Journal of Forensic and Legal Medicine 34 (2015) 73–80

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Light microscopy can reveal the consumption of a mixture of psychotropic plant and fungal material in suspicious death

Patricia E.J. Wiltshire [a, b, *], David L. Hawksworth [b, c, d], Kevin J. Edwards [a, e]

[a] Department of Geography and Environment, School of Geosciences, University of Aberdeen, Elphinstone Road, Aberdeen AB24 3UF, UK
[b] Mycology Section, Royal Botanic Gardens, Kew, Surrey TW9 3DS, UK
[c] Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, Madrid 28040, Spain
[d] Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
[e] Department of Archaeology, School of Geosciences, University of Aberdeen, Elphinstone Road, Aberdeen AB24 3UF, UK

ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form
24 May 2015
Accepted 25 May 2015
Available online 4 June 2015

Keywords:
Ayahuasca
Cannabis
Papaver
Psilocybe
Palynology
Mycology

ABSTRACT

Light microscopical examination of plant and fungal remains in the *post mortem* gut may be capable of demonstrating the ingestion of unexpected natural psychotropic materials. This is demonstrated here in a case in which a 'shaman' was accused of causing the death of a young man. The deceased had participated in a ceremony which involved the drinking of ayahuasca in order to induce a psychotropic experience. Ayahuasca is an infusion of *Banisteriopsis caapi* (ayahuasca vine), which produces a monoamine oxidase inhibitor, and one or more additional tropical plants, generally *Psychotria viridis* (chacruna) which produces dimethyltryptamine (DMT). The monoamine oxidase inhibitor prevents DMT from being broken down in the gut, so enabling its passage into the bloodstream and across the blood/brain barrier. Toxicological tests for DMT demonstrated the presence of this compound in the body. The deceased was reported to be in the habit of using *Psilocybe semilanceata* (liberty cap). This fungus (popularly called magic mushroom) contains psilocybin which is hydrolysed in the gut to psilocin; this compound mimics a serotonin uptake inhibitor, and also invokes psychotropic experiences. Microscopical examination established that the ileum and colon contained spores of *Psilocybe* and, in addition, pollen of *Cannabis sativa* and seeds of *Papaver* cf. *somniferum* (opium poppy). Both the plant species yield psychotropic substances. Palynological and mycological analysis of containers from the deceased person's dwelling also yielded abundant trace evidence of pertinent pollen and spores. The police had requested analysis for DMT but there was no screening for other psychotropic substances. Investigators were surprised that a mixture of hallucinogenic materials had been consumed by the deceased. The charge was modified from manslaughter to possession of a 'Class A' drug as the deceased had been consuming psychotropic substances not administered by the 'shaman'. Where death involving drugs from plants or fungi is suspected, microscopical examination of samples from the gut can provide a rapid and effective method for assessing, in a temporal context, the presence of ingested materials that may not have been previously suspected. The example presented here also demonstrates the need for caution in interpreting toxicological results where screening for unusual compounds has been limited.
© 2015 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.

1. Introduction

In cases of suspicious death by poisoning, the presence of one or more toxic substances is tested for by various techniques of chemical analysis of body fluids and other material from the corpse. Initial screening is usually limited to the most commonly-encountered toxins, and the library of reference samples to which the toxicologist has access. Plants and fungi produce a vast array of potentially toxic compounds, and in the absence of special intelligence, the process of identification of specific substances could be prolonged and costly. The light microscope is rarely used where poisoning is involved, but in the case of ingested material, gut contents and faeces can be examined directly, and plant and fungal material can be identified, often to species. This is illustrated here by the death of a healthy young man in south-west England in 2008, where microscopical analysis of gut contents yielded information that might otherwise have been missed; the findings had implications for

* Corresponding author. Department of Geography and Environment, School of Geosciences, University of Aberdeen, Elphinstone Road, Aberdeen AB24 3UF, UK. Tel./fax: +44 1372 272087.
E-mail address: patricia.wiltshire1@btinternet.com (P.E.J. Wiltshire).

http://dx.doi.org/10.1016/j.jflm.2015.05.010
1752-928X/© 2015 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.

