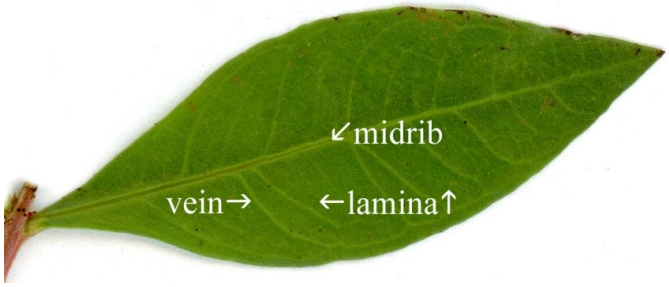
Chapter 4: Science and Microscopy: Microscopy of Henna

The microscopic structures of a henna leaf

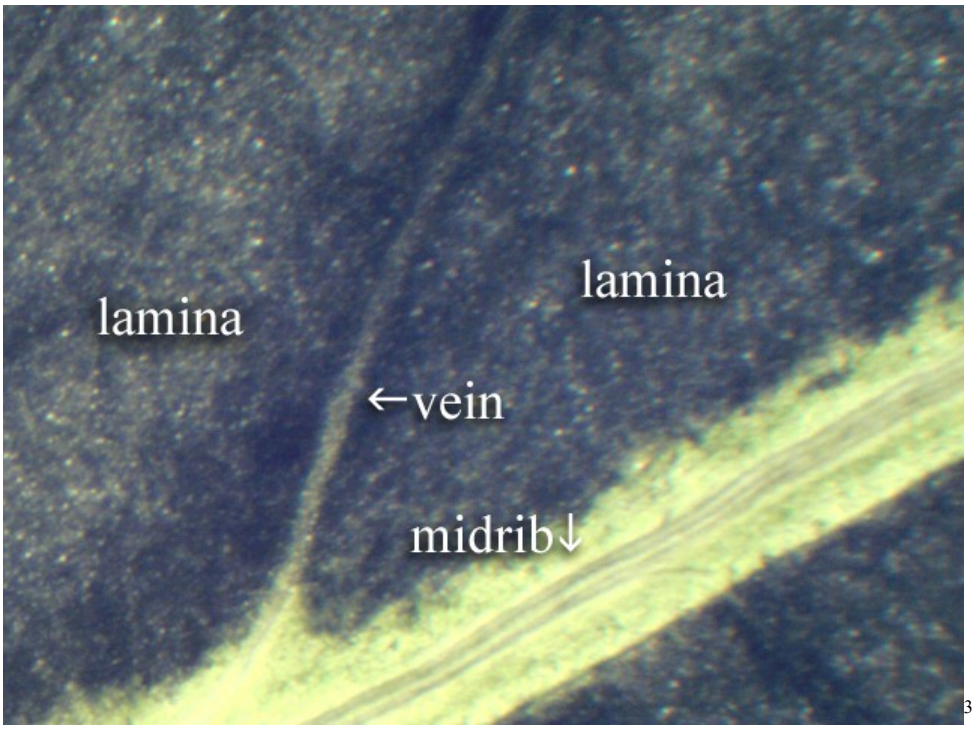
This is a quantitative microscopy survey of different brands of henna hair dye products. Henna products in the marketplace frequently contain unlisted additives and adulterants, and the milling and sifting varies with particles from 0.2 mm to over 3 mm. Consumers become frustrated by henna products' coarse sift, sand, problematic interactions with chemical dyes, and unpredictable, fading results. The public understanding of what is henna is further misinformed by false advertising claims made by by exporters and retailers. Investigation into henna's agricultural, industrial, and marketing processes was part of my Master's and PhD dissertations, in contrast to the usual anthropological and folkloric investigations on henna. Through that research, I amassed years of henna, indigo and cassia analysis from a certified independent laboratory, over one hundred henna hair dye products, and declarations by henna exporters. This chapter shows quantitative microscopic comparisons of products sold as 'henna.'



The above image is are macroscopic view of the ventral side of a 38 mm long henna leaf, 1.5 inches, with the midrib, vein, and lamina indicated.[1]

The above image shows the dorsal side of four paired young henna leaves; the larger leaves are 20 mm (about 0.8") long. The above images are scanned, so show natural color from reflected light.[2]

1 Henna plants raised by Catherine Cartwright-Jones PhD
2 Epson Perfection V600 Photo scanner 1200 DPI



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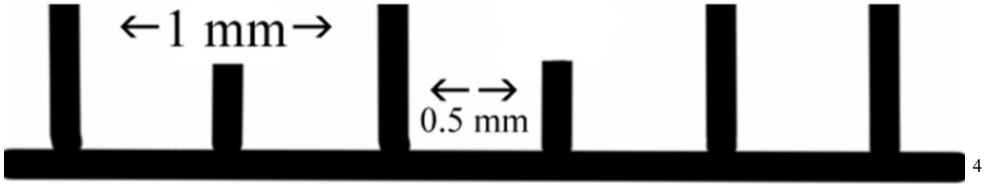

[4]

The above is a microscopic view a henna leaf section at 40x magnification with the midrib, vein, and lamina indicated. The section of this leaf is about 3 mm, 0.1 inches

The above image is a section of a single leaf of henna flattened between two microscope slides, illuminated from the base and magnified at 40x. The henna leaf colors appear different from the scanned leaf images because base light records opacity and translucency in a microscope image, rather than reflected light. Where the leaf is relatively opaque, the color appears darker, and structures which are relatively translucent appear lighter than one would see in a naked eye view of a leaf growing on a henna plant.

The midrib is the large structurally strong vein along the middle of the henna leaf. Fragments of midrib are often visible in henna powder, recognizable by interior parallel structures. A vein splits off from the midrib. Veins and netted veins diverge in a range of 50° to 70° angles. The broad sections filling in the space between the veins and netted veins are lamina.

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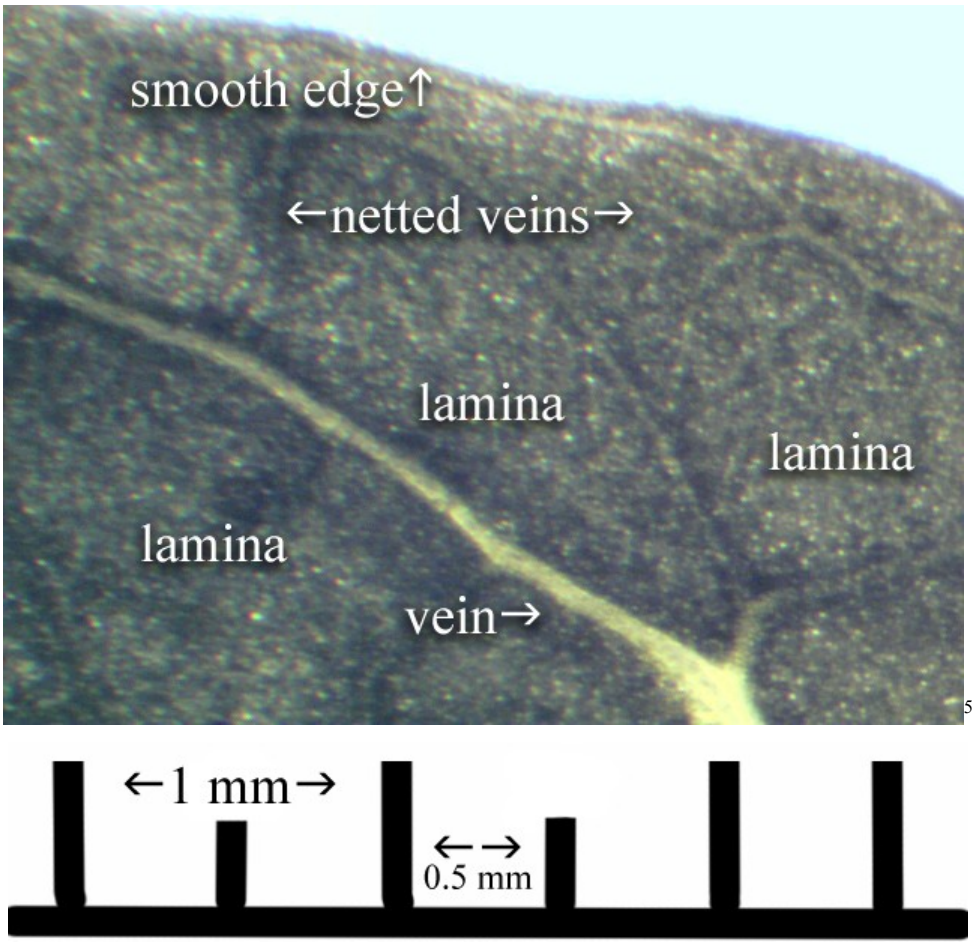
3 Henna leaf imaged at 40x under a AmScope MD35 0.3MP Digital Microscope by Catherine Cartwright-Jones PhD

4 This micrometer scale will be added to images to assist the reader to easily ascertain the size of the objects in the images. The micrometer scale will always be adjusted to the image.



Vein, netted veins, lamina, and smooth edge of henna leaf

Henna leaves after drying, crushing, milling and sifting

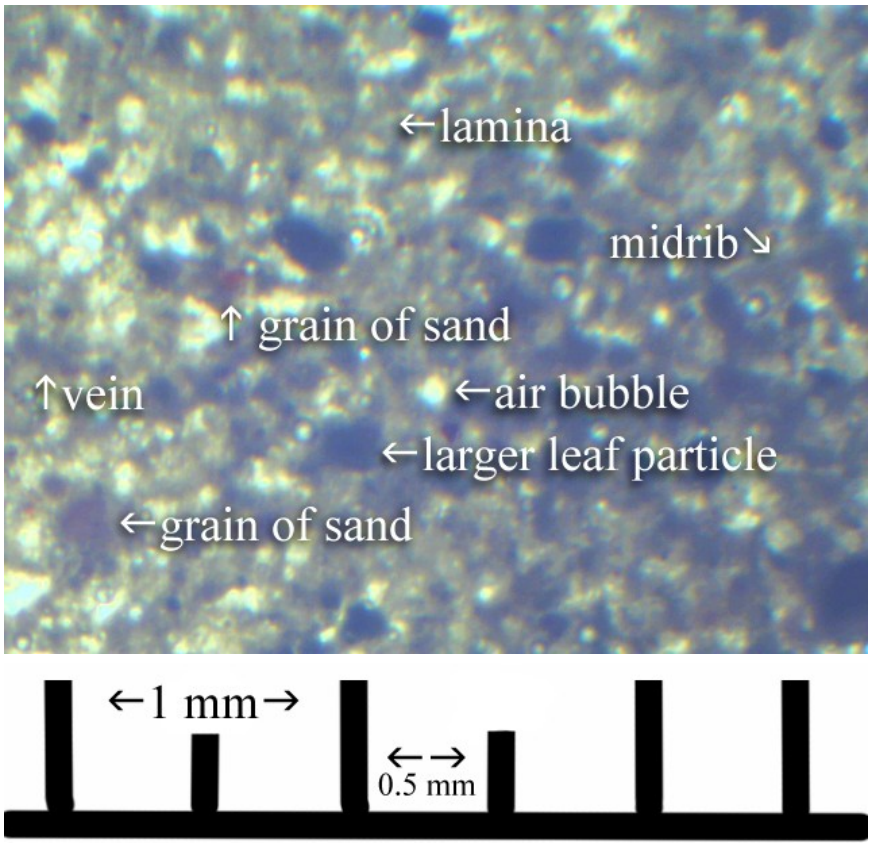
The same henna leaf structures exist in henna powder as the whole leaf: midrib, veins, netted veins, and lamina, though they are fragmented and randomized by milling and sifting. Midrib particles are often the largest particles in henna powder, because they are structurally strong and resist crushing and milling. The larger leaf particles are often have a 50° to 70° angular side, reflecting the intersections of the netted veins which support the lamina. Finely powdered lamina constitutes a large proportion of henna powders, as lamina is proportionally the largest area of leaf.[6]

5 40x magnification

6 The natural parts of henna studied in most scientific papers are 1,4-naphthoquinone, tannins, gallic acid, flavonoids, lipids, sugars, triacontyl tridecanoate, mannitol, xanthones, coumarins (5- alkyloxy 7-hydroxycoumarin), 2-3% resins, 5-10% tannic ingredients and up to 2% Lawsone (2- hydroxy-1,4- naphthoquinone). None of these are visible at the microscopic resolutions in this survey. Only the physical botanical structures are visible at 60 x resolutions.

[7]

When dried and crushed, the structures of the lamina, veins, and midrib are still recognizable.



