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The Filtering Effects of Various Household Fabrics on the Pollen Content of Hash Oil (Cannabis Extract)

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ABSTRACT: Hash oil samples were analyzed for pollen before and after filtration through 12 different household fabrics, to determine to what extent such samples can be shown to have come from the same source despite having undergone these different treatments. Unfiltered hash oil samples extracted from the same batch of cannabis leaf material showed similar pollen values. An unstirred portion of the extraction solution showed differences in some pollen values to those of stirred samples, suggesting differential rates of pollen settling. However, the presence of some of the same uncommon pollen types in unstirred and stirred samples suggests a common source. Of 12 filter fabrics, ten (a bath towel, two tea towels, a bedsheet, two pillowcases, three stockings and a t-shirt) had a minor effect on the pollen content of the hash oil by slightly reducing the frequencies of some of the larger sized pollen types. Only two of the fabrics had a major effect on the pollen content of the hash oil. The nappy markedly reduced the proportion of the larger sized pollen types resulting in a marked increase in the proportion of some smaller pollen types whereas the calico filtered out virtually all pollen. Illicit hash oil samples recovered from different people or places may therefore in many cases be compared to determine a common source despite samples from the same batch having undergone different filtration treatments and despite differential settling rates of pollen. Also, hash oil samples may be compared to samples of untreated cannabis leaf material to establish a common source.

KEYWORDS: forensic science, pollen analysis, palynology, substance abuse, cannabis, hash oil

The leaves and especially the flowering heads of the plant cannabis (*Cannabis sativa*) have microscopic resin glands. One of the ingredients of this resin is the psychoactive agent tetrahydrocannabinol (THC). Because users prefer to smoke flowering heads, containing higher levels of THC, growers are left with surplus lower grade leaf (disparagingly referred to as "cabbage") and stalk material. This has led to the proliferation of simple hash oil laboratories to process such material into marketable hash oil.

The plant material is soaked in a solvent to extract the resin. Isopropanol is commonly used for this. Extraction usually takes three or four days, although warming the solution can reduce this to one day. To ensure a clean product, the leaf material is either contained within a filter in the solvent or the solution is filtered after extraction. Household items, such as bedding, clothing, and curtains, are commonly used as filters (1). The solvent is then evaporated leaving the dark brown colored extract, commonly known as hash oil. Unlike the more sophisticated procedures used elsewhere, no further processing is undertaken. The oil may be taken in a variety of ways, e.g., by smearing the oil on a cannabis or tobacco cigarette prior to smoking, or by "spotting," i.e., heating the oil on aluminum foil or a knife blade over a flame and inhaling the fumes.

Because of its concentrated form, hash oil is commonly distributed in small containers. Pharmaceutical capsules, for example, are often used [K. Bedford, unpublished data] because of their convenient size and because the two halves make a snug fit to contain the oil.

Law enforcement agencies may seek to determine a link between illicit drug samples found on different people or at different places. Pollen and spore analysis of the samples may provide this link (2–5).

Having a sticky and rough surface due to the presence of resin and microscopic hairs, cannabis leaves and flowering heads are effective pollen traps. Pollen settling on these surfaces may be trapped indefinitely. As the different vegetation of various localities is generally reflected in the local pollen rain (6–8), cannabis plants grown in different areas will usually have different amounts and types of pollen adhering to them. The non cannabis pollen on different samples of cannabis leaves and flowering heads can be compared to determine whether or not the samples were grown in the same location [M. Horrocks, unpublished data]. Hash oil samples could presumably be compared in the same manner to determine whether or not they are from the same extraction.

Recently, an attempt to analyze the pollen in a batch of illicit hash oil in our forensic laboratory resulted in finding virtually no pollen within the samples. The samples were also devoid of other microscopic material, such as leaf hairs and resin glands. This result was surprising considering that abundant pollen is routinely found on samples of cannabis leaf material [M. Horrocks, unpublished data], and that a relatively large quantity of leaf material is required to produce a small amount of hash oil.