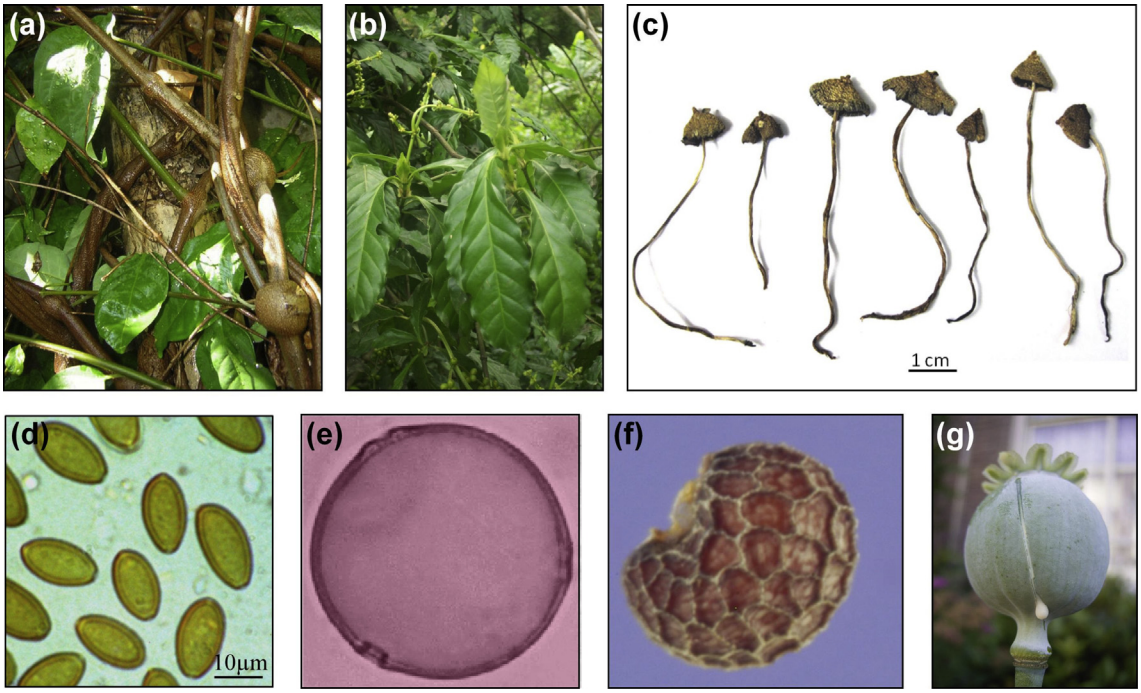

Fig. 1. (a) *Banisteriopsis caapi* (part of climbing stem with leaves; both are used to obtain active substances); (b) *Psychotria viridis* (part of plant showing stem, leaves, flowers, and unripe fruit); (c) *Psilocybe semilanceata* sporophores; (d) *Psilocybe semilanceata* spores; (e) *Cannabis sativa* pollen grain (19 μm in diameter); (f) *Papaver somniferum* seed (longest axis ~0.7 mm); (g) *Papaver somniferum* fruit (capsule): latex-containing opiates oozes from a cut. (a) Courtesy of Rafael Guimarães dos Santos, PhD; (b), (f), and (g) courtesy of Wikipedia Commons; (c), (d), and (e) photographs obtained from reference collections.

criminal charges and sentencing of a defendant. This approach to the detection of psychotropic substances throughout the length of the gut of a corpse, and items associated with the deceased, represents a new application of botany, palynology (the study of palynomorphs, i.e. pollen, many kinds of spore, and other microscopic entities), and mycology (the study of fungi). They were applied to the identification of a fungal sporophore (spore-bearing body), fungal spores, pollen grains, and seeds, in a variety of containers, as well as in the deceased man's gut. The disciplines provide a powerful approach in forensic investigation[1] and, in this case, provided intelligence which affected the final court case and its outcome.

2. Background

2.1. Psychotropic substances

Plants and fungi have been exploited for their psychotropic effects[1] since prehistoric times. A shaman, witch doctor, priest, or 'wise elder' would usually be responsible for dispensing and supervising their consumption, and this tradition continues today. The tropical rainforest has a species-rich and highly diverse flora, with many plant families yielding arrays of complex compounds possessing medicinal and/or psychotropic properties (e.g. mescaline, cardiac glycosides, ergot alkaloids, morphine, tryptamines, cocaine, caffeine, coumarins).[2] Many of the substances provide feelings of well-being and euphoria, and these may contribute to their therapeutic value,[3] while others elicit religious and hallucinogenic experiences, and may have played a role in human history.[4] The tropics are the main sources of psychotropic plants and fungi, but some occur naturally and are imported into, or cultivated in, temperate regions where they are used as 'recreational' drugs.

South American tribes have long been combining infusions of one or more species (or varieties of species) for healing and for inducing hallucinations and religious experiences. They also have extensive knowledge of individual plant species which are used for particular purposes. Depending on the recipe, stem, bark, and leaves of the required plants are cut into appropriate pieces and steeped in boiling water for various lengths of time. The resulting infusions, containing medicinal and/or psychotropic substances, are termed 'ayahuasca'. At least 44 families, 87 genera, more than 113 species of plant, as well as some that have eluded identification, are known to be components of various kinds of ayahuasca.[2] Experienced shamans may use as many as 100 admixtures to obtain their desired and specific effects.[2] Recipes differ, depending on tribe and geographical area, but the stem and bark of the lianes, *Banisteriopsis* species (Malpighiaceae) (Fig. 1a), known as ayahuasca vines, form the basis for all ayahuasca recipes. *Banisteriopsis caapi* is the most commonly used liane, and it contains harmala alkaloids which are powerful monoamine oxidase inhibiting beta-carbolines: harmine, harmaline, and tetrahydroharmine. The former two selectively and reversibly inhibit monoamine oxidase A, and tetrahydroharmine is a serotonin uptake inhibitor. The leaves of *Psychotria viridis* (Rubiaceae) (Fig. 1b),[2,3] 'chacruna', are invariably added when a strong psychotropic experience is desired. The leaves of this liane contain harmine and other beta-carboline alkaloids, but also, significantly, the alkaloid dimethyltryptamine (DMT). The monoamine oxidase inhibitors in *B. caapi* prevent gut enzyme activity, so that any endogenous DMT, or that from *P. viridis*, passes unchanged from the gut into the bloodstream. It can then cross the blood-brain barrier to activate receptor sites and cause hallucinations and other psychotic effects.[5]

Various 'magic mushrooms' have also long been used for inducing hallucinations and religious experiences, and representations of them appear in Palaeolithic cave paintings in Siberia and Algeria; more recently, a pre-Neolithic site in Spain showed that mushrooms were of cultural significance.[6] Species of *Psilocybe* produce the alkaloid psilocybin (4-phosphoryloxy-N, N

dimethyltryptamine), which is hydrolysed to psilocin in the gut. These are serotonin mimics and have similar effects to DMT and other hallucinogenic compounds.[1]