Henna leaves milled and sifted for Ancient Sunrise® for use as henna hair dye[8]

The white orbs in the above image are air bubbles. Air bubbles are not an intrinsic part of henna powder, but when henna is mixed with water on a slide and the slip cover applied, there will be air bubbles. These are irrelevant and may be ignored.[9]

Every henna powder has some wind-blown desert sand; henna grows in semi-arid regions and sand is unavoidable. Sand in henna can be confirmed through microscopy by the appearance of translucent crystalline silica particles which do not dissolve in water. This

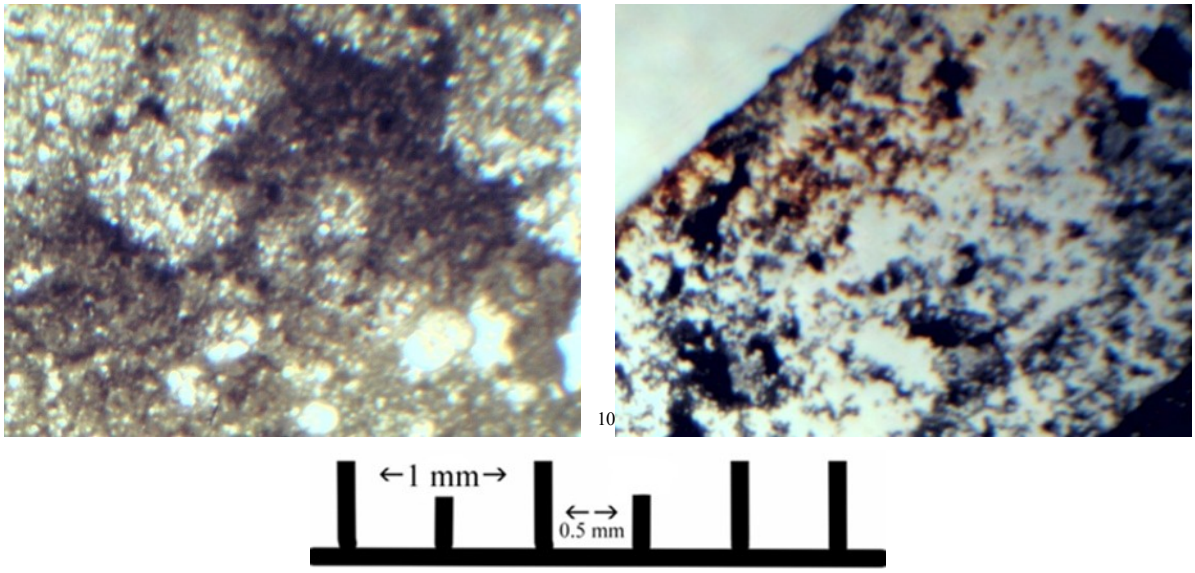
7 Henna leaf dried, crushed and scanned at 1200 DPI by author

8 Ancient Sunrise® Rajasthani Twilight Henna from mehandi.com 40x magnification, certified to be pure henna by Alkemist Laboratories

9 I mention this because it is my experience that bubbles have confused people who were not familiar with the random artifacts which may appear in a microscope image.

desert sand is usually tinged with iron oxide, as henna is most often grown in iron-rich sandy soils. A small proportion of sand does not interfere with henna, though a large amount of sand can leave a mess in your bathtub when you rinse the henna from your hair. This sand is usually too finely pulverized and sparse to be naked-eye visible, but if you mix water into henna and hear gritting between the bowl and spoon that sound is probably sand.

Powdered pure lawsone



The images above are powdered 98% pure lawsone mixed with water. After three minutes, lawsone particles form opaque clumps; there is no aqueous dispersion of lawsone.

Pure lawsone is a curry-yellow powder; the particles are small, less than 0.01 mm. There is no evidence of plant material in this sample of lawsone; it is 98% pure lawsone chemical powder. Lawsone is semi-standard non-polar and does not disperse in water; after several minutes the powder forms opaque clumps under the cover slip.

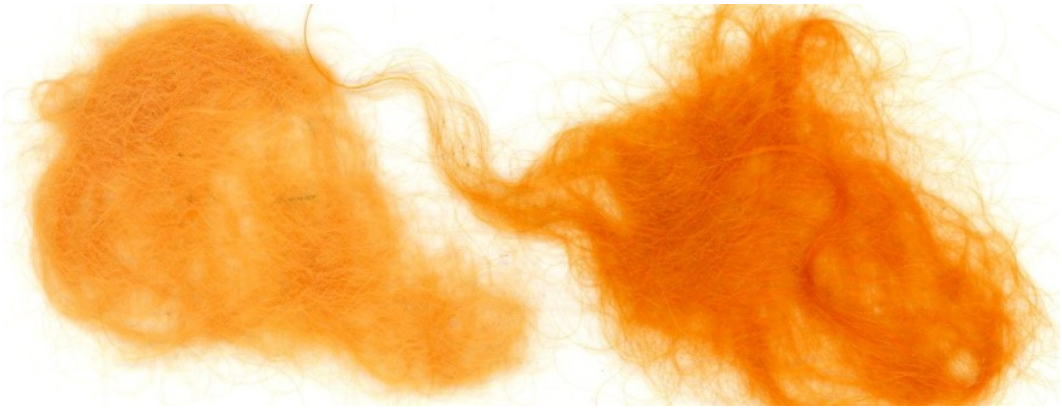
Lawsone cannot be directly observed in a henna leaf or henna leaf powder. Lawsone (2-hydroxy-1,4-naphthoquinone) is in a glycosidic bound in a henna leaf, which is cleaved by acidic hydrolysis of the glycosidic hennosids and autooxidation of aglucons.[11] It cannot be directly observed under a microscope at 40x, so any orange coloration in henna must be attributed to another source.

Lawsone is not normally claimed as an additive to henna for hair products. If a hair dye product has added pure lawsone to their henna powder, the stain will not be improved; hair is more

10 Alfa Aesar, Thermo Fisher Scientific

11 SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS, SCCP, Opinion on Lawsonia inermis (Henna) COLIPA N° C169 EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL Directorate C - Public Health and Risk Assessment C7 - Risk assessment Adopted by the SCCP during the 6th plenary meeting of 13 December 2005

effectively dyed with the aglycone precursor to lawsone in the henna leaf, rather than the lawsone itself. Also, hair dyed with henna darkens, whereas the hair dyed with lawsone does not darken over time as seen in the images below.

 [12]

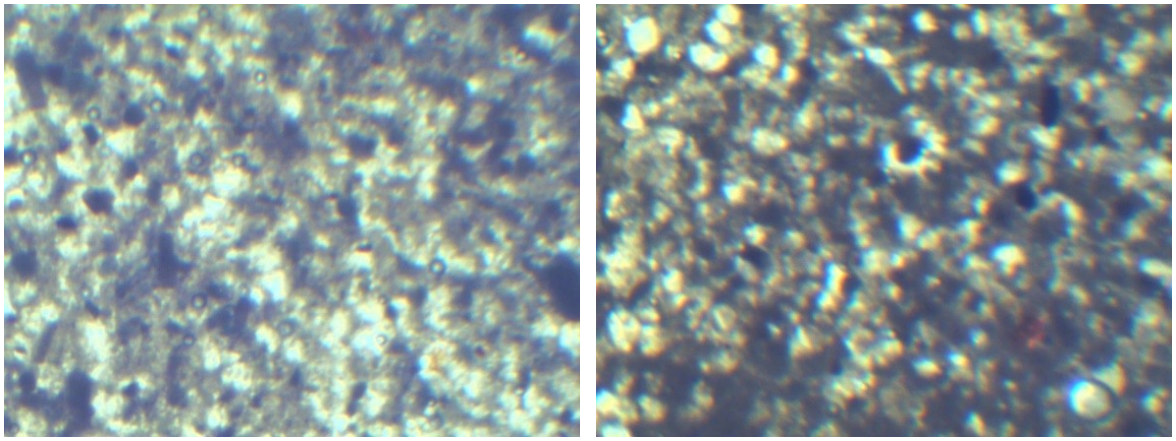
Left: White hair dyed for 12 hours with 98% pure lawsone
Right: henna with 1.5% lawsone.
Application done October 30, 2018, scanned November 2, 2018

The scan above compares naturally white hair dyed for 12 hours with 98% pure lawsone,[13] to henna with 1.5% lawsone, both mixed in a mildly acidic medium, rinsed, then scanned three days after dye application. Isolated lawsone is not as effective a dye as the henna aglycone precursors to lawsone. In the subsequent image, the sample dyed with henna has darkened considerably over a sixty-day interval.

Left: White hair dyed for 12 hours with 98% pure lawsone;
Right: henna with 1.5% lawsone.
Application done October 30, 2018 and scanned December 30, 2018

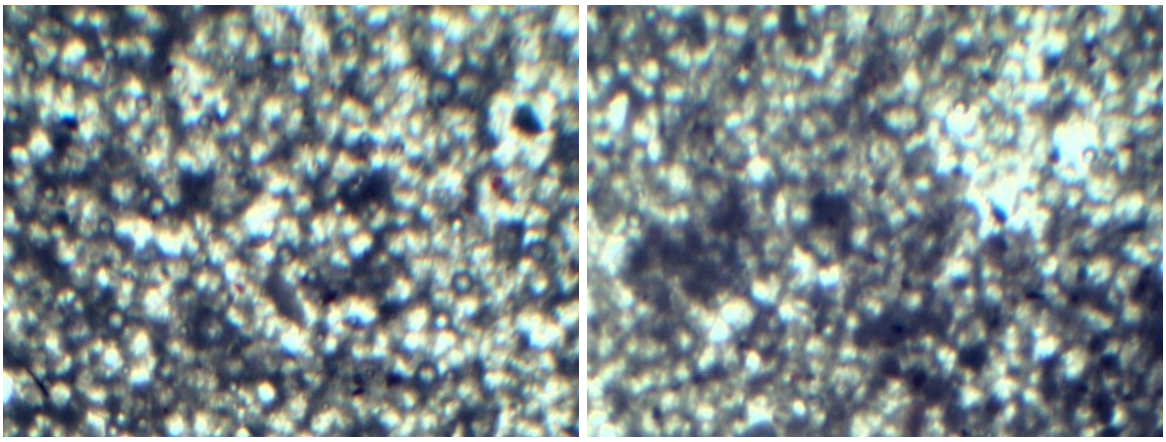
12 Pure lawsone was mixed with lemon juice and water, the henna was mixed with lemon juice. The hair is natural white mohair, unbleached and undyed. The hair was left to soak in both the lawsone and the henna mix for 12 hours and then rinsed. The results were scanned at 300 dpi against a white background by Catherine Cartwright-Jones PhD

13 Lawsone (2- hydroxy-1,4- naphthoquinone) from Alpha Aesar, Thermo Fischer Scientific. 2-hydroxy – 1,4 – napthoquinone 98% https://www.alfa.com/en/catalog/A11880/



The image above shows two laboratory certified pure henna leaf powders, milled and sifted for use as Ancient Sunrise® henna hair dye, magnified 40x.[14]

To evaluate henna products for hair under a microscope, it is necessary to have a known, verified example for comparison. The above Ancient Sunrise® hennas have been certified to be dried, powdered henna leaves of lawsonia inermis. Both contain a few particles of wind-blown sand. The henna at the left has a slightly coarser sift: the largest particles are less than 0.4 mm; most particles are 0.2 mm or less. This sift makes a creamy paste that can be easily washed out of hair. The henna at the right is a finer sift, with no particles larger than 0.2 mm, a body artist using a jac bottle would not be troubled with clogs with this sift.



Cassia ovobata leaves milled and sifted for use as Ancient Sunrise® hair dye.[15]
These cassia powders are 40x magnification, there is minimal sand, particles are all less than 0.3 mm and are certified to be pure cassia obovata leaf by Alkemist Laboratories.