A further three different batches of hash oil were then tested. Two of these produced the same negative result but one batch contained abundant pollen, including large-sized pollen types. The most likely explanation for the pollen-free hash oil seemed to be that the filter material used during extraction had removed the pollen along with the leaf material. Also, the pollen in the extraction solution may have settled and the solution may not have been stirred prior to decanting some of the solution into smaller vessels for evaporation. Destruction of the pollen by hash oil extraction processes was ruled out as pollen is extremely durable. For example, treatment with acids as strong as 40% hydrofluoric, which are

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commonly used for extracting pollen from soil, does not adversely affect pollen (9).

The smallest pollen grains measure about 5 μm whereas the largest may measure >200 μm (9). The use of filters with a mesh size falling within or below this range will obviously prevent some pollen from passing through. Another factor may be the amount and length of fine fibers which may snag pollen in the filter material. Pollen grain morphology may also have an affect. For example, many of the pollen types of the daisy family (Asteraceae) have spines which may hinder their passage through filters, whereas the smoother surfaced and spheroidal pollen of the grass family (Poaceae) would be expected to pass more easily through filters.

The following questions are raised when attempting to determine a link between hash oil samples found on different people or at different places: 1) do hash oil samples from the same batch show similar pollen values? 2) if so, how are these values affected by the use of different types of filters during the extraction process? and 3) what are the effects of pollen settling during extraction? The aim of the study was thus to determine whether or not hash oil samples could be shown to have come from the same source, despite having undergone different treatments during manufacture. A batch of hash oil was produced in our laboratory and analyzed for pollen before and after filtering through various household textiles.

Methods

Five hundred grams of cannabis leaf material was soaked in 10 L of isopropanol in a sealed 20 L plastic bucket at 20°C for 12 days. The leaf material was unconstrained within the solution, forming a loose suspension.

A total of 15 samples, each 0.25 L of the solution, was prepared. One of the samples was unfiltered and taken from the top of the clear, undisturbed solution to determine the extent of pollen settling. The solution was then thoroughly stirred and two samples were taken unfiltered from the solution. One of these contained abundant coarse leaf material whereas the other contained very little.

Each of the remaining 12 samples of the solution were then filtered through one of the following materials: Bath towel (cotton), thin tea towel (cotton cloth or napkin used to dry dishes), thick tea towel (cotton), bedsheet (65% cotton, 35% polyester), pillowcase (cotton), flannelette pillowcase (cotton), stocking (sheerness 1) (95% nylon, 5% elastane), stocking (sheerness 3) (95% nylon, 5% elastane), stocking (sheerness 5) (65% cotton, 30% nylon, 5% elastane), t-shirt (cotton), nappy (cotton diaper or baby napkin), and calico (cotton cloth).

The materials were selected as common household fabrics. As there are many different types of such fabrics and as wear changes fabric structure, no attempt was made to determine relative fabric characteristics, such as yarn structure and weave type, and how these might affect filtering. During filtration, the stock solution was constantly agitated to maintain homogeneity. Filtration was not assisted or hindered, the materials were one thickness and rested in a funnel approximately 16 cm wide and 13 cm deep. The sheerness index of the stockings, as given by the manufacturer (Legalong, Hilton Bonds NZ Ltd, Porirua, New Zealand), is on a scale of 1–5. The lower the number, the more sheer or more finely woven the stocking. Only the calico and stockings were new.

To minimize pollen contamination, all of the filtering materials were washed in warm water and detergent, tumble-dried, and stored in separate sealed plastic bags before use. Also, all utensils were rinsed with distilled water immediately before use. Pollen traps showed that airborne pollen contamination in the laboratory during preparation was not significant.

The filtered solutions were evaporated down and the resulting hash oil samples were analyzed for pollen using the standard acetylation method (9). Samples were mounted in glycerine jelly.