Acetaldehyde, the primary metabolite of ethanol, reacts with endogenous biogenic amines, producing tetrahydroisoquinolines and b-carbolines (tryptolines) which also act as monoamine oxidase inhibitors. Alcohol can thus facilitate the passage of DMT into the blood and, given the widespread consumption of alcohol, this is an important consideration. Further, tobacco smoking is associated with a reduction of monoamine oxidase in the brain and in the rest of the body, and such reduction may prolong and strengthen any psychotropic effects. The combined use of tobacco and *Cannabis* has been reported to induce psychoses,[7] and much is now known of the mode of action, and the effects on neural pathways, of some classes of mind-altering chemicals.[8] There is little information, however, as to potential synergistic and/or antagonist impacts on humans if different naturally occurring drugs are taken in combination. Anecdotal evidence from users is accumulating,[9] and information on the chemistry, activity, effects, and social implications of multiple drug use and abuse is becoming available. There may be synergisms between various compounds, and it is possible that, for the recipient, the concentration of active compounds is more critical than just presence. This paper highlights a potential and previously unreported danger in the consumption of a combination of psychotropic natural substances.

3. The case

Four days before his death, the deceased attended a ceremony organised by a 'shaman' who gave all the participants doses of ayahuasca. The man became highly agitated, hallucinatory, and had to be restrained; the rest of the participants experienced no adverse effects. The man was taken away by friends who stated that he had become comatose and incontinent and, although they had managed to get him to drink orange juice, he had not eaten since the ceremony. Police considered that the shaman could have been responsible for the man's death and took him into custody.

Investigators needed to confirm that the man had ingested the compounds present in ayahuasca and, accordingly, biochemical analysis of gut contents was carried out to establish the presence of DMT. There was also an issue regarding a single mature sporophore of a basidiomycete fungus (Fig. 1c) found in the man's bedroom. Police wished to establish if it were the liberty cap 'magic mushroom' (*Psilocybe semilanceata*) as his friends stated that he was in the habit of drinking infusions of this fungus.

The cause of death was not determined definitively and, at the beginning of the case, the police only suspected that ayahuasca and possibly magic mushrooms might have played a role in his demise. It was suggested to them that palynological analysis of flasks, plastic containers, and drawers from the deceased man's room might give additional information. However, to confirm ingestion of any materials found in them, it was also necessary to examine the contents of his gut.

During the four days after taking ayahuasca, the deceased had defecated into 'nappies', indicating that peristalsis had continued for some time after he had become comatose. It was likely, therefore, that any psychotropic substances (or materials from which these had been derived), solids, and microscopic particulates, present in the digestive tract at the time of the ceremony, would have moved to the lower intestine. Consequently, as well as the routine sample of stomach contents obtained at the *post mortem*, samples of the contents of the ileum, proximal and distal colon, and rectum were requested for microscopical analysis (Fig. 2).

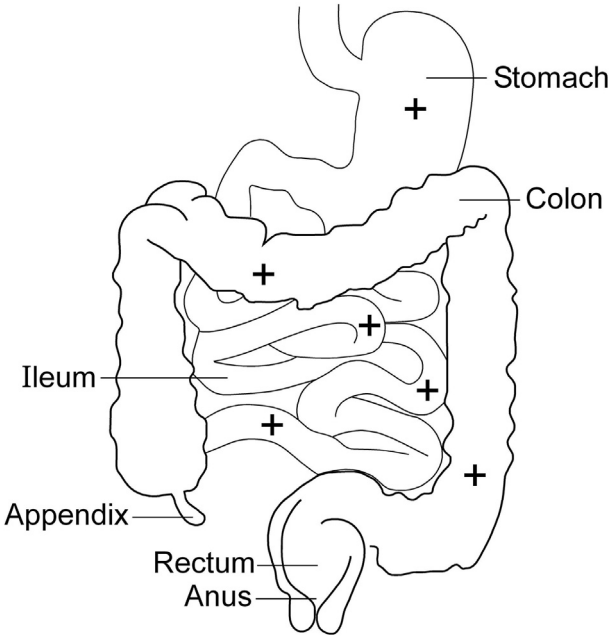

Fig. 2. Schematic representation of the human gut. Crosses indicate the approximate areas from which gut samples were obtained.

4. Laboratory methods

Chemical analysis of stomach contents for DMT was carried out by spectroscopic methods by a third party toxicologist at the request of the police. No toxicological tests for other drugs were requested.

The classes of material obtained from the exhibits and the gut that required description and microscopical analysis were: (a) macroscopic remains, (b) fungal spores, and (c) other palynomorphs.

A record of continuity of possession of items was kept for all the procedures.

4.1. Palynological processing

Standard palynological preparation was used.[10] This involved treatment with sodium hydroxide, hydrochloric acid, glacial acetic acid, acetolysis mixture (acetic anhydride and concentrated sulphuric acid), and hydrofluoric acid. Where appropriate, samples were stained with 0.5% aqueous safranine and permanently mounted in glycerol jelly. Potential sources of contamination were checked by processing reagent blanks alongside samples, and setting bench traps (exposed slides coated with glycerol jelly) at all stages.

4.2. Microscopy and comparison

All macroscopic remains were viewed at ×10 and ×40 magnification, as appropriate, with a Nikon stereo-magnifier. Pollen and other remains were examined, with a Zeiss phase-contrast microscope at ×100, ×400, and ×1000 magnification as necessary. Fungal spores were examined with an Olympus BH-2 microscope, with Normarski differential interference contrast optics at ×600, and ×1500 where appropriate.