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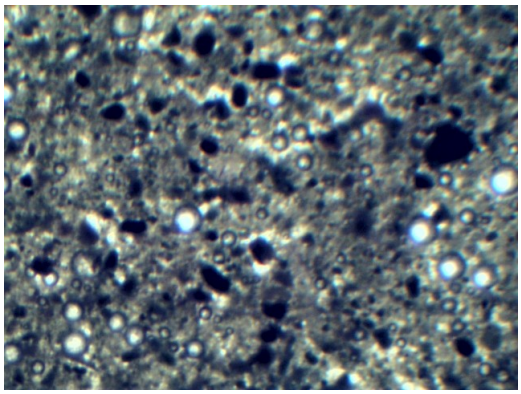
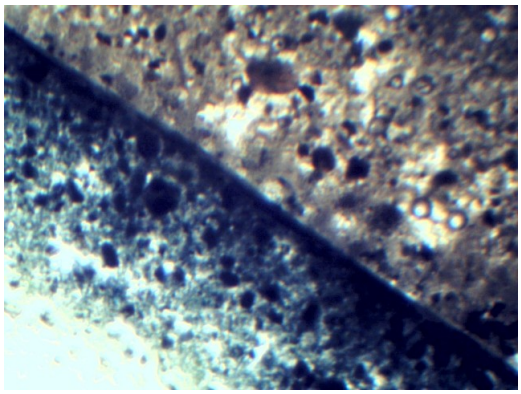
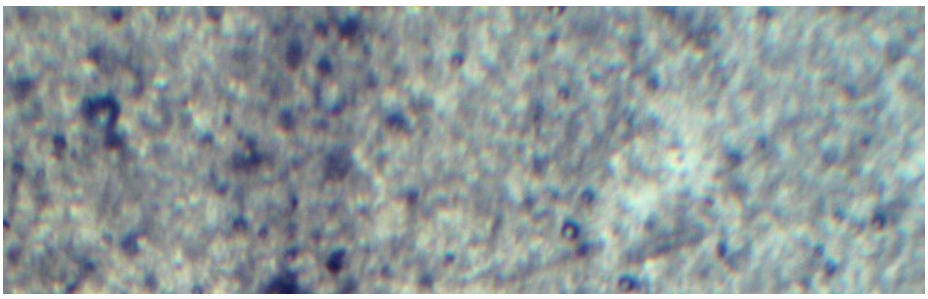
14 Left: Ancient Sunrise® Rajasthani Monsoon Henna from mehandi.com Right: Ancient Sunrise® Rajasthani Jasmine Henna. Both at 40x magnification and certified to be pure henna by Alkemist Laboratories.
15 Left: Ancient Sunrise® Zekhara Cassia obovata from mehandi.com. Right: Ancient Sunrise® Rajasthani Sudina Cassia. Both magnified at 40x and been certified to be pure Cassia obovata by Alkemist Laboratories.

Cassia Obovata has a similar appearance to henna under a microscope. Chrysophanic acid (chysophanol), a golden yellow anthraquinone molecule, (1,8-Dihydroxy-3-methylanthraquinone), is not visible in the leaf powder or paste at 40x for the same reason that lawsone (2-hydroxy – 1,4 –napthoquinone) is not visible in henna. They both exist in the leaf in the precursor state, and are released through acidic hydrolysis over a period of several hours.



[16]

Left: Ancient Sunrise® Indigofera tinctoria, partially fermented, dried, milled and sifted for hair dye[17]
Right: indoxyl changing to indigo in contact with oxygen outside of the edge of the cover slip.

Indigo is prepared for hair by partial fermentation in an alkaline environment; this transforms the indigene in the leaf to indoxyl. When water is added to the indigo, the indoxyl reacts with oxygen to produce blue indigo. It is possible to see this process in about four minutes as the wet indigo powder seeps out from under the cover slip as is seen at the image on the right, above. There is no comparable quick release for henna or cassia powders.



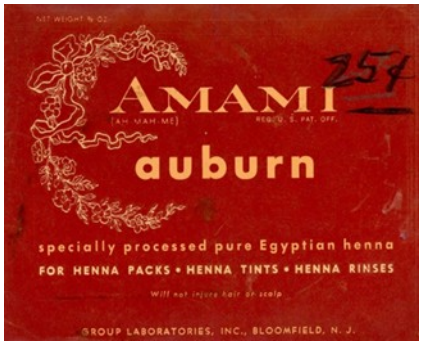
Ancient Sunrise® Amla powder (emblica officinalis)[18] fruit dried, milled, and sifted. The largest particles are 0.1mm, image at 40 x. Amla fruit is dried, milled, and sifted. Amla is in many mixed henna hair dye products from India as the acid for dye release.

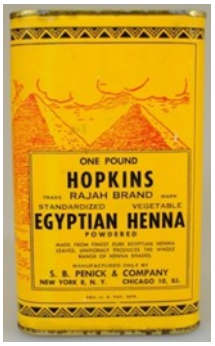
16 Magnification 40x, largest plant particles are 0.3 mm.
17 Left: Ancient Sunrise® Zekhara Indigo from mehandi.com at 40x magnification, certified to be pure fermented Indigofera tinctoria by Alkemist Laboratories.
18 Ancient Sunrise® Amla (emblica officinalis) from mehandi.com at 40x magnification , certified to be pure by Alkemist Laboratories.

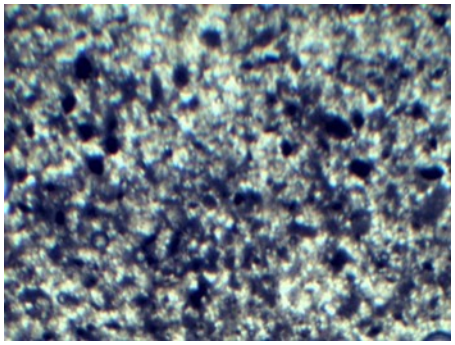
Identifying the contents of packages of henna sold as hair dye

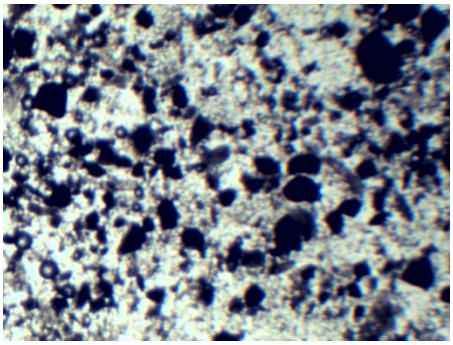
Henna in the USA in the Mid-20th century

Some henna products available in the early to mid-20th century were well-sifted unadulterated henna. The amount of sand was average for henna and the particles were less than 0.5 mm. The term 'henna' was loosely applied to a variety of hair care and coloring products, but there was some pure henna available and the processing seems to have been pure, clean, well-milled henna.







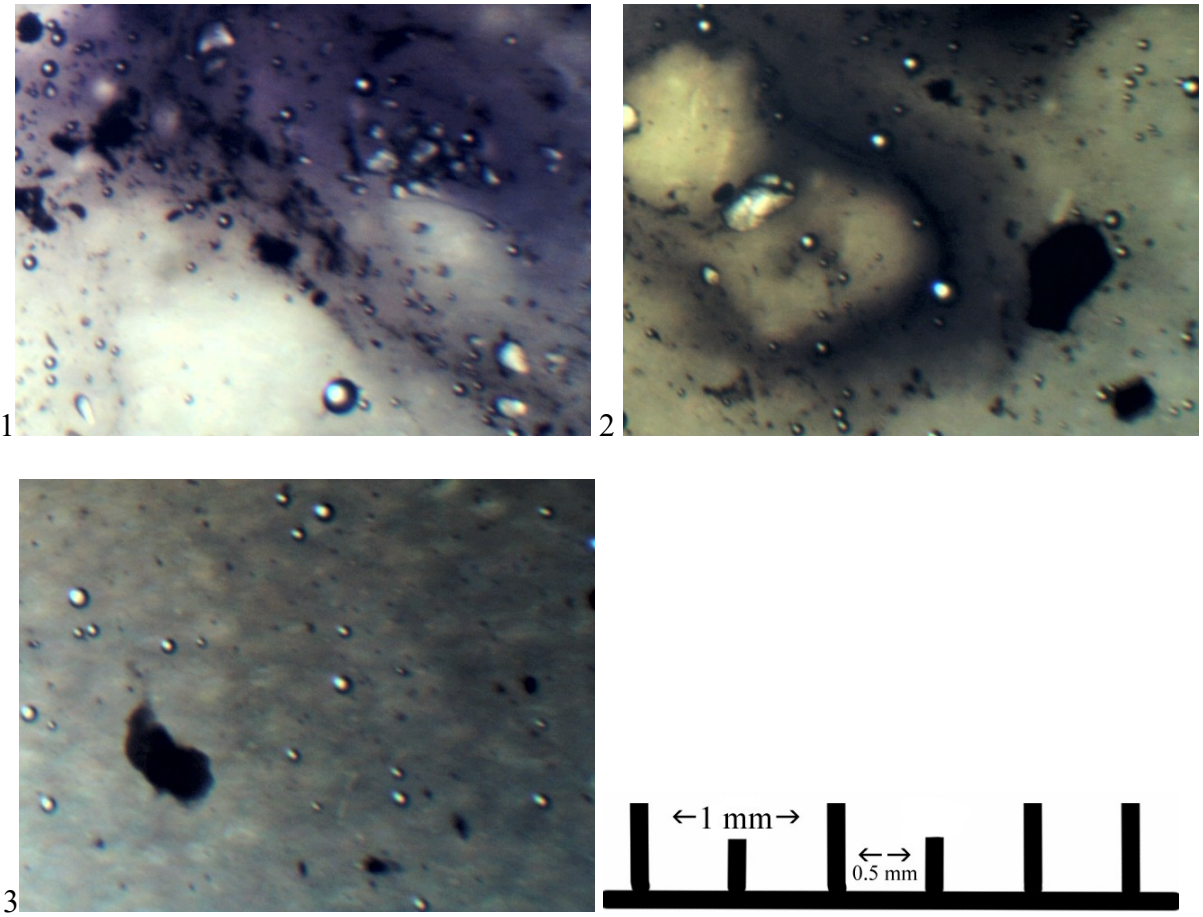


Henna for hair products from Egypt available 1930 – 1950 in the USA,[19] henna produced in Egypt. All particles are consistent with henna. Products are declared to be henna.

19 Vintage henna in the author's collection, powders imaged at 40x, largest plant particles are 0.5 mm. There is no indication of added dye. There is no indication of added plant species.

“Black Henna” containing para-Phenylenediamine

By the 1970’s, a product marketed as ‘black henna’ was produced in India. This product contained para-phenylenediamine (PPD) with some henna powder mixed in for ease of application in hair or on skin. These powders are still on the market and contain much higher levels of PPD than are considered safe for hair dye by the USFDA.[20] Because these products were sold as ‘black henna,’ consumers were persuaded that there was a ‘henna plant’ which could quickly, easily dye skin and hair black.

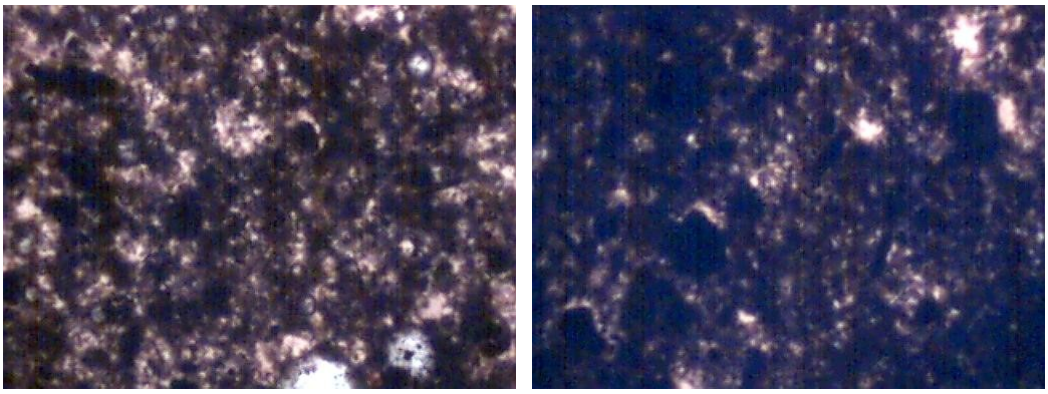


Sequential images of para-phenylenediamine dispersing in water over a period of one minute from a PPD/henna product, images are magnified 40x.

The powder in the above image sequence was initially pale gray (the color of para-phenylenediamine) and dispersed dye immediately after wetting with water. The color dispersing was at first purple and then changed to black as it came in contact with oxygen. Once dispersed, there remained some visible plant particles, though sparse in proportion to the amount

20 Weiyang Chena, Thobile. A.N. Nkosia, Sandra Combrincka, Alvaro. M. Viljoena, Catherine Cartwright-Jones. (2016) “Rapid analysis of the skin irritant p-phenylenediamine (PPD) in henna products using atmospheric solids analysis probe mass spectrometry” *Journal of Pharmaceutical and Biomedical Analysis*, Volume 128, 5 September 2016, Pages 119–125

of the first ten declared chemical cosmetic ingredients.[21] Though the packages of para-phenylenediamine/henna hair dye products usually emphasize the 'nourishing,' 'ammonia free,' 'sacred ayurvedic' henna and other plant ingredients declared in their mixtures, these are potentially dangerous products, and often contain little or no plant material.[22]



The above images are of "black henna" products from India sold for use as hair dye and black temporary tattoos. These show mixtures of plant powder and para-phenylenediamine dye. The powders were black, acquired in 2001 and 2002, imaged at 60x.