In the pollen diagram, the pollen types were assigned to the following three groups: (1) conifers, (2) flowering plants, and (3) ferns and others. The first two groups comprise pollen-producing plants whereas the third comprises plants that produce spores. Spore types are included in the term "pollen types." Common names of pollen types are used where possible. [Scientific names are given in Table 1.] The kinds of pollen with more than one "type" (e.g., daisy types 1–4) are numbered in order of increasing size. The pollen sum, from which the percentages in the pollen diagram are calculated, is shown on the right of the diagram. The pollen sum is comprised of all pollen and spores except cannabis. The software package TILIA (E. Grimm, personal communication) was used to construct the pollen diagram.

Sizes of pollen grains and spores are from Clarke & Jones (10), Lieux (11), Punt & Malotaux (12), Large & Braggins (13), and

TABLE 1—*Scientific names of pollen types in pollen diagram (Fig. 1).*

Kahikatea	*Dacrycarpus dacrydioides*
Macrocarpa type	*Cupressaceae/taxodiaceae*
Matai/miro	*Prumnopitys taxifolium/P. ferruginea*
Pine	*Pinus spp.*
Rimu	*Dacrydium cupressinum*
Tanekaha	*Phyllocladus spp.*
Totara	*Podocarpus spp.*
Beetroot family	*Chenopodium spp.*
Birch	*Betula spp.*
Bulrush	*Typha spp.*
Buttercups	*Ranunculus spp.*
Cabbage tree	*Cordyline spp.*
Carnation family	Caryophyllaceae
Clover	*Trifolium spp.*
Daisy type	Asteraceae
Dandelion type	Cichoreae
Docks	*Rumex spp.*
Grass family	Poaceae
Himalayan honeysuckle	*Leycesteria formosa*
Hinau/pokaka	*Elaeocarpus dentata/E. hookerianus*
Kamahi/towhai	*Weinmannia racemosa/W. silvicola*
Lily family	Liliaceae
Manuka/kanuka	*Leptospermum scoparium/Kunzea ericoides*
Palm family	Arecaceae
Pea family	Apiaceae
Pigeonwood	*Hedycarya arborea*
Plantains	*Plantago spp.*
Pohutukawa/rata	*Metrosideros spp.*
Reeds & sedges	Cyperaceae
Restionads	Restionaceae
Rewarewa	*Knightia excelsa*
Southern beech	*Nothofagus fusca, N. solandri, N. truncata*
Tutu	*Coriaria spp.*
Walnut	*Juglans spp.*
Wattle	*Acacia spp.*
Willow	*Salix spp.*
Wineberry	*Aristotela serrata*
Bracken	*Pteridium esculentum*
Cyathea type 1	*Cyathea dealbata, C. colensoi, C. cunninghamii, C. medullaris*
Cyathea type 2	*Cyathea smithii*
Dicksonia type 1	*Dicksonia lanata, D. squarrosa*
Water fern	*Histiopteris incisa*
Cannabis	*Cannabis sativa*

Moar (14) and are for acetolyzed pollen mounted in glycerine jelly. For non spheroidal pollen types, two measurements are given: That of the narrowest part that would allow the entire grain to pass through a filter, and that of the greatest length or width along one axis. Variable size within a pollen type may be due to that pollen type encompassing more than one species or to normal intra specific morphological variation. Also, immature fern spores are smaller than mature spores (13). Acetolysis affects the size of pollen grains to some extent (9) but this is not considered significant for the purposes of this study.

Results

Sizes of pollen grains and spores in this study ranged from 13–14 by 13–15 μm (hinau/pokaka) to 52–67 by 74–95 μm (pine). Pollen analysis results are shown in Fig. 1. Except for the calico sample, which contained virtually no pollen, cannabis was as expected by far the most abundant pollen type in all samples. Cannabis pollen measures 21–27 by 25–30 μm.