Pollen grains and plant spores were identified and counted. The main objective was to determine whether *Psilocybe* spores and *Cannabis* pollen were present and, once this had been established, counting was restricted to approximately 25 transects per slide preparation.

Table 1
Pollen, plant spore, fungal, and other palynomorph counts in various containers obtained from the deceased man's home. Plus signs (+) indicate presence; increasing numbers of plus signs indicate increasing abundance of palynomorphs (subjective assessment).

Contents of sample	Common description	Flasks		Other containers		Drawers
Macroscopic remains		Black	Large	Biscuit tin	Plastic box	Middle & bottom
Plant debris (unidentifiable)	General fragments of tissue	+	+	+	+	+
Angiosperm protoxylem	Young wood cells	+			+	+
Angiosperm fibre bundles	wood elements	+			+	
Angiosperm vessels and tracheids	Wood fragments					+
Epidermal cells	Outer tissue of plant					+
Fibres	Unidentified	+				+
Leaf fragments	Unidentifiable				++	+
Sphagnum leaf	*Sphagnum* moss					+
Trichomes	Plant hairs					+
Fungal spores						
Psilocybe semilanceata	Liberty cap (magic mushroom)		+++++	+++++	++++++	++
Tree/shrub pollen						
Corylus	Hazel					1
Fagus	Beech					1
Picea	spruce					1
Pinus	Pine	1				1
Quercus	Oak					1
Ferns						
Pteropsida monolete	Undifferentiated ferns					1
Pteridium	Bracken					1
Herb pollen						
Cannabis-type	Hemp/hop	++++				2
Aster-type	Daisy, ragwort and others	4				
Apiaceae	Hogweed family	3				
Artemisia	Mugwort	3				
Cerastium-type	Mouse-ear and others	3				
Cerealia-type	Wheat/barley/oats	2				
Papaver	Poppy	2				
Poaceae	Grasses	2				2
Amaranthaceae	Goosefoot family	1				
Ranunculus-type	Buttercups	1				
Rumex	Docks	1				1
Urtica-type	Nettle	1				1
Geranium	Geranium, stork's bill and others					1

The numbers of palynomorphs counted in the 25 transects of each slide are given in Tables 1–3.

Fungal spores were compared with those on microscopic preparations made from reference material of *Psilocybe* species held in the fungarium at the Royal Botanic Gardens Kew, and pertinent literature (Fig. 1d). The fungal spores, and *Cannabis* pollen (Fig. 1e), were given a subjective scale of abundance presented as a series of plus signs (+) in Tables 1–3. A single plus sign indicates either a single occurrence, or relative rarity within the assemblage.[11] Multiple plus signs (e.g. +++) indicate a subjective abundance assessment whenever it was impractical to count individual components of the assemblage because of their large numbers.

The mushroom specimen from the deceased man's bedroom was compared with reference specimens of hallucinogenic *Psilocybe* species maintained in a secure section of the fungarium at the Royal Botanic Gardens Kew, following procedures authorised by the Home Office.

4.3. Containers

All exhibits, except the black flask (which contained liquid), were washed vigorously with a hot (approximately 50 °C) dilute surfactant (Teepol) solution. The washings were sieved through a 180 μm mesh and centrifuged. The liquid in the black flask contained a suspension of small leaf fragments, and an object which resembled a tea bag. The contents of the 'tea-bag' and the liquid contents of the flask were sieved separately and then centrifuged. The pellets resulting from this process were all subjected to standard palynological processing (see below).

4.4. Gut contents

The stomach sample consisted of a yellow, opaque liquid. Boiling with sodium hydroxide cleared the opacity of the fluid and it was then sieved through micromesh (mesh 120 μm). The sieved fluid was centrifuged to obtain a pellet. The samples from the ileum and the colon were treated identically. About 20 ml of hot (approximately 50 °C), dilute surfactant solution was added to each, agitated to disaggregate the solids and smaller particulates, and then sieved through micromesh as above. The sievings were examined microscopically before further processing. After sieving, the liquid part of each sample was centrifuged, and pellets were subjected to standard palynological processing after which standard slide preparations were made from the embedded suspension of palynomorphs. Preparations of the sieved material were photographed where appropriate and the microscope slides retained so that they would be available for examination by defence experts.

5. Results and discussion

The results of the microscopical examinations are shown in Table 1 (containers), Table 2 (gut samples), and Table 3 (summary of colon contents). The mixture of plant debris, pollen, and spores found in the containers provided investigators with a degree of intelligence as to the kind of place from which the *Cannabis* and magic mushroom had been collected. The distribution of plant debris, pollen, and spores in the gut samples afforded some support for the statement, made by the friends of the deceased, that the man remained alive for some days after the ceremony.

Table 2
Pollen, plant spore, fungal, and other palynomorph counts in the deceased man's gut. Plus signs indicate presence; increasing numbers of plus signs (+) indicate increasing abundance of palynomorphs (subjective assessment).