"Polished" henna with green sand

Most henna samples originating in India and available in the US markets in 2003 contained sand dyed azo green.[23] This green dye is recognizable by its rapid dispersion when the henna powder is mixed with water, often coloring the entire sample green within the first four minutes.

The Essential Oil Association of India stated, "Major contaminants /adulterants in henna leaves are stems, dirt, plant waste and other leaves. However in case of henna powder admixture of dyed sand is observed. It has been reported that for adulteration, finely ground local sand is used. It is first dyed with auramine yellow (C.I. No. 41000) and then green with diamond green (C.I. No. 20440). This is then mixed with pure henna powder. The extent of adulteration is variable in accordance with the price of the powder reflected therein.

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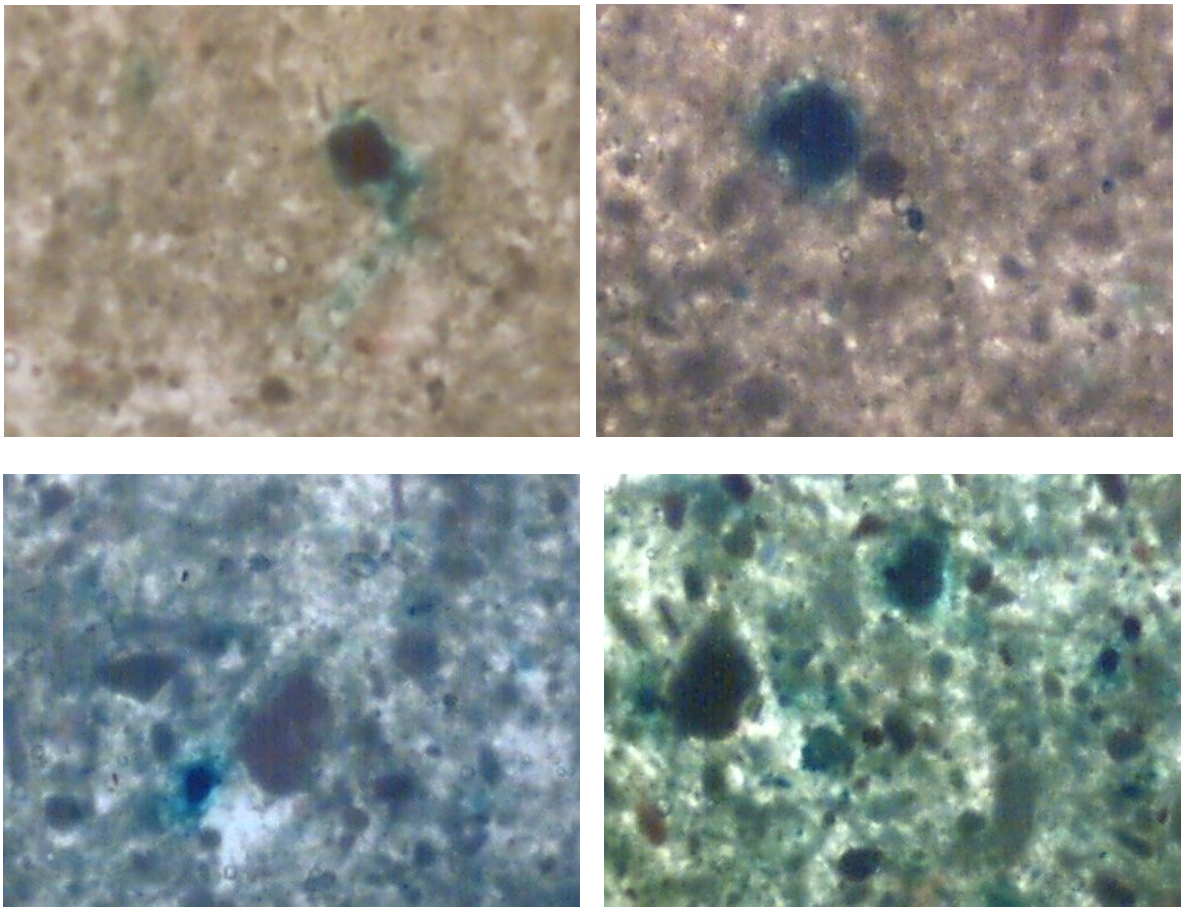
21 Declared ingredients of black hair dye product: Sodium perborate monohydrate, p-phenylenediamine, Sodium sulphate anhydrous, Cellulose gum, Sodium lauryl sulphate, Resorcinol, Citric acid, 2,3-diaphenoxyethanol HCI, Tetrasodium Pyrophosphate, Maltodextrin, Hibiscus rosa-sinensis, Lawsonia inermis, Trigonella, foenum-graecum, Phyllanthys emblica, Indigofera tinctoria, Eclipta alba, Dimethicone, Acrylamidopropyltrimonium chloride/Acrylamide copolymer, Cetyl hydroxyethylcellulose, BHT and Perfume.

22 In this survey, I opened only one package of 'black henna' containing para-phenylenediamine, as I have become severely allergic to the chemical from coming into contact with it during the writing of my PhD dissertation: Cartwright-Jones, C. "The Geographies of the Black Henna Meme Organism and the Epidemic of Para-phenylenediamine Sensitization: A Qualitative History." PhD dissertation. 2015. Kent State University, Kent, Ohio. Accession Number: kent1425412566

23 Chourasia, Sardar, Patil, Mathew,Kanpur, (1989) "Study of Quality Characteristics of Henna", *Essential Oil Association of India.*

"Added azo dyes were not found in henna leaf samples, but yellow and green coal tar dyes were observed in powdered samples. As mentioned earlier, this may be due to the presence of (the afore mentioned dyed sand). Unlike Lawsone, the natural color of henna, these added synthetic azo-dyes used for dyeing the sand or for polishing the leaves may have an adverse effect on the skin. It is, therefore, necessary to ensure that these artificial dyes are not there in the product marketed."

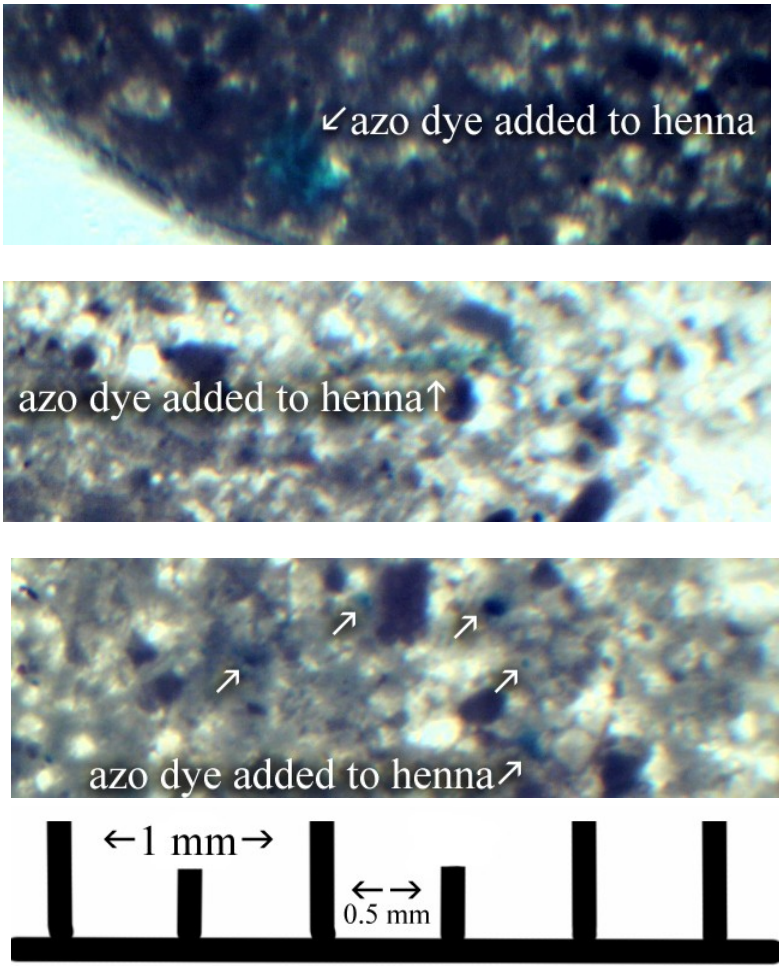


Azo green dyed sand in henna products in collected in 2001- 2, dispersing green dye when the paste was wetted and imaged at 60x.

The particles of green dye are often so large as to be visible to the naked eye. This green dye 'polish' was added henna to make it appealing to consumers. Consumers were told that greener henna was better henna, then green dye was added and the price was raised.[24] Higher priced henna powders contained more green dye. In 1989, the Essential Oil Association of India stated, "added synthetic azo-dyes used for dyeing the sand or for polishing the leaves may have an adverse effect on the skin. It is, therefore, necessary to ensure that these artificial dyes are not there in the product marketed." In 2002, most Indian henna powders for use on skin and hair still

24 I have never found henna with green dyed sand from any country other than India, though when companies in other countries buy henna from India, rebrand and resell it, the green dye remains. These rebranded green-dyed powders have been found in henna products from Turkey, Syria, and Morocco.

contained azo green dye. The practice of adding green-dyed sand to henna seems to have decreased by 2018, though it still can be found in products meant for hair dye. The azo dye is never in the ingredient declaration. The azo dyes do not seem to be hazardous, but the dye is misleading not only about freshness and quality of henna, but they mask dye release. Henna dye release normally makes the paste slightly brown whereas the green dyed paste remains bright green, confusing customers about when the henna paste is ready to use.



Henna samples containing unlisted green azo dye from India, acquired in 2013 and 2018[25]

Milling and sifting henna

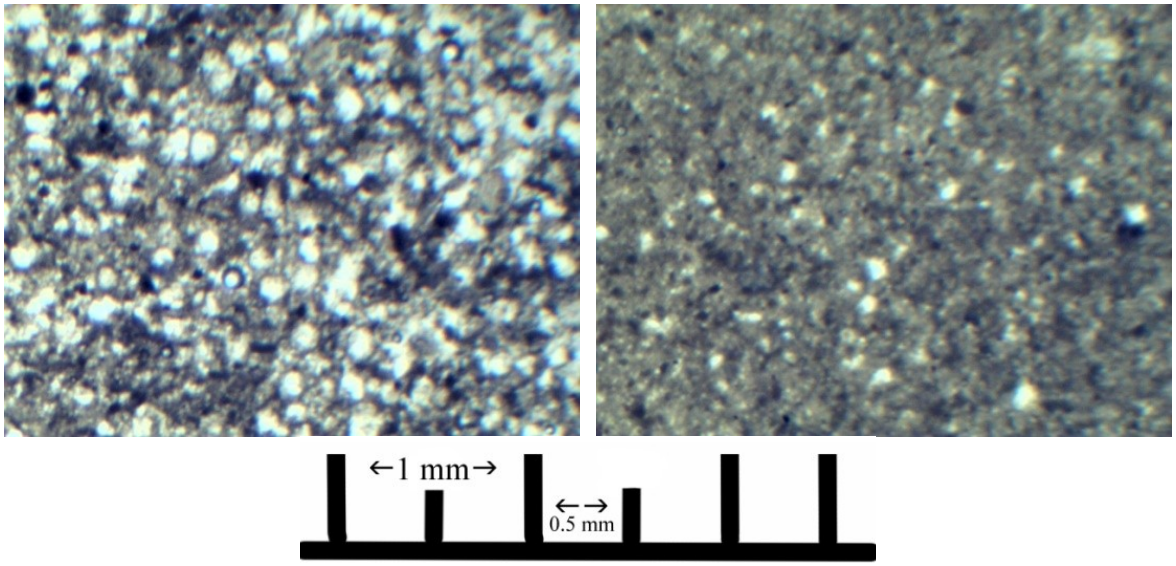
Henna arrives at henna processing factories[26] as bags of crushed leaves, often purchased at auction, though some sellers contract farmers to raise henna to their specifications or own their own land. First, the henna is cleaned in a rotary drum having sieves of three different grades where dust and fine particles get removed. After sieving, air is blown through the machines to

25 Henna products sourced from Middle Eastern and South Asian markets, Amazon.com and Ebay.com in 2013 and 2018.

26 In 2005, there were 25 major henna processing factories in Sojat.

separate debris from leaf: soil and berries fall near the fan separating them from clean leaves. Small, clean, lightweight leaf fragments are blown farther, and collected. The cleaned leaves, 'choori,' are put through a thresher. After each step of milling and sifting, the larger particles and debris are removed. This process removes 12% to 18% of the rough henna product, usually dust, fruits, stems, branches and seeds. Most of the milling is done at night to keep the temperature low; if the equipment heats up, the quality of the henna is adversely affected.[27]

In microscopic images, the largest particles indicate the sieve opening size, and therefore the sieve designation number. Sieved henna tends to cling to the sieve material, especially if the air is humid, so more there is more effort in getting fine henna through a sieve than coarse henna. Henna which is easy to apply and rinse from hair is sieved at about #50 mesh or finer. Ancient Sunrise® henna products are sifted at #50, #60, and #70 mesh..[28] The particle sizes[29] observed for other henna products for hair are 0.177 mm particles which pass through a No. 80 seive, 0.250 mm particles will pass through a No. 60 sieve, 0.354 mm particles will pass through a No. 45 sieve, and on through progressively larger sieves, with coarsely sifted henna particles of 4.00 mm passing through a No. 5 sieve.