The two stirred, unfiltered samples had generally similar proportions of non cannabis pollen and were dominated by grasses (13 and 14%), rewarewa (9 and 11%), bracken (15 and 16%) and *Cyathea* type 1 (10 and 17%). The unstirred, unfiltered sample showed differences for some pollen types, such as grasses (21%) and *Cyathea* type 1 (6%). The unstirred sample also had approximately 3–5 times more cannabis pollen relative to the other pollen types than the two stirred samples. In addition, the unstirred sample had a higher proportion of *Coprosma* pollen (6.7%) than the two stirred samples (1.5 and 1.9%). Although these differences may partly be the result of a smaller pollen sum for the unstirred sample, higher cannabis and *Coprosma* proportions suggest that these pollen types take longer to settle in fluids (or at least in isopropanol) than some of the other pollen types found in this study.

Of the filtered samples, all except the calico sample (see below) contained abundant pollen. Except for the nappy sample, these samples were dominated by three of the four dominant pollen types present in the two unfiltered, stirred samples, i.e., grasses, rewarewa, and bracken. However, grasses showed higher pollen proportions (19–32%) in the filtered samples, mainly at the expense of *Cyathea* type 1 (1.5–9.0%) and to a lesser extent, pine (down to 0.7–3.7% from 6.3 and 4.5%). Rewarewa (12–20%) and plantain pollen (up to 6.1% from 1.5 and 2.7%) showed marginal increases in proportion. As the pollen values are percentages, any decrease in a relatively abundant type will obviously result in an increase in other types.

The proportional decrease in *Cyathea* type 1 and pine pollen is most likely due to their relatively large sizes. After pine (52–67 by 74–95 μm), *Cyathea* type 1 spores were the next largest pollen type in this study (excluding trace amounts of a few larger types), measuring approximately 35–50 by 50–75 μm. Also, the outer covering (perine) of these spores was often torn and splayed, further increasing spore size and perhaps providing a filter snag. However, perine tearing may have occurred after filtering.

The proportional increase of grass pollen is most likely due to its generally smaller size (approximately 25–40 μm), relatively