Contents of sample	Common description	Stomach	Ileum			Colon	
Macroscopic remains		Contents	Upper	Middle	Terminal	Proximal	Distal
Parenchyma cells (crushed)	Plant cells, common in many tissues	+++	+	+			
Papaver somniferum seeds	Opium poppy		+			+	++
Citrus sinensis seed	Orange pip						+
Comminuted debris	Unidentifiable plant material		+	+	+	++	+
Lens culinaris cf. testa	Lentil seed coat cf.						+
Plant debris (unidentifiable)	General fragments of tissue					+	+
Black fragments	Unidentifiable				+		
Fungal spores							
Psilocybe semilanceata	Liberty cap (magic mushroom)					+++	++++
Puccinia	Rust fungus (urediniospore)					+	
Smut	Unidentified						+
Tree/shrub pollen							
Rosaceae (*Crataegus*-type)	Hawthorn/rowan					6	2
Rosaceae indet.	Hawthorn/bramble/rose and others					4	30
Acer	Sycamore and maples					1	4
Ligustrum-type	Privet/lilac					1	2
Eucalyptus	Gum					1	
Rosaceae (*Rosa* cf.)	Rose						1
Fraxinus	Ash						1
Quercus	Oak						1
Betula	Birch	1					1
Herb pollen							
Brassicaceae indet.	Many members of rape & cress family					12	9
Vicia-type	Vetches					4	1
Poaceae	Grasses					2	1
Papaver	Poppy					2	2
Geum	Wood avens					2	
Stachys sylvatica-type	Woundwort and others					2	
Boraginaceae (*Borago* cf.)	Borage					1	8
Cerealia-type	Wheat/barley/oats	1				1	5
Ranunculus-type	Buttercups					1	1
Convolvolus	Bindweeds					1	
Melampyrum cf.	Cow-wheat					1	
Cannabis-type	Hemp/hop						13
Trifolium-type	Clovers and trefoils						6
Mentha-type	Mint and others						4
Fabaceae indet.	Pea/bean/bird's foot trefoil and others						2
Geranium	*Geranium*,stork's bill and others						1
Lathyrus-type	Vetchlings and sweet peas						1
Amaranthaceae	Goosefoot family						1
Mercurialis	Dog's mercury						1
Total pollen counted		**2**				**42**	**98**

5.1. Containers

5.1.1. Black flask

This flask held liquid containing fragments of leaf material, and a 'tea bag' which smelled of *Mentha* (mint). It also contained a concentrated suspension of *Cannabis*-type pollen; this was so abundant that no attempt was made at its quantification, and the results are shown as a series of 'plus' signs in Table 1. *Cannabis* pollen is similar to that of *Humulus lupulus* (hop) so that it can only be identified reliably to '*Cannabis*-type'. However, hops are dioecious (with male and female flowers on the different plants), and it is the female that is grown for commercial use and making beverages. Only the male produces pollen, and male hop plants are occasionally found in hedgerows and on garden fences, where they are treated as weeds; they are rarely grown for aesthetic purposes. The likelihood of someone collecting male hop flowers to make an infusion is very low, whereas *Cannabis* is widely used as a narcotic. It is highly likely that the pollen in the black flask was of *Cannabis sativa* rather than hop.

Other pollen retrieved from the container supported the pollen identification as *Cannabis* because no pollen of other hedgerow plants was represented, as would have been likely if the pollen had been of hop. Instead, there was a range of ruderal weeds commonly found on wasteland, verges, field edges, disturbed ground, and neglected gardens, including Apiaceae (hogweed family), *Artemisia* (mugwort), *Rumex* (docks), and *Papaver* (poppy). The assemblage indicated that the *Cannabis* was likely to have been grown in Britain or north-west Europe. The virtual lack of Poaceae (grass) pollen (two grains were found in the black flask and two in the drawers), could further suggest that the plants had been grown in a paved, weedy garden where there was very little, if any, grass, or where the grass was regularly mown so that flowering was prevented.

5.1.2. Large flask

The large flask contained no liquid but washings obtained during palynological processing yielded large numbers of *Psilocybe semilanceata* spores. This suggested that the container had held an infusion of the fungus.

5.1.3. Plastic box and biscuit tin

These contained some leaf fragments, plant fibres, and very large numbers of *Psilocybe semilanceata* spores. No spores of any other fungal species were found in the samples.

Table 3
Summary table of percentages of palynomorphs found in the deceased man's colon. Plant families typically collected by bees (Rosaceae, Brassicaceae, and Fabaceae) are each totalled for convenience. Palynomorphs are expressed as percentages of total palynomorphs found in the colon. Plus signs (+) indicate presence; increasing numbers of plus signs indicate increasing abundance of palynomorphs (subjective assessment).

Macroscopic remains	Description	Colon
Papaver somniferum seeds	Opium poppy	++
Citrus sinensis seed	Orange pip	+
Comminuted debris	Unidentifiable plant material	++
Lens culinaris cf. testa	Lentil seed coat cf.	+
Plant debris (unidentifiable)	General fragments of tissue	+
Taxa favoured by bees		
Rosaceae (total)	Hawthorn/bramble/rose and others	30.7
Brassicaceae indet.	Many members of rape and cress family	15.0
Fabaceae (total)	Vetches, clovers, vetchlings, sweet peas	8.6
Boraginaceae (*Borago* cf.)	Borage	6.4
Ligustrum-type	Privet/lilac	2.1
Stachys sylvatica-type	Woundwort and others	1.4
Geum	Wood avens	1.4
Geranium	*Geranium*, stork's bill and others	+
Melampyrum cf.	Cow-wheat	+
Taxa here associated with drugs		
Psilocybe semilanceata	Liberty cap (magic mushroom)	++++
Cannabis-type	Hemp/hop	9.3
Papaver	Poppy	2.9
Mentha-type	Mint and others	2.9
Adventive taxa		
Cerealia-type	Wheat/barley/oats	4.3
Acer	Sycamore and maples	3.6
Poaceae	Grasses	2.1
Ranunculus-type	Buttercups	1.4
Betula	Birch	+
Eucalyptus	Gum	+
Fraxinus	Ash	+
Quercus	Oak	+
Amaranthaceae	Goosefoot family	+
Convolvolus	Bindweeds	+
Mercurialis	Dog's mercury	+
Total palynomorphs counted		**140**