Particles less than 0.2 mm, sifted through a No. 80 sieve

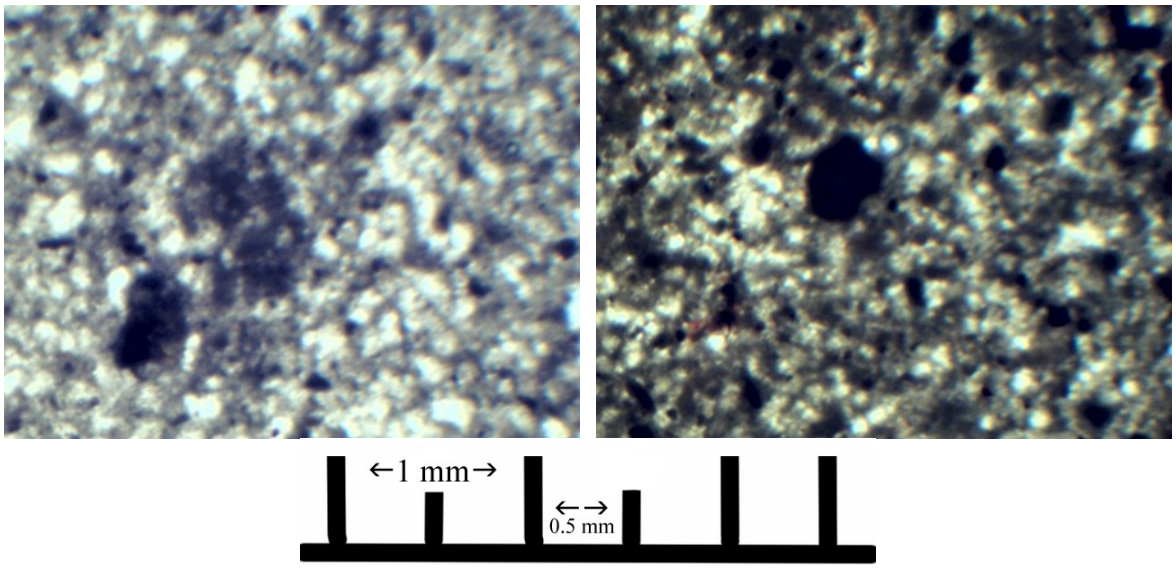
The images above are two brands of henna which have maximum particle size less than 0.2 mm. There is only lamina visible in the powder and little or none of the larger, sturdier structures such as midrib and vein.

27 Singh, M., Jindal, Z. K., Kavia, B. L.,Chand, J. and K. (2005) "Traditional Methods of Cultivation and Processing of Henna." *Henna Cultivation, Improvement and Trade*, Central Arid Zone Research Institute, Jodhpur, India. p. 24

28 Sieve size confirmed in testing by Alkemist Pharmaceuticals.

29 Millipore Sigma Particle size/sieve mesh conversion chart https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/particle-size-conversion.html

Henna powders put through a sequentially finer meshes are designated by the marketing term 'triple sifted.' Each sift separates out more waste. There is also more labor required so factories charge more for finer sifts. Exporters often quadruple the price of the finest sift and market it to artists in wealthy countries and sell their coarser sifts in markets unwilling or unable to pay higher prices. The highly sifted hennas are favored by people doing body art because it is easy to get the paste through a jac bottle tip or syringe without clogging. A #3 jac bottle has a 0.3 mm metal tip; if there is a 0.4 particle stuck, the artist has to stop and pick the clog out of the tip with a needle. Rolled polypropylene film cones are more forgiving: a small clog can be pinched out. These #80 mesh hennas are very malleable for body art work, and are favored in Morocco by artists who apply henna with syringes. Time spent digging out clogs means money not earned doing art. However, these #80 mesh hennas do not necessarily have the highest lawsone content.[30] Since the higher lawsone content crops of rain-fed henna are harvested in October, then dried and crushed, the henna leaves must wait six months to be milled during the driest season to be sifted through the finer sieve or be harvested during a period of lesser quality irrigated henna crops.[31]



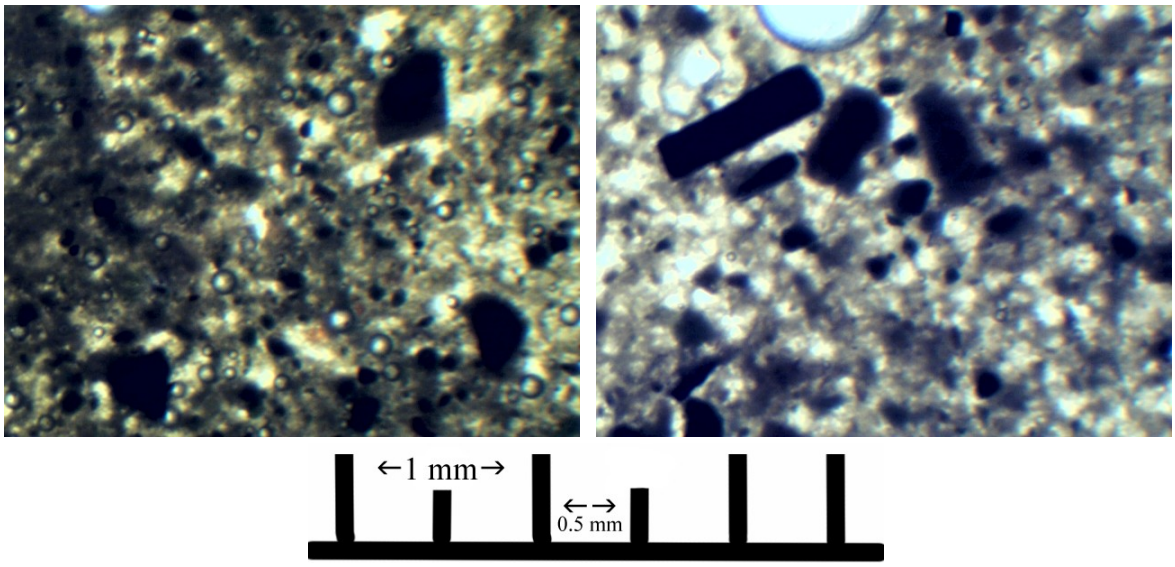
The above hennas have particles up to 0.5 mm that would pass through a #30 mesh, would clog an artist's jac bottle unless strained through a stocking, and would take some persistence to rinse from hair.

30 One exporter claimed that every time his henna was sifted, the proportion of lawsone was higher until his finest sift had nearly 100% pure lawsone. This claim was simply not true; Lawsone occurs in the henna plant leaves in the form of glycosidic precursors and sifting would not change that. Also, HPLC tests of these highly processed hennas do not show any lawsone content improvement over comparable henna products of coarser sift. (Source Alkemist Laboratories, testing for TapDancing Lizard® LLC 2008 – 2018)

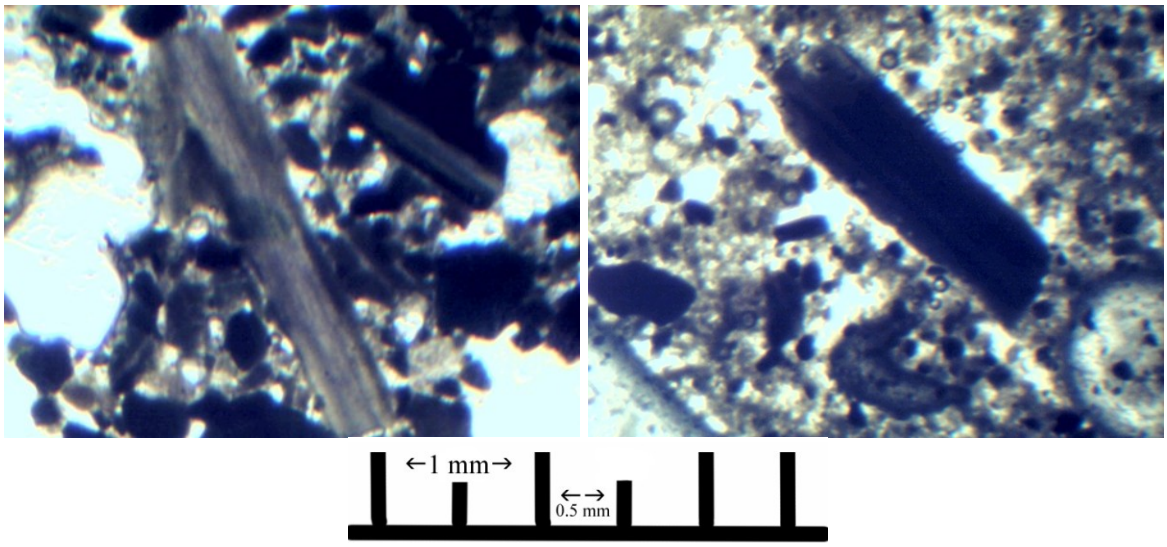
31 Roy, P. K.. Singh. M., Tewari, P. (2005) "Composition of Henna Powder, Quality Paramters and Changing Trend in its Usage" *Henna Cultivation, Improvement and Trade*, Central Arid Zone Research Institute, Jodhpur, India. p. 39, table 1

Pali district June harvest average henna lawsone content: 2.38%

Pali district October harvest average henna lawsone content: 2.82%



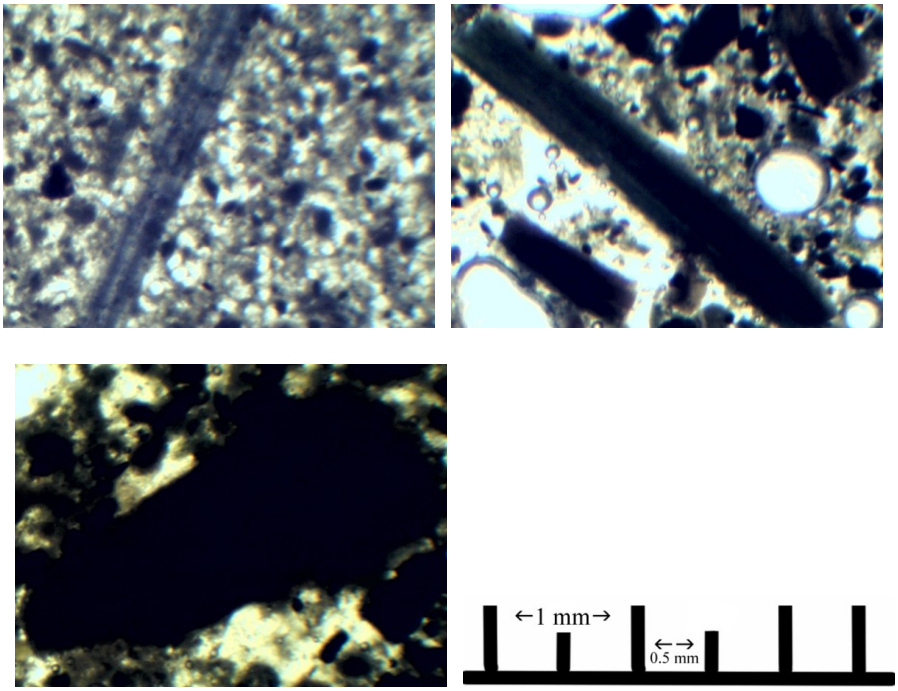
The above hennas have particles up to 0.7 mm which would pass through a #20 mesh. These cannot be used with a jac bottle for henna body art, would cause clogs in a polypropylene cone, and would be difficult to rinse from hair.



The above image has 2 mm to 2.5 mm segments of henna leaf midrib that would pass through a #10 mesh. These would leave debris in hair that might take several rinses and generous application of conditioner to remove.

