated with the total sediment fraction with time in
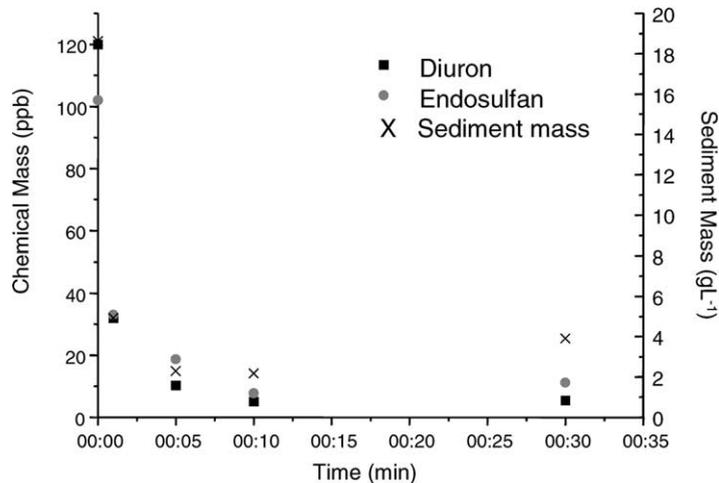

Fig. 4. The total mass of endosulfan and diuron collected in run-off over the period of the irrigation plotted together with the total sediment in each sample.

run-off is shown. Visual observations of turbidity made during the course of the experiment indicated that the amount of suspended sediment decreased markedly during the experiment after the first minute and the data presented in the figure supports this observation.

The first sample collected (1 l), simulating a 'first flush' sample, contained a total of 18.5 g of sediment. After this heavily loaded initial sample the total mass transported fell significantly to 4.9 g l^{-1} collected in the minute after the first flush sample. A steady decline in suspended sediment concentration and associated pesticide concentration was observed throughout the plot.

3.2. Fractionated sediment analysis

The results for the fractionated sediments collected at different times during the laboratory run-off experiment are shown in Table 2. A mass balance on the centrifugal SPLITT fractions was performed to check the accuracy of the procedure. The mass of particles in each cut-off fraction for each sample should add to give the value reported for the 'less than' 25 μm (within error margins). The results in Table 2 show a satisfactory agreement between the addition of fractions and the <25 μm fraction and hence the data is considered to show a good mass balance.

The results for the sieving are clear-cut, the majority of the mass is associated with the large aggregates of sediment in the first sample and the first flush is evident from the sharp drop of a factor of two in the following sample (T1). The smaller particles, those greater than 25 μm and less than 62 μm, fell by a factor of 10. The

Table 3
The mass of diuron (μg) in fractions generated from samples collected over time during the irrigation of the verosol[a]

Sample	T0	T1	T5	T10	T30
Bulk	120	33	10.3	5.0	5.4
<62	92	32	9.2	4.5	3.8
<25	96	14	6.2	–	4.5
SPLITT fractions					
>10	–	1.8	0.9	nd	nd
<10	118	17.8	–	3.0	2.2
>2	38.2	0.8	0.7	2.0	1.2
<2	88.2	9.1	6.0	5.1	1.9
>0.5	41.0	2.3	1.7	2.4	0.6
<0.5	58.8	10.5	7.6	3.1	2.1

[a] nd: not detected.

next sample (T5) then fell by a factor of two in both fractions.

It appears that the mass collected in the greater than 62 μm fraction and that collected for the 25 μm fraction are not related. This is evident further in the final sample collected (T30) where there is an increase in greater than 62 μm fraction and a decrease in greater than 25 μm fraction.

3.3. Fractionated chemical analysis

The results obtained for the analysis of diuron in each fraction following SF are shown in Table 3. It is immediately obvious that a large percentage of the mass of diuron is associated with the dissolved and fine colloidal fraction as the 'less than' fractions consistently show a higher mass.