5.1.4. Drawers

The two drawers yielded various kinds of macroscopic debris, which indicated that they had contained whole plant material at some time. Identifiable leaves of *Sphagnum* moss were amongst the debris. The presence of *Psilocybe semilanceata* spores, and two grains of *Cannabis*-type pollen, suggested that the fungus and the plant (or objects carrying the spores and pollen of these) had been placed in the drawers. The assemblage of other pollen and plant spores indicated that the palyniferous (containing or coated in palynomorphs) materials had probably been collected from at least two different places. One probably had acidic soils, where there were trees in the pollen source area, while the other indicated weedy ground which was either grazed or mown.

5.2. Gut samples

There was no microscopical evidence of *Psychotria viridis* or *Banisteropsis caapi* in the gut samples, but the ingestion of ayahuasca was inferred by the presence of DMT in the toxicological analysis. Table 2 shows the distribution and abundances of all plant debris, pollen, and fungal spores in the gut samples.

5.2.1. Stomach

The yellow, opaque liquid, in which there were floating fat globules, smelled of orange. Clearing the liquid with sodium hydroxide allowed microscopical examination and the results showed only aggregations of collapsed parenchyma (undifferentiated cells, probably from juice vesicles). These findings were consistent with the deceased having ingested either fresh orange, or juice of whole orange, a short period before he died. Single pollen grains of *Betula* (birch) and Cerealia (wheat, barley, or oats) were also found; these could have been residual from previous food having been grown nearby, or present in the fruit juice.

5.2.2. Ileum

The three samples taken from the small intestine were virtually devoid of solid material except for some collapsed parenchymatous tissue, and a single seed of *Papaver somniferum* (opium poppy) (Fig. 1f) in the mid-ileum. This suggests that most of the pollen, spores, and other organic material, had moved down to the colon, and that the tissue and seed were residual in the mucosa.

5.2.3. Colon

The colon samples contained much comminuted plant debris; a single testa of cf. *Lens culinaris* (lentil); a single seed of *Citrus sinensis* (orange); fairly abundant *Papaver somniferum* seeds, and abundant microscopic fragments of fibres, protoxylem elements (first formed wood in a stem), and other plant debris. The results of the colon analyses are provided in Table 3. The abundance of *Papaver somniferum* (opium poppy) seeds was surprising. The seeds contain a small but detectable amount of active alkaloid, but most is contained in the latex within the tissues of the seed capsule (Fig. 1g). The plant is a rich source of benzylisoquinoline alkaloid narcotics which affect the central nervous system, and which include morphine (and its derivative heroin), thebaine, codeine, papaverine, and noscapine.[12]

When the plant tissue is cut, the latex containing these compounds is easily drawn-off for processing or consumption. Opium poppy seeds are commonly dusted onto bread rolls, but it was stated that he had not eaten any solids during the four days before his death, and this was supported by the absence of any substantial bulk material in the colon. In any case, bread rolls would seem to be an unlikely choice of food for a comatose person. It is also conceivable that the seeds in the gut had been ingested directly from *Papaver somniferum* seed capsules.

Pollen of *Papaver* was also found, and this may have been opium poppy pollen which had been residual on the seed capsule. It is not possible to differentiate the pollen of poppy species by light microscopy, and it is feasible that the poppy pollen, found in the black flask and colon, was not from opium poppy but from a ruderal (plant of waste ground) species. In that case, it might be part of the same assemblage as other weedy species detected, and originated from the places where the opium poppy, *Cannabis*, and *Psilocybe* were collected.

In Table 3 the palynomorphs are grouped to indicate their relevance. The most abundant taxa retrieved from the colon were of Rosaceae (includes rose, hawthorn, rose, blackthorn, and others), and these were combined for ease of comparison. All taxa of Brassicaceae (rape family; includes cress, rape, and charlock), and Fabaceae (pea, bean, and vetch family; includes clover, vetches, vetchlings, sweet pea, and bird's foot trefoil) were also each combined. These two taxa contain species which are attractive to bees and so they were grouped with other plants that are also highly favoured by bees: *Borago* cf. (borage); *Stachys sylvatica*-type (hedge woundwort and others); and *Ligustrum*-type (privet/lilac), *Geranium* (geranium, storks bill and others), and *Melampyrum* (cow-wheat). Because bees often visit these plants preferentially, their pollen is often highly abundant in honey in the UK.

Psychotria viridis is very bitter, and honey is sometimes added to reduce the bitterness. Since ancient times, honey has been added to sweeten such herbal remedies, and similar palynological profiles have been obtained from medicine administered by Iron Age 'doctors'.[13] Honey is usually heavily laden with pollen, and the pollen assemblage of anything sweetened with honey will be biased towards 'bee' plant pollen. These 'bee' plants could also be contaminated with pollen falling on them from other sources, and bees can inadvertently pick up adventive pollen (pollen which arrives at a site, or on a surface, fortuitously). It is not surprising, therefore, that a wide range of pollen taxa could be retrieved from such a food in the human gut. The pollen profile in this case suggests that the deceased had eaten honey at least four days before he died, and perhaps as an ingredient of the ayahuasca.