These coarse henna samples are not from obscure suppliers; they are in well-known brands frequently found in natural food stores in the USA and Europe. The products were purchased from Amazon.com and Ebay in 2013 and 2018. This coarseness has contributed to the consumer rejection of henna hair dye in western countries. These roughly sifted products also contributed to the confusion around the emergence of henna into western culture at the end of the 1990's. People who aspired to do henna body art in its first surge of western popularity

purchased ‘henna for hair’ products and found them entirely unsuitable. Bigen black hair dye was a black hair dye with a fine sift, and could be used to create the elusive ‘black henna’ temporary tattoos shown in Madonna’s music video of “Frozen.” Unfortunately, the dry Bigen powder has a 12.5% para-phenylenediamine content and other ‘black henna’ hair dyes from South Asia have up to 30% para-phenylenediamine dry cotent.[32] The sensitizations caused by this misunderstanding of henna products gave rise to the current epidemic of para-phenylenediamine sensitization.[33] This sensitization has now ironically created a demand for high quality henna for hair as those the recipients of ‘black henna’ temporary tattoos have become allergic to oxidative hair dye.



The above images have 2.5 to 3 mm segments of henna leaf that would pass through a #5 or #6 mesh.

Ingredient declarations on henna for hair products in the late 20th century

In the 1970’s Clairol and other major hair dye companies abandoned henna. Henna had always been safe for home hair dye application, but Clairol introduced their home hair-coloring oxidative dye product "Miss Clairol" in 1956 which quickly outpaced henna in the marketplace

32 Weiyang Chena, Thobile. A.N. Nkosia, Sandra Combrincka, Alvaro. M. Viljoen, Catherine Cartwright-Jones (2016) “Rapid analysis of the skin irritant p-phenylenediamine (PPD) in henna products using atmospheric solids analysis probe mass spectrometry” J*ournal of Pharmaceutical and Biomedical Analysis*, Volume 128, 5 September 2016, Pages 119–125

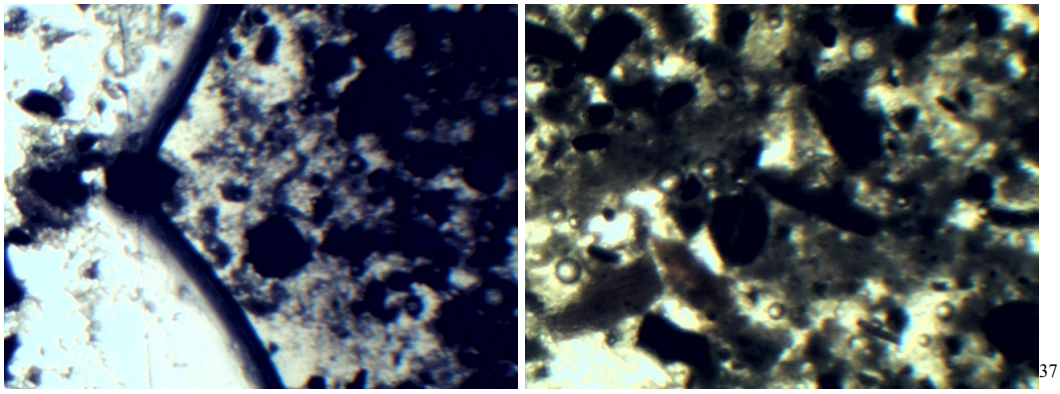
33 Cartwright-Jones, C. (2015) “The Geographies of the Black Henna Meme Organism and the Epidemic of Para-phenylenediamine Sensitization: A Qualitative History.” PhD dissertation. Kent State University, Kent, Ohio. Accession Number: kent1425412566

for customer acceptance and products with their "Does she...or doesn't she?" slogan.[34] Within six years of that ad campaign, 70% of all adult women were coloring their hair at home with oxidative hair dye. There was no longer any reason for the major corporations to dabble in what they considered dirty, unreliable henna, which, if it contained metallic salts, interfered with the oxidative dye and perm products that were so profitable for them.

In the 1970's, smaller henna companies began marketing henna, partly in response to the 'hippie-back-to-nature movement. The New York Times released an article on henna hair dye products in 1977, which reveals some of the problems created by the deliberate misinformation or deception promulgated by the new henna companies. A henna merchant 'revealed the secrets' of his henna to a journalist,

> "He took the visitor into the salon storeroom and opened three drums of henna. Red henna—actually a mustard color in its powdered form-comes from the leaves of the plant, he said, and, imparts the lighter red colors. Black henna (light green in powder) comes from the roots of the plant; is used on dark hair for deep tones, and is sometimes mixed with strong coffee to darken it further. Then there is neutral henna (it looks like fine sand) which is made from the plant stems and gives no color, but adds shine and body to the hair." [35]

These falsehoods about henna were received as truth by customers and several companies repeated them as ingredient declarations: "Pulverized leaves, roots and stems of the Lawsonia plant family: Lawsonia Inermis, Lawsonia Elba and Lawsonia Spinoza.When these 3 basic shades (of henna); Red, Black and Natural are mixed in various proportions, you are able to achieve 13 shades of henna."[36]

 [37]

Ingredient declaration for the above Chestnut Brown henna color product states, "Lawsonia Inermis, Lawsonia Elba and Lawsonia Spinoza" leaves, bark and roots. The sample smelled strongly of indigo. In the slide at the left there are indoxyls oxidizing to indigo blue.

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34 In 2 004, Clairol sold $1.6 billion in hair dye products.

35 Taylorjan, A. (1977) "All About: Henna, for Hair With a Shine" *The New York Times* January 19, 1977, Page 52 https://www.nytimes.com/1977/01/19/archives/all-about-henna-for-hair-with-a-shine.html

36 Avigal Henna ingredient declaration.

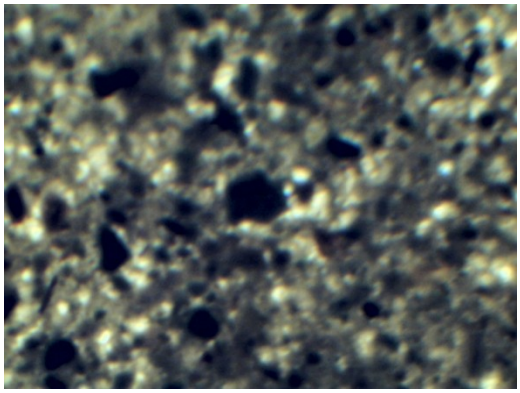
37 40x magnification. Plant particles up to 0.8 mm

Henna hair dye ingredient declarations in the current marketplace

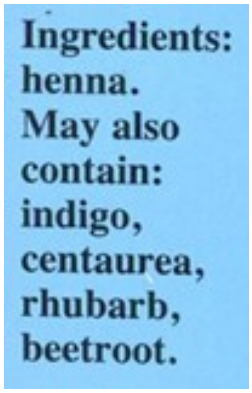
Since 2003, henna hair dye companies selling in North America have been under pressure by the FDA and consumers to properly declare ingredients. The FDA requirements for labeling are:

> *21 CFR 101.4(a)(1) states that ingredients shall be listed by common or usual name in descending order of predominance by weight.* The declaration shall be presented on any appropriate information panel in adequate type size, without obscuring design, vignettes, or crowding. The entire ingredient statement shall appear on a single panel of the label.
>
> The Food and Drug Administration will not object to "shotgun" ingredients labeling, labels which are designed for more than one product.

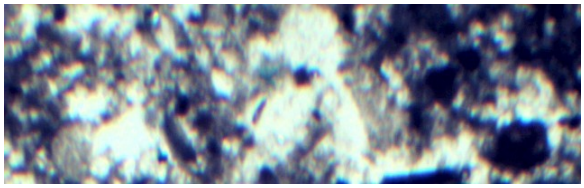
Though it is not possible to confirm the identity of botanicals in a henna product simply by viewing the powder at 40x, one can at least see if the declaration is consistent or inconsistent with the visible material. More precise confirmations would require complex lab testing.



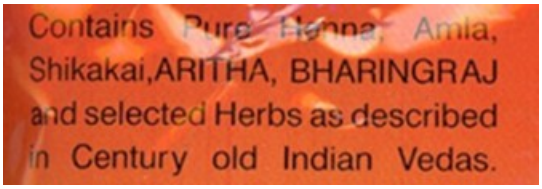
38



The above 'Chestnut Henna' powder has particles consistent with henna and indigo, but there is no evidence of beetroot. The odor of the powder consistent with henna. This is an example of a 'shotgun' ingredient declaration.



39



The above henna hair dye has particles consistent with henna and other herbs, but does not mention the azo green dye particle or the identity of other 'selected Herbs as mentioned in Century old Indian Vedas,' so does not comply with FDA ingredient declarations.

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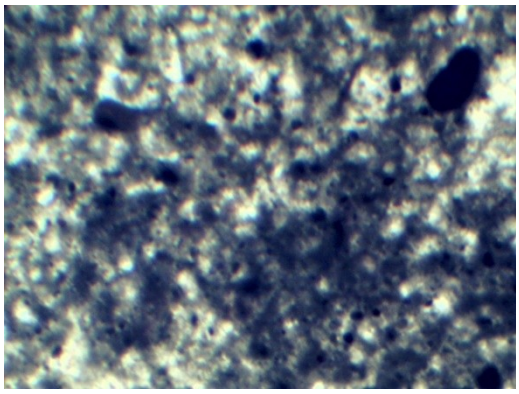
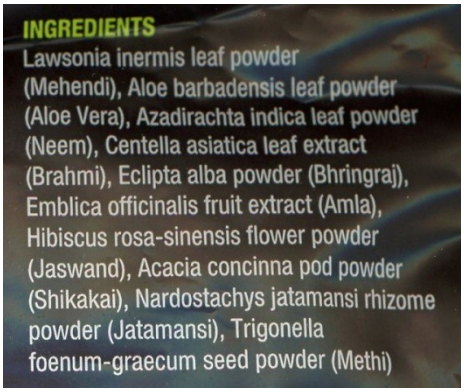
38 40x magnification. Plant particles up to 0.5 mm
39 40x magnification. Plant particles up to 0.6 mm



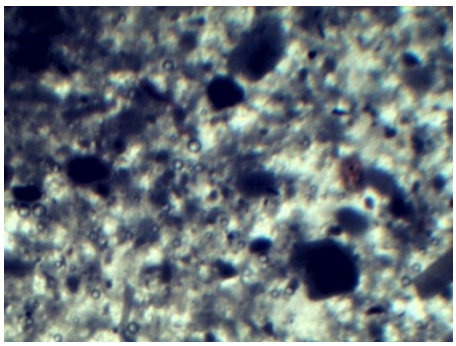
40

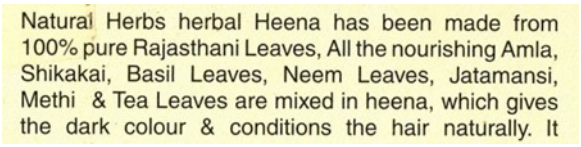
The above 'Light Brown Henna' powder has particles consistent with indigo, henna, cassia, and amla. The powder smells like henna. There was is visible indigo dye release.



41

The above henna powder has particles consistent with henna and other declared plant material. The large red particle is consistent with Acacia concinna (shikakai).





42

The above henna powder has particles consistent with henna and other declared plant material. The large red particle is consistent with Acacia concinna (shikakai). The odor of the powder was consistent with henna and basil.

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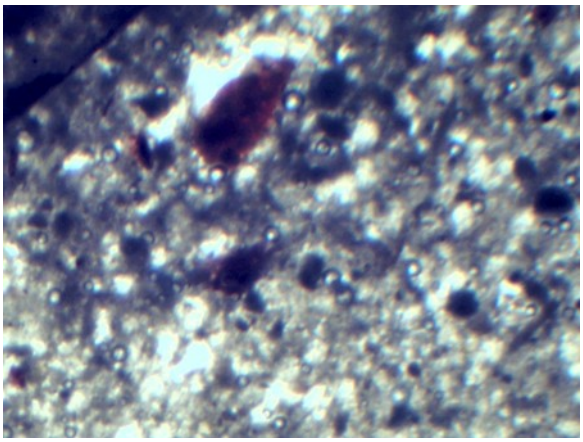
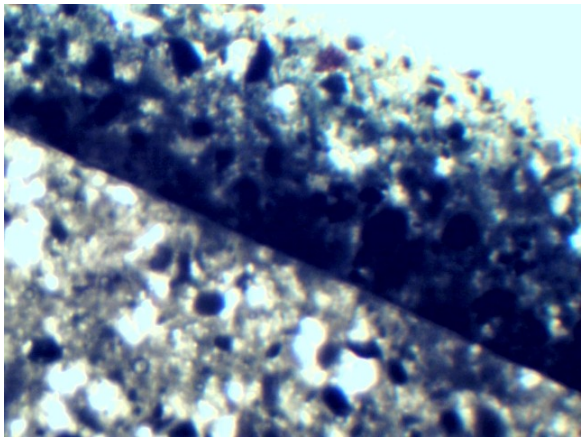
40 40x magnification. Plant particles up to 0.5 mm
41 40x magnification. Plant particles up to 1.5 mm
42 40x magnification. Plant particles up to 0.8 mm



Ingredients:
Henna, Amla, Kali Harar, Tulsi, Brahmi, Bahera, Lodh, Jamun, Neem, Chandan, Kattha, Shikakai, Bhringraj

43

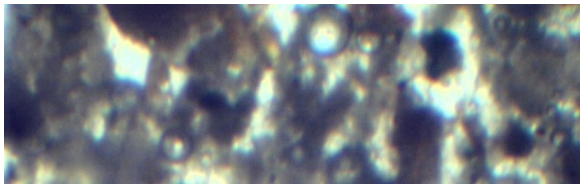
The above henna powder has particles consistent with henna and other declared plant material. The large red particle is consistent with acacia concinna (shikakai).