Table 2
The mass of sediment of each fraction in run-off; three different techniques are used to obtain dataset

Method	Sample	T0	T1	T5	T10	T30
Residue from wet sieve	>62	9.224	4.208	1.955	1.799	3.487
	>25	2.90	0.291	0.119	0.197	0.141
Dried sub-sample	<25	6.37	0.42	0.22	0.12	0.28
SPLITT fractions						
Mass determined from turbidity	>10	–	0.06	0.0048	0.019	0.024
	<10	4.61	0.27	–	0.080	0.12
	>2	3.24	0.19	0.092	0.041	0.083
	<2	1.93	0.13	0.029	0.041	0.072
	>0.5	5.51	0.24	0.098	0.13	0.17
	<0.5	0.27	0.03	0.023	0.023	0.012

The sample collected as flow emerged from the end of the flume (T0) is representative of a first flush sample. It has a different distribution of diuron as compared to the following samples. There appears to be a significant association of about 25% of the diuron with the larger particles, those greater than 62 μm. A two-part explanation for this can explain this result. Firstly, there is a large percentage of large aggregates of soil removed in this first flush (see Table 2), due to the headwater removing all detached soil aggregates. Also, as the aggregated material was found on the surface of the self-mulching soil, these particles are likely to be directly sprayed with pesticide during the application which had little time for subsequent diffusion.

The results for endosulfan detected in the fractions generated from the samples collected during the irrigation can be found in Table 4. The initial similarity to the results detected for diuron is immediately obvious.

It was initially surprising to find that the results for distribution of endosulfan and diuron appeared so similar. Based upon their different physical properties, it was anticipated that diuron would be detected more in the aqueous fractions whereas endosulfan would more likely be associated with organic matter in the sediment fractions. However, based upon the results presented, this conclusion is not validated. A more detailed consideration leads to the conclusion that differences would be more likely to show up in details of the distribution rather than the bulk results.

For example, one of the major characteristics of endosulfan is its volatile nature [20]. It is presumed that this is the main cause for the lack of the detection
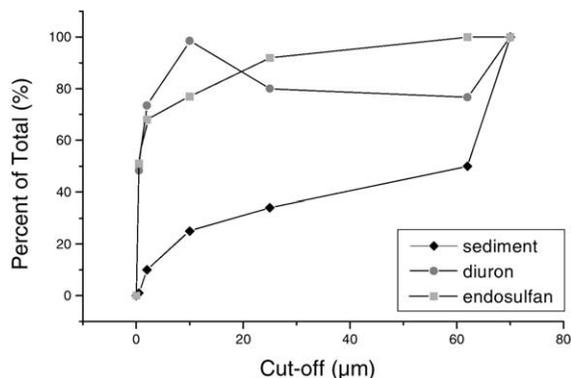

Fig. 5. The cumulative percentage of the total sediment, endosulfan and diuron for each fraction for the initial surface-flow sample (T0).

of endosulfan in section of results for smaller sizes at later times. Based on the diuron results, a detectable concentration would be expected. Greater volatility could have resulted in deeper penetration into the bulk soil, thus, reducing the average concentration in the surface fractions. It is a feature of SPLITT that such detail in the relative behaviour of different chemicals can be examined.

3.4. Percentage total cumulative analysis

To further explore the relationship between the distribution of pesticide and sediment a cumulative analysis is useful. Figs. 5–7 show plots of the percentage cumulative totals for samples collected from surface flow initially and after 1 min and the initial subsurface flow, respectively. The results obtained are interesting for the overall trends rather than values of individual points. Further studies will investigate whether the variation of some data points is due to the analytical methods (e.g. ELISA or irreproducible SPLITT fractionation) or whether the experiment is approaching the limit of sensitivity.