Cannabis accounted for 9.3% of the total pollen found in the colon. If the deceased had taken the drink before attending the shamanistic ceremony, the bulk of the pollen might have already been passed with faeces while he was in a coma. *Mentha*-type amounted to 2.9% of the total pollen, and *Stachys*-type was also present. The family of plants to which these belong (Lamiaceae) are also 'bee' plants, and some *Mentha*-type could have come from the honey. However, as there was a smell of mint in the black flask (along with a 'tea-bag'), it is probable that the pollen had come from a suspension of *Cannabis* in an infusion of mint tea.

The other pollen taxa detected could have been introduced into the gut as adventives or in food. Some were of common weeds and occur naturally in the British Isles, and may well represent plants growing near the sites of collection of the mushroom or where the *Cannabis* was grown. Little pollen of Poaceae (grasses) was found in any of the samples, and *Psilocybe semilanceata* sporophores are generally collected from long-established short turf (such as lawns or mown or grazed grasslands) where grass flowering is reduced. Communities of tall, ruderal herbs (plants of disturbed ground) also often inhibit the ready dispersal of grass pollen. The paucity of grass pollen may also be because commercial kits are marketed for magic mushroom cultivation, or the *Cannabis* may have been grown in a weedy garden. In any event, except for the *Cannabis*, and probable associated plant taxa (which were not otherwise known to be involved in this case), the pollen assemblage from the gut was consistent with the testimony of witnesses.

Even though the deceased was known to have passed faeces before he died, microscopic analysis revealed an abundance of *Psilocybe semilanceata* spores in both colon samples, showing that not all the material ingested before (or possibly shortly after) the shamanistic ceremony had been expelled from the gut.

5.3. Cause of death

The cause of death in this case was inconclusive. Police officers had assumed that the drinking of the ayahuasca infusion was responsible; it was known that the deceased man had taken it, and DMT was found in his gut. However, other participants in the ceremony had consumed similar amounts to the dead man and had exhibited no ill effects. The police found a whole mushroom in the man's room and, when it was confirmed to be *Psilocybe semilanceata*, they requested analysis of all the various containers. The plastic box, biscuit tin, large flask, and the drawers all contained spores of the fungus, but investigators were surprised when *Cannabis* pollen was also found in the drawers and the black flask (Table 1).

The mere possession of psychotropic material is not evidence of consumption and this needed to be confirmed by analysis of the gut. It was suggested to the police that, considering the deceased was reported to have lived for four days after the ceremony, particular attention needed to be paid to the lower part of the digestive system. If the faeces and the contents of the rectum had been available, palynological and mycological analyses would have provided evidence of any solids and suspended material that had been eaten prior to the ceremony. The presence of orange juice in the stomach confirmed the friends' statement that they had fed him the juice after the ceremony. Continued gut function, for possibly several days, was evidenced by the absence of solid food in the stomach and ileum, the presence of a citrus seed in the colon (presumably from orange juice given to him after the ceremony), and the concentration of pollen, spores, and seeds in the colon. Again, this was confirmation that the man had lived for some period after taking ayahuasca.

The abundant magic mushroom spores, *Cannabis* pollen, and opium poppy suggested that the psychotropic substances from all these were likely to have been in the man's body at his death. Presumably, he would have been simultaneously affected by DMT from the ayahuasca, psilocin from the magic mushroom, cannabinoids from the *Cannabis*, and opioids from the poppy. Consumption of each of these individually can have pleasant and even positive effects and, amongst certain user-groups, it is also common to combine experimentally various psychotropic compounds to induce particular and specific experiences. However, DMT can cause death if the dosage is too high[1,4]; and, where herbal preparations are made, the concentrations of the active compounds are not easily quantified or controlled. The preparatory techniques for ayahuasca are crude and, as some individual plants and varieties have higher yields of active compounds than others, it would be impossible to control their concentration in any particular batch. Furthermore, as noted above, the effects may be exacerbated by consumption of alcohol and inhalation of tobacco smoke. It is unlikely that most users of 'recreational drugs' realise the possible harmful synergies between the various substances they use.

6. Conclusions

There are factors which should be kept in mind where natural substance abuse is suspected to be involved in illness or death. In particular, they demonstrate the kinds of unexpected information that can be gleaned from mycological and palynological analyses.

1. Chemical analysis is not the only method that can be useful in identifying material which contains psychotropic substances. Botanical, mycological, and palynological examination of macroscopic and microscopic organic materials and particulates can provide valuable intelligence for many kinds of investigation. Such analyses can be carried out relatively quickly and cost-effectively, and can demonstrate the potential presence of compounds which are otherwise unsuspected; these can then be investigated toxicologically.
2. Seemingly unpromising objects can yield powerful trace evidence that can have rich information content. In this case, even when empty, analysis of flasks, a tin, a plastic box, and some drawers showed that the deceased had possessed an illicit plant and fungus. The pollen and spore profiles of the flasks demonstrated the way in which the deceased had prepared and consumed the substances, and trace evidence from other plants found in the containers gave information on the habitats from which the plants and fungi had been collected. Reconstruction of locations from palynological analysis has been shown to be successful for locating human remains and other items[14]. Even with very few pollen grains, it was possible to make some inference about the kind of place where the *Psilocybe* mushrooms were collected and/or where *Cannabis* was grown. A great deal of pertinent information can be generated by these methods, even without exhaustive analyses of numerous samples. In other investigations, fuller analysis and consequent construction of more comprehensive profiles has been used to locate sites.[15] The approach

used here merits more attention in tracing the places of origin or cultivation of illegal plants and fungi.