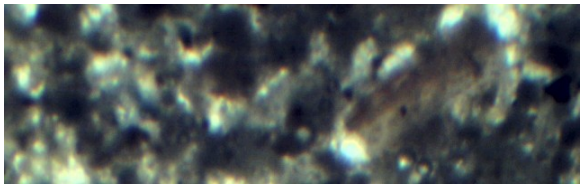


Ingredients: Indigofera tinctoria, Lawsonia Inermis, Emblica officinalis , Eclipta alba, Azadirachta indica, Bacopa monnieri, Vetiveria zizanioides.

44

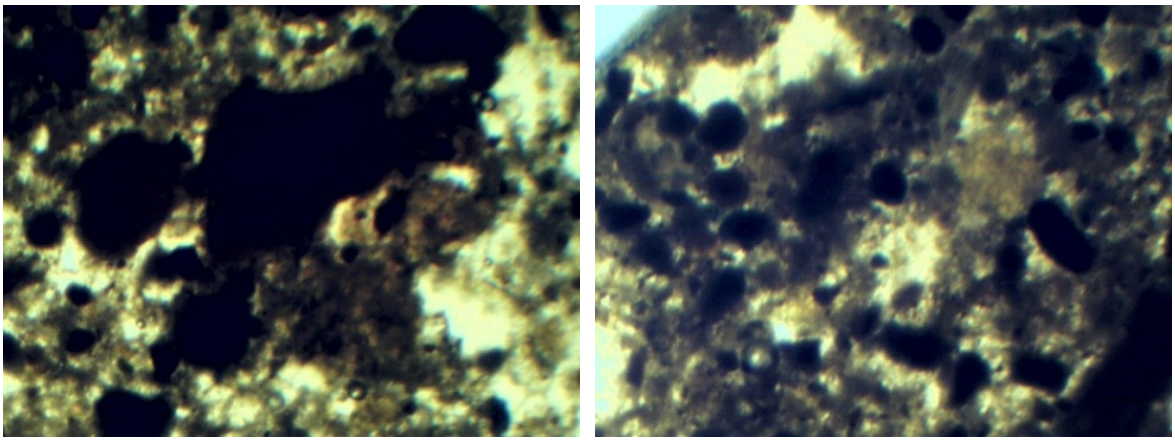
The above 'black' henna powder contains particles consistent with indigo, henna, and other declared plant material. At the edge of the slip cover, indoxyls oxidize to blue indigo within four minutes of being wetted and coming into contact with air.



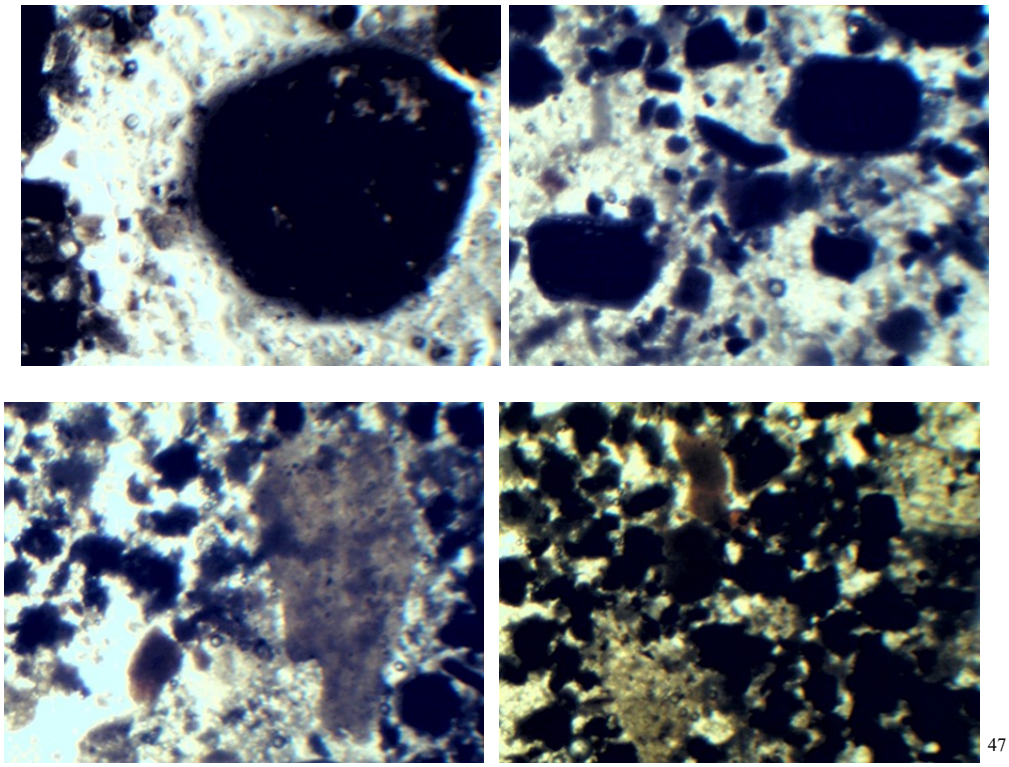


The above light green powder, marketed as 'Marigold Blond Henna' is declared to be cassia obovata (neutral henna) (sic), marigold flowers, and chamomile.[45] Plant particles consistent with the declaration are visible.

43 40x magnification. Plant particles up to 1 mm
44 40x magnification, plant particles up to 3 mm
45 40x magnification, plant particles up to 1.5 mm

 [46]

The above image is of olive green powder marketed as 'henna and natural herbs' to be used for hair dye. Some particles are consistent with pulverized henna leaf, there is no visible evidence of artificial dye, there is sand in the powder, and there are particles of undeclared, unspecified plants.

 [47]

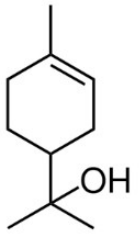
The above henna powder product contained two packets: one of henna (upper two images above) and one of herbs (lower two images above). There was no ingredient declaration. There are particles consistent with henna and other plant material as well as large particles of sand.

46 Magnification 40x, plant particles up to 1.5 mm
47 Magnification 40x, plant particles up to 3 mm

Terpineol

There are henna powders which are brownish and have a strong pine scent like Pine-sol cleaner. The factories which produce and sell these products state that the smell is from powdered terpineol, though I have never found terpineol listed in the ingredient declaration. Some of these products declare that they are henna, citric acid, and "Henna Oil, Mu grade." Henna essential oil smells like henna leaves, not terpineol, so something other than henna essential is used. This formula is used to make henna that only needs to have water stirred into it and can be applied immediately to skin, with no wait for dye release. The skin stain is quick, but tends to also fade quickly, not lasting as long a stain with a slow release of aglycones and longer application.

Terpineol is a monoterpene alcohol that has been isolated from a variety of sources such as tea tree essential oil, eucalyptus oil, cajuput oil, pine oil, and petitgrain oil. Anhydrous Terpineol, which seems to be what is being added to these henna powders,[48] is the terpineol alpha-isomer, a white crystalline powder.[49] Though many plants produce terpineol, the powder is more often produced from hydration of alpha-pinene or turpentine oil with aqueous mineral acids to give crystalline cis-terpin hydrate (mp 117 deg C), followed by partial dehydration to alpha-terpineol.[50]



Alpha-terpineol: $C_{10}H_{18}O$. Alpha-terpineol is a terpineol that is propan-2-ol substituted by a 4-methylcyclohex-3-en-1-yl group at position 2.

It is not unusual for artists using henna for body art to add essential oils containing high levels of terpineol and other monoterpenes[51] to their paste to achieve a superior stain on non-keratinized skin. Palms and soles are highly keratinized and stain more easily than backs of hands and feet, arms and legs. In the case of body art, the henna is usually dye-released before the addition of the essential oils. The crystalline form of alpha-terpineol is not an unusual component of perfume and cosmetics, but is not easily available to regular consumers.[52]

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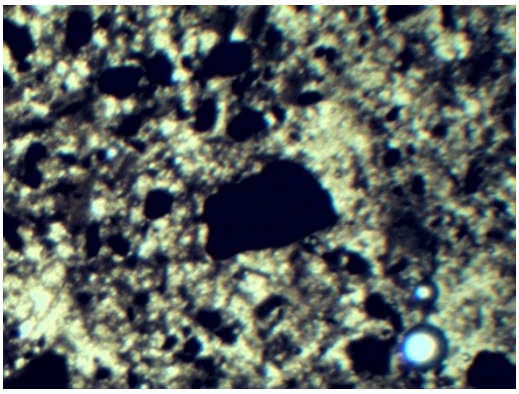
48 Since I have not found this in any ingredient declaration, only in discussions with henna exporters, this is my best etimate.

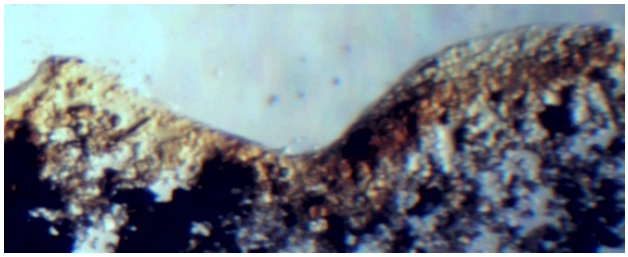
49 Alpha-TERPINEOL; Terpineol. *Pub Chem Open Industry Database*. National Center for Biotechnology Information https://pubchem.ncbi.nlm.nih.gov/compound/alpha-TERPINEOL

50 Fahlbusch K-G et al; Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry 7th ed. (1999-2015) NY, NY: John Wiley & Sons.

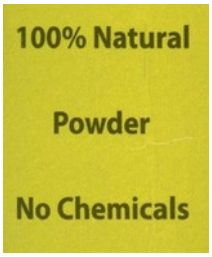
51 The term 'terps' as used in henna body art was invented by myself, Catherine Cartwright-Jones PhD in 2000, because I did not want to type 'essential oils with high monoterpene alcohol content' hundreds of times on the hennapage.com forum and in websites and research papers. This term abbreviated for convenience, 'terps,' has since passed into regular usage among henna artists and businesses.

52 In 30 years of studying henna, I have not heard of any henna artist using crystalline terpineol alpha-isomer in their paste; most use essential oils with high monoterpene alcohol contents.

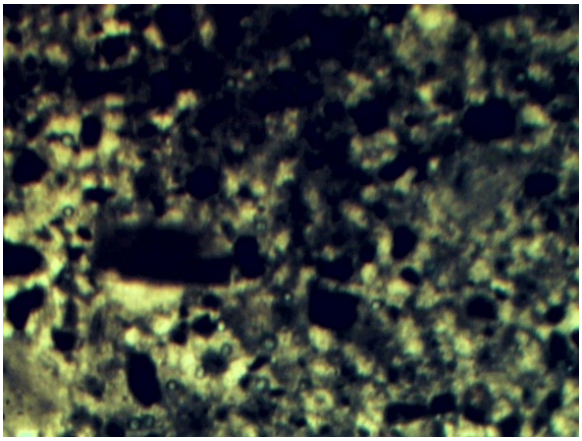


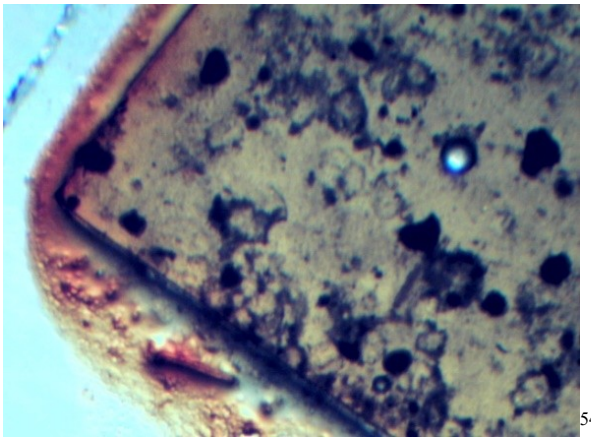


53



The brownish henna powder shown above has the odor of terpineol, so the claim of 'no chemicals' is questionable. The packaging shows color has leached through the interior packet, staining the white cardboard box. Under the microscope there is a visible reaction within two minutes as the henna and water paste releases color in contact with air at the margins of the slip cover.