The association of pesticide with the fractions less than 25 μm is clear from the plot given in Fig. 5. This plot shows that 90% of the total endosulfan and about 80% of the total diuron is associated with particle smaller than 25 μm. However, these fractions only accounted for 34% of the total mass of sediment in the sample. In all, 50% of the total sediment mass was contained in the fraction greater than 62 μm and only

Table 4
The mass of endosulfan (μg) in fractions contained in run-off samples collected over time during the irrigation of the vertosol[a]

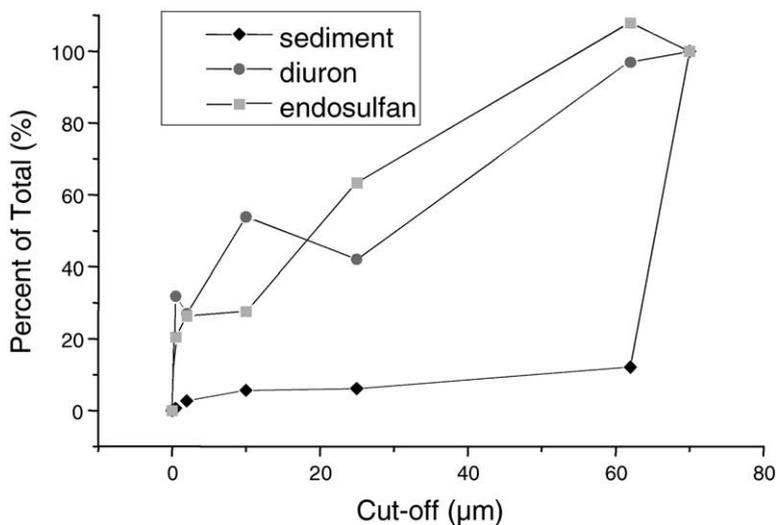| Sample	T0	T1	T5	T10	T30
Bulk	–	33.0	18.7	7.8	11.1
<62	102.4	35.6	18.6	8.7	3.7
<25	93.7	20.9	10.3	6.8	7.0
SPLITT fractions					
>10	–	3.9	0.6	1.1	nd
<10	78.5	9.1	–	3.2	2.6
>2	34.6	1.4	1.6	nd	nd
<2	69.5	8.7	4.1	3.1	3.3
>0.5	45.3	0.8	2.3	1.2	nd
<0.5	52.4	6.8	nd	nd	nd

[a] nd: not detected.

Fig. 6. The cumulative percentage of the total sediment, endosulfan and diuron for each fraction for the sample collected after 1 min (T1) of surface flow.

a small percentage of pesticide is associated with this fraction.

The cumulative analysis of the sample collected after 1 min (see Fig. 6) shows a slightly different trend to Fig. 5 with a similar overall outcome. The majority of sediment, at least 80%, is found in the sediment of greater than 62 μm. This fraction does not account for

more than 10% of the total pesticide concentration. Approximately, 50–60% of total diuron and 63% of total endosulfan is associated with the fractions less than 25 μm.

A very different distribution is observed for the sub-surface sample (Fig. 7) when compared to the previous samples. The trends for sediment and pesticide
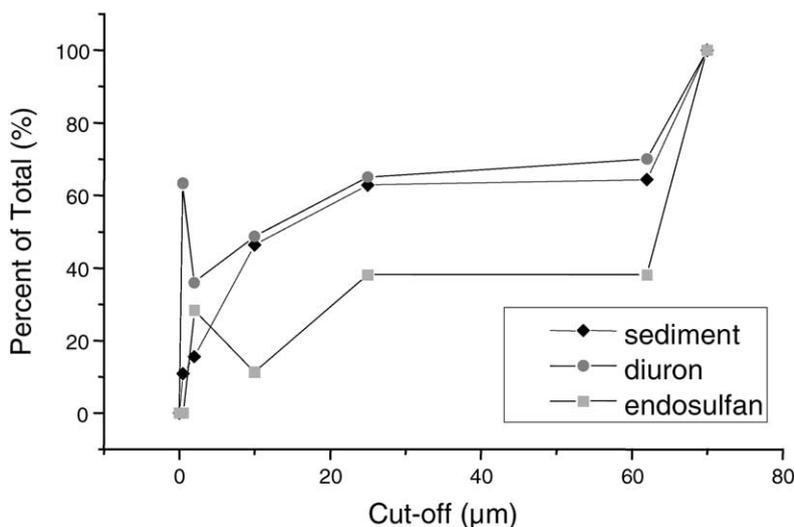

Fig. 7. The cumulative percentage of the total sediment, endosulfan and diuron for each fraction for the initial sub-surface sample (G0).

in the ground water sample (G0) are similar, with 64% of the total mass of sediment collected being less than 62 μm. About 40% of the total endosulfan and 65% of the total diuron is associated with sediments less than 25 μm, this fraction of sediment accounts for 62% of the total mass of sediment.