3. As the deceased was the only person who suffered ill effects after consumption of ayahuasca at this particular event, the simultaneous presence of other psychotropic substances may well have been a contributory factor in his death. Screening for a range of substances, and a more comprehensive toxicological analysis than usual, may need to be requested in future cases of such unexpected deaths. This problem in toxicology has long been recognised – for example in 1916 by Agatha Christie. The author, who trained in pharmacy, describes a case in which analysts were requested to report on the presence of strychnine where foul play was suspected, and narrates how a compound that had failed to be considered proved to be the fatal one.[16]
4. The immediate *pre-* and *post-mortem* history of a deceased person, including when they last ate, should be obtained so that appropriate parts of the digestive tract are sampled. If a deceased person had lived for any period after a drug-taking session, sampling of the lower gut (ileum and various parts of the colon and rectum), in addition to the standard collection of stomach contents, is advisable.
5. Plant material contains unpredictable concentrations of mind-altering substances, and the mass of stem and leaf of *Banisteriopsis caapi* and *Psychotria viridis* (and others) is calculated crudely during preparation of the mixture. If a number of psychotropic substances were present in a person's system simultaneously, synergy between compounds and possible high concentrations could be lethal.

In summary, microscopical analysis can show that a range of plant materials which produce mind-altering substances are present in the digestive system of a corpse. In the case exemplified here, none of the plant and fungal materials would have been ingested in regulated doses, and combined with an unknown concentration of DMT they may have been injurious. Police originally considered the shaman to be responsible for the man's death. However, the deceased had independently consumed large numbers of *Psilocybe* spores and *Cannabis* pollen, before taking ayahuasca, and the accusation made by the police became unsound. In consequence, the shaman did not stand trial for manslaughter, and instead was convicted for the possession of a Class A drug. On appeal, he received a 15 months custodial sentence.

Conflict of interest

There are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the preparation of this article.

Ethical approval

No invasive studies were carried out by us. The Coroner concerned granted permission to publish the results presented here. Names and places have been anonymised at the Coroner's request.

Acknowledgements

We are indebted to the Coroner for granting permission to publish the results of this investigation, and to Dr Begoña Aguirre-Hudson for enabling the examination and photography of material in the neurotropic *Psilocybe* reference collections held at the Royal Botanic Gardens Kew, under conditions permitted by the Home Office regulations in force. Audrey Innes and Alison Sandison are thanked for assistance with laboratory processing and artwork respectively, and we thank anonymous referees for their comments.

References

1. Wiltshire PEJ, Hawksworth DL, Webb JA, Edwards KJ. Palynology and mycology provide separate classes of probative evidence from the same forensic samples: a rape case from southern England. *Forensic Sci Int* 2014;**244**:186–95.
2. Rätsch C. *The encyclopaedia of psychoactive plants: ethnopharmacology and its applications*. Rochester, Vermont: Park Street Press; 1998.
3. Wink M, van Wyk B-E. *Mind-altering and poisonous plants of the world*. London: Timber Press; 2008. Portland.
4. Stamets P. *Psilocybin mushrooms of the world. An identification guide*. Berkeley: Ten Speed Press; 1996.
5. Sklerov J, Levine B, Moore KA, King T, Fowler D. A fatal intoxication following the ingestion of 5-methoxy-N, N-Dimethyltryptamine in an ayahuasca preparation. *J Anal Toxicol* 2005;**29**:838–41.
6. Akers BP, Ruiz JF, Piper A, Ruck CAP. A prehistoric mural in Spain depicting pyschotropic *Psilocybe* mushroom. *Econ Bot* 2011;**65**(2):121–8.
7. Gastel van WA, MacCabe JH, Schubart CD, Vreeker A, Tempelaar W, Kahn RS, et al. Cigarette smoking, and cannabis use are equally strongly associated with psychotic-like experiences: a cross-sectional study in 1,929 young adults. *Psych Med* 2013;**43**:2393–401.
8. McKenna DJ, Callaway JC, Grob CS. The scientific investigation of ayahuasca: a review of past and current research. *Heffter Rev Psychodelic Res* 1998;**1**:65–76.
9. Gable RS. Risk assessment of ritual use of oral DMT and harmala alkaloids. *Addiction* 2007;**102**:24–34.
10. Moore PD, Webb JA, Collinson ME. *Pollen analysis*. 2nd edn. Oxford: Blackwell Scientific Publications; 1991.
11. Wiltshire PEJ, Hawksworth DL, Edwards KJ. A rapid and efficient method for evaluation of suspect testimony: palynological scanning. *J Forensic Sci* 2015 [in press].
12. Kapoor L. *Opium poppy: botany, chemistry, & pharmacology*. New York: Haworth Press; 1995.
13. Wiltshire PEJ. Palynological analysis of the organic material lodged in the spout of the strainer bowl. In: Crummy P, Benfield S, Crummy N, Rigby V, Shimmin D, editors. *Stanway: an élite burial site at Camulodunum. Britannia Monograph Series 24*. The Society for the Promotion of Roman Studies; 2008. p. 394–8.
14. Wiltshire PEJ. Hair as a source of forensic evidence in murder investigations. *Forensic Sci Int* 2006;**163**:241–8.
15. Wiltshire PEJ. Forensic ecology, botany, and palynology: some aspects of their role in criminal investigation. In: Ritz K, Dawson L, Miller D, editors. *Criminal and environmental soil forensics*. Dordrecht: Springer; 2009. p. 129–49.
16. Curran J. *Agatha Christie's murder in the making: stories and secrets from her archive*. London: Harper Collins Publishers; 2011.