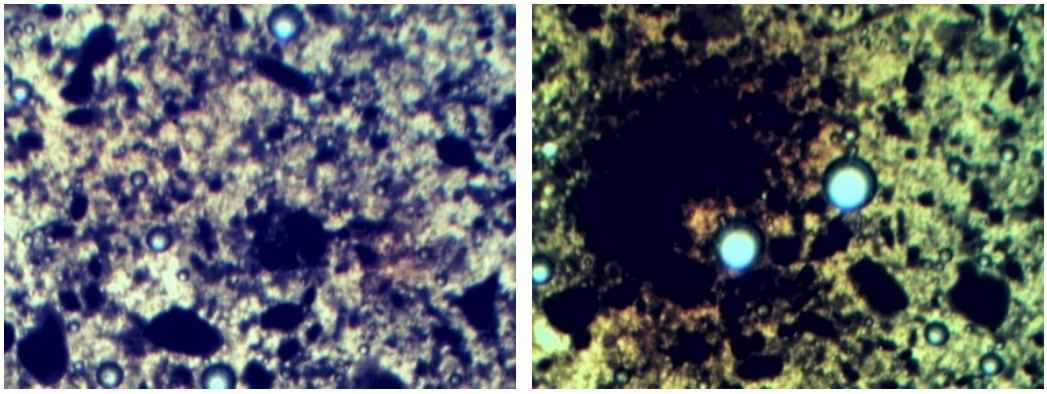




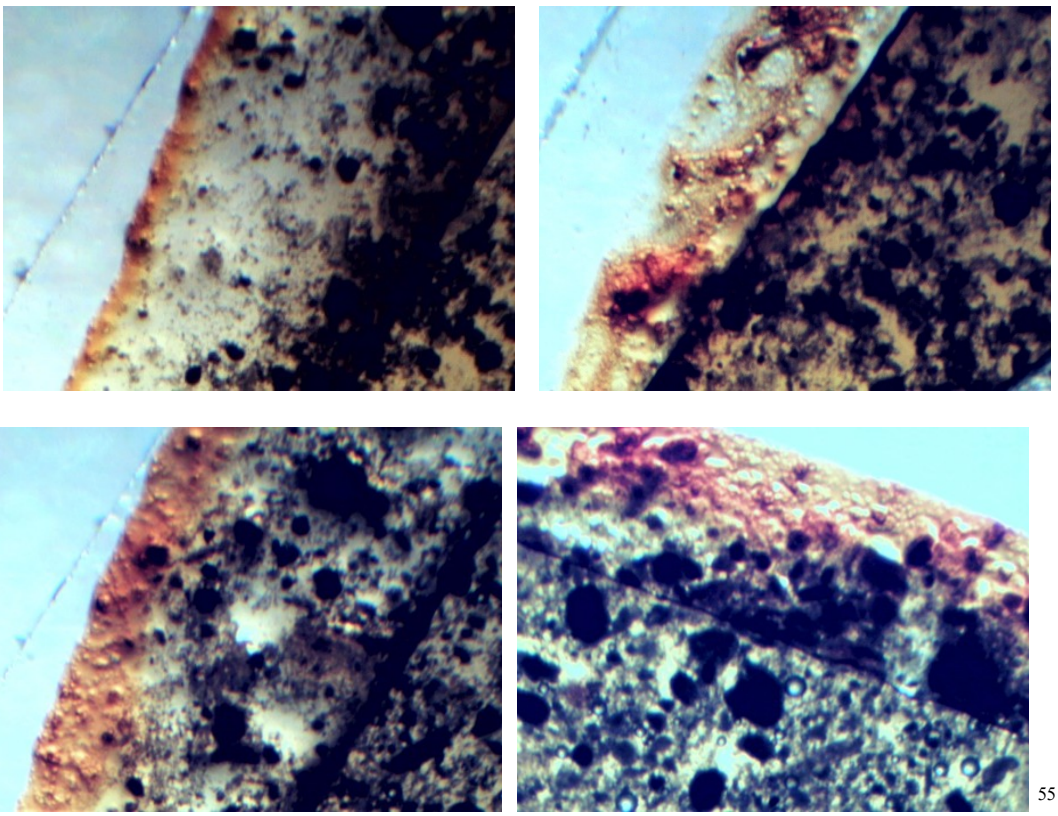
54

The above images show a henna powder which strongly smells of terpineol after mixing with water. Two minutes after mixing, the liquid leaking from under the cover slip forms red pigments in contact with air. The color of the powder is a brownish curry color. The product has no ingredient declaration.

53 40x magnification. Plant particles up to 0.9 mm
54 40x magnification. Plant particles up to 0.6 mm

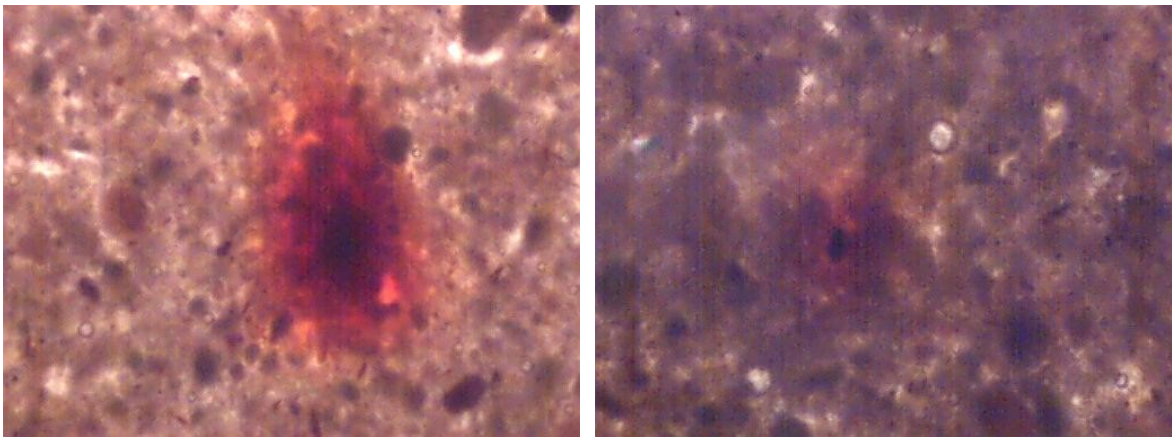


The above images are of an 'instant henna' powder that strongly smells of terpineol releasing orange color during the first minute after adding water, with the orange reaction spreading towards the margins of the microscope slide.



These sequential images show the progress of orange release from a henna product. After two minutes, liquid from the wetted product leaks from under the edge of the cover slip; when in contact with the air, the liquid becomes become vivid red-orange. The plant particles are consistent with henna, but henna alone would not produce this result when wetted for two minutes.

55 40x magnification. Plant particles up to 0.8 mm

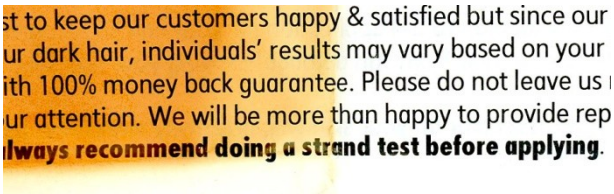
 [56]

Terpineol has been added to henna products to produce 'instant henna' in Pakistan for at least twenty years. The above images are from products labeled as henna and showing henna body art, acquired in 2000.

Red clay

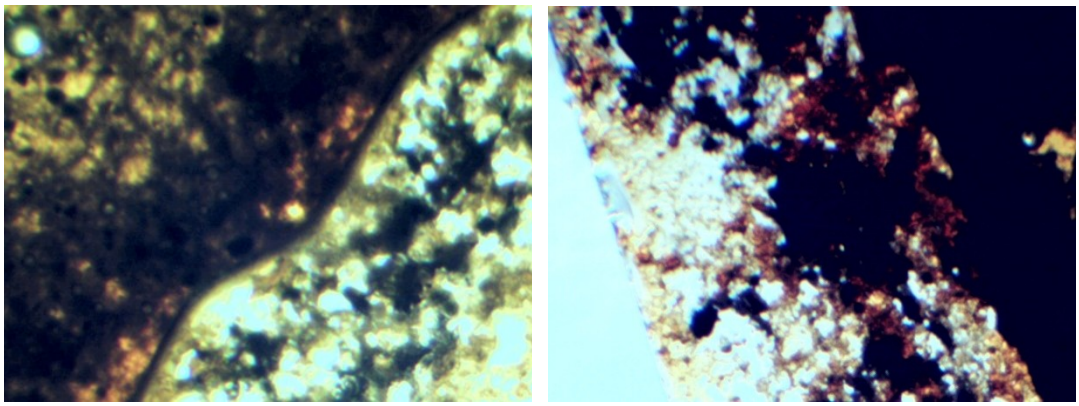
Red, yellow, white, blue, and black clays have been pulverized and used as cosmetic paints by nearly every human group, and were also used by Neanderthals. These clays are fairly harmless on skin except for those which contain lead. Some henna products contain red clay[57] to make hair appear fuller and redder, though the color fades from hair over a few washings. Red clay is not a permanent hair dye. Henna powders with red clay smell like damp red earthenware clay used in pottery class, or a damp red patch of clay on a river bank.

Red clay is a kaolinite;[58] kaolinite itself being white or pale gray dioctahedral phyllosilicate clay. The various colors of clay come from iron and other oxides in the. Clay dust is difficult to expel from lungs, so people who use clay products in their hair should wear a dust mask when mixing.[59] The red clay ochre will leach pigment through an interior plastic bag as seen below, and stains the paper instruction sheet orange; red clay stains cellulose fibers more readily than keratin. While the red will quickly fade from hair, the iron oxide will stain paper, white towels, and shirts.


st to keep our customers happy & satisfied but since our
ur dark hair, individuals' results may vary based on your
ith 100% money back guarantee. Please do not leave us
ur attention. We will be more than happy to provide rep
lways recommend doing a strand test before applying.

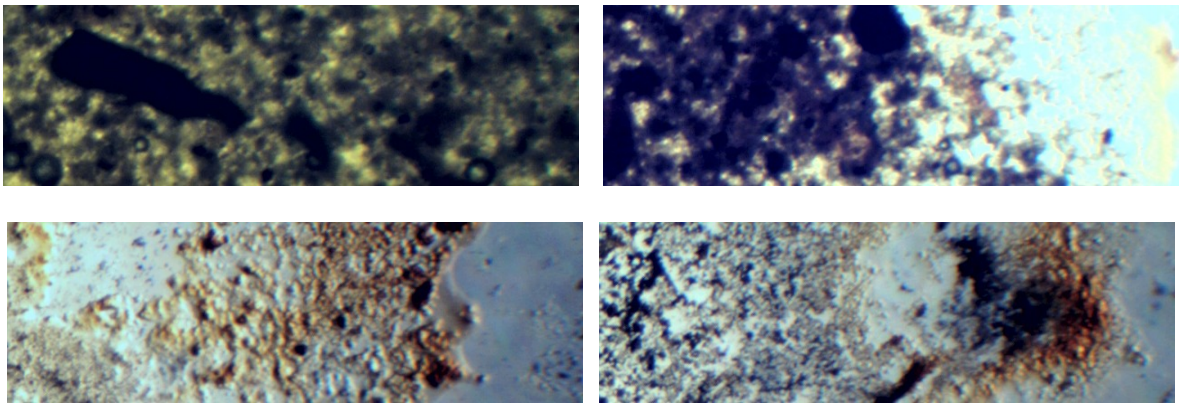
56 'Instant henna' product acquired in 2000. 60x magnification. Plant particles up to 0.2 mm
57 Other kaolin clay products are rassoul, bentonite, montmorillonite or illite. These clays are drying to skin and should not be used more than once per week or by people with sensitive or dry skin.
58 $Al_2Si_2O_5(OH)_4$.
59 Long term long term inhalation of clay dust can cause silicosis, or "potter's rot."

The following henna hair dye product claims 3% red clay in the ingredient declaration; the color of the powder is yellowish olive, but turns red as soon as water is added



The images above show a rapid dispersion of red kaolin in a henna product when mixed with water during a period of one minute. The henna and other plant particles are up to 0.4 mm; the red clay particles are smaller.