The trend for sediments in the initial samples collected from the surface (T0) and sub-surface (G0) flow are similar with the exception of the greater than 62 μm fraction. This may be explained by the larger aggregates, collected in the surface flow (T0) and absent from the sub-surface flow (G0), being "filtered" by the soil bed. Yet the remainder of the sediments, those less than 25 μm, are carried with the first flush flow in both cases. It is likely that the proportion of sediment would remain high in the sub-surface samples collected over time due to the large contact that percolating water would have with the soil bed and a relatively limitless source of fine soil.

3.5. Assessing the possibilities for remediation

This study shows clearly that detailed information on chemical distribution can be gained using the combination of SF and ELISA. Usually results obtained when measuring the chemical or nutrient associated with run-off is a report of the totals contained in the whole sample [17] similar to the plot in Fig. 4.

The information obtained gives insight into the distribution of chemical within each fraction. This can be beneficial for determining the best solutions for remediation. Based upon Fig. 4, it may be concluded that the pesticide transported within run-off is mainly associated with the sediment. Therefore, if most of the sediment mass was detained, in a sediment trap or settling tank, it would be expected that the pesticide could also be detained. However, based upon the information given by SPLITT fractionation, 60% of the mass of the sediment is associated with particles greater than 25 μm whereas 80–90% of both endosulfan and diuron is associated with sediments below 25 μm. Therefore, removal of more than half of the sediment mass, in a settling tank where the largest particles settle first, could not significantly reduce the concentration of pesticide in the run-off. Techniques able to remove the finer fractions, such as flocculents, are obviously needed for both these chemicals under the conditions of this study.

The other major advantage of the SPLITT system is the potential for application to field studies. In principle, the SPLITT system may be relatively mobile, making transport possible. The complete system, including pumps, balances, motor and channel can be easily transported within a small trailer (assembled and operational). This allows for in situ field trials, which would eliminate a number of processes involved in fieldwork that can reduce the reliability of collected samples. Researchers from the Department of Chemistry, Water Studies Centre, Monash University, have undertaken some preliminary trials to separate pond and river water in situ.

The results shown here suggest that this system could be used to fractionate run-off water in situ with the chemical analysis being readily used in the field. The immunoassays used in this project have already been rigorously field-tested [18,19]. A greater insight and more real information would be obtained by assessing run-off in situ and this would greatly benefit assessment for the purpose of remediation.

4. Conclusion

It has been shown that using ELISA to analyse SPLITT-fractionated sediment samples for diuron and endosulfan, a greater insight into the mechanism of transport of pesticides can be obtained. SPLITT fractionation has facilitated the collection of sediment fractions on a preparative scale (approximately, 120 ml of suspended sediment is collected in a 30 min run). Although a larger sample is collected using SPLITT as opposed to other FFF techniques, difficulties still remain when trying to assess the concentration of pesticides at field levels. The use of ELISA to analyse SPLITT fractions for pesticides overcomes the problems associated with a limited sample size. This study has shown that the combination of these two relatively new analytical techniques can be complementary. The results obtained are useful for assessing the mechanism of pesticide transport and provide different viewpoints from those normally generated.

The versatility of both of these techniques opens up a broad area of analytical chemistry within the environment. There are many immunoassays becoming available for a wide variety of environmental toxicants [21]. Similarly, SPLITT fractionation is a very

versatile technique that can be used to fractionate many different environmental flows.

Acknowledgements

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