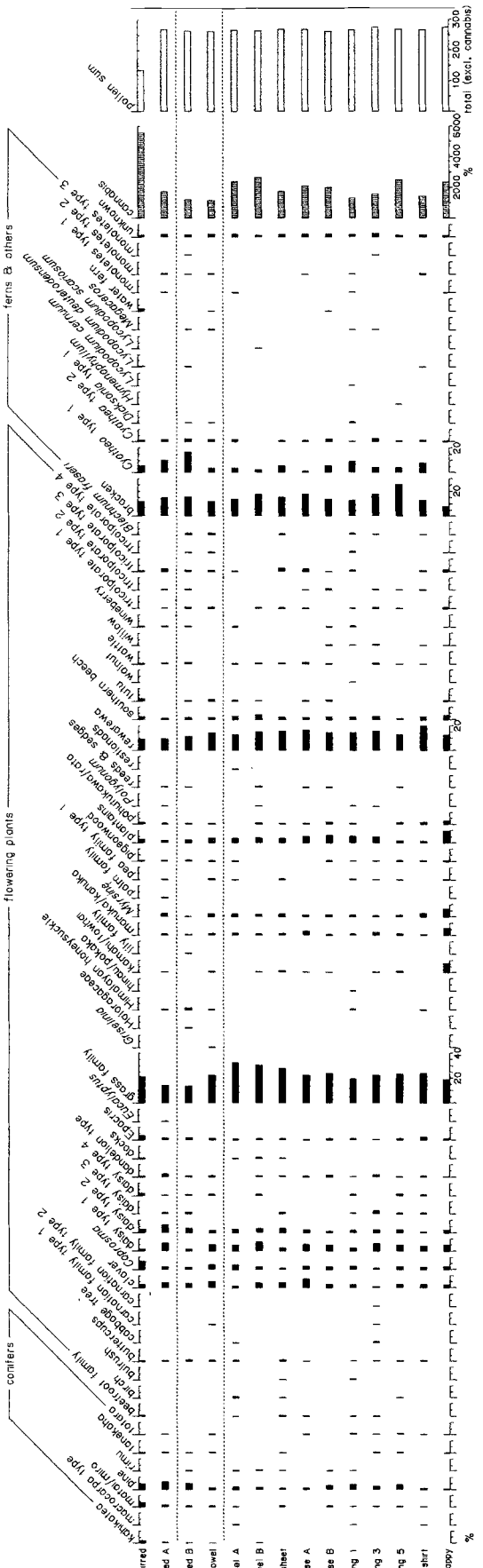

FIG. 1—*Pollen diagram of hash oil samples (stirred A = abundant plant material, stirred B = little plant material, teatowel A = thin tea towel, teatowel B = thick tea towel, pil case A = pillow case, pil case B = flannelette pillow case, stocking numbers = sheerness index).*

smooth surface and spheroidal shape. The same applies to plantain pollen (19–32 μm). Although not spheroidal in shape, rewarewa pollen is of a similar size (25–29 by 40–47 μm) and would likewise be expected to show an increase in relative proportion.

Bracken was the only relatively abundant pollen type not to show a similar general increase in proportion in the filtered samples, despite having a similar size (24–33 by 29–39 μm) to those that did. This is probably due to the tendency of bracken spores to split easily thus increasing their size and perhaps snagging on the filters. As with *Cyathea* type 1 spores, however, splitting could have occurred after filtering.

The nappy sample was also dominated by grass (19%) and rewarewa pollen (12%). However, this sample recorded higher proportions than the other filtered samples for kamahi/towhai (7.7%), manuka/kanuka (6.2%) and plantains (9.9%). These higher values were at the expense of bracken (8%) and *Cyathea* type 1 (zero count) and are due to the relatively small sizes of kamahi/towhai, manuka/kanuka, and plantain pollen. Kamahi/towhai measures 11–16 by 14–18 μm and manuka/kanuka measures 9–14 by 15–22 μm. Unlike the other filtered samples, the nappy sample (and especially the calico sample) were seen to restrict considerably the flow of the solution during filtering.

The calico sample contained virtually no pollen. Other microscopic material, such as cannabis leaf hairs and resin glands, was also not found in this sample. The mesh size of the weave in this fabric is obviously smaller than the smallest pollen grains recorded in this study.

Discussion and Conclusions

Results show that unfiltered hash oil samples extracted from the same batch of cannabis leaf material have similar pollen values. However, an unstirred sample of the extraction solution recorded some different pollen values to the stirred samples, suggesting differential pollen settling. Davis & Brubaker (15) and Pocknall (6) also found evidence for differential pollen flotation and settling rates. Nevertheless, the presence of some of the same rare pollen types in unstirred and stirred samples (e.g., buttercups and pigeonwood) suggests a common source.

Filtration through a wide range of common household fabrics mainly had the effect of slightly reducing the frequencies of the two largest pollen types (pine and *Cyathea* type 1). Fabrics having only a minor effect on the pollen content of hash oil were thus a bath towel, two tea towels, a bedsheet, two pillowcases, three stockings and a t-shirt.

Only the nappy and the calico filters had a major effect on the pollen content of the hash oil. The nappy sample showed a marked increase in the proportion of several smaller sized pollen types at the expense of larger types whereas the calico filtered out virtually all pollen and other microscopic material.

Comparisons of the pollen in illicit hash oil samples recovered from different people or places may therefore be used to determine a common source. This is the case, despite samples from the same batch having undergone different filtration treatments and despite possible differential settling rates of pollen during extraction. In addition, hash oil samples may be compared to samples of untreated cannabis leaf material to establish a link. However, caution should be used when comparing samples. For example, a hash oil sample with fewer larger pollen types may have been filtered with a nappy or may simply have come from an area which did not have a supply of larger pollen types. The degree to which samples share the same rare pollen types will help to determine whether or not samples are from a common source.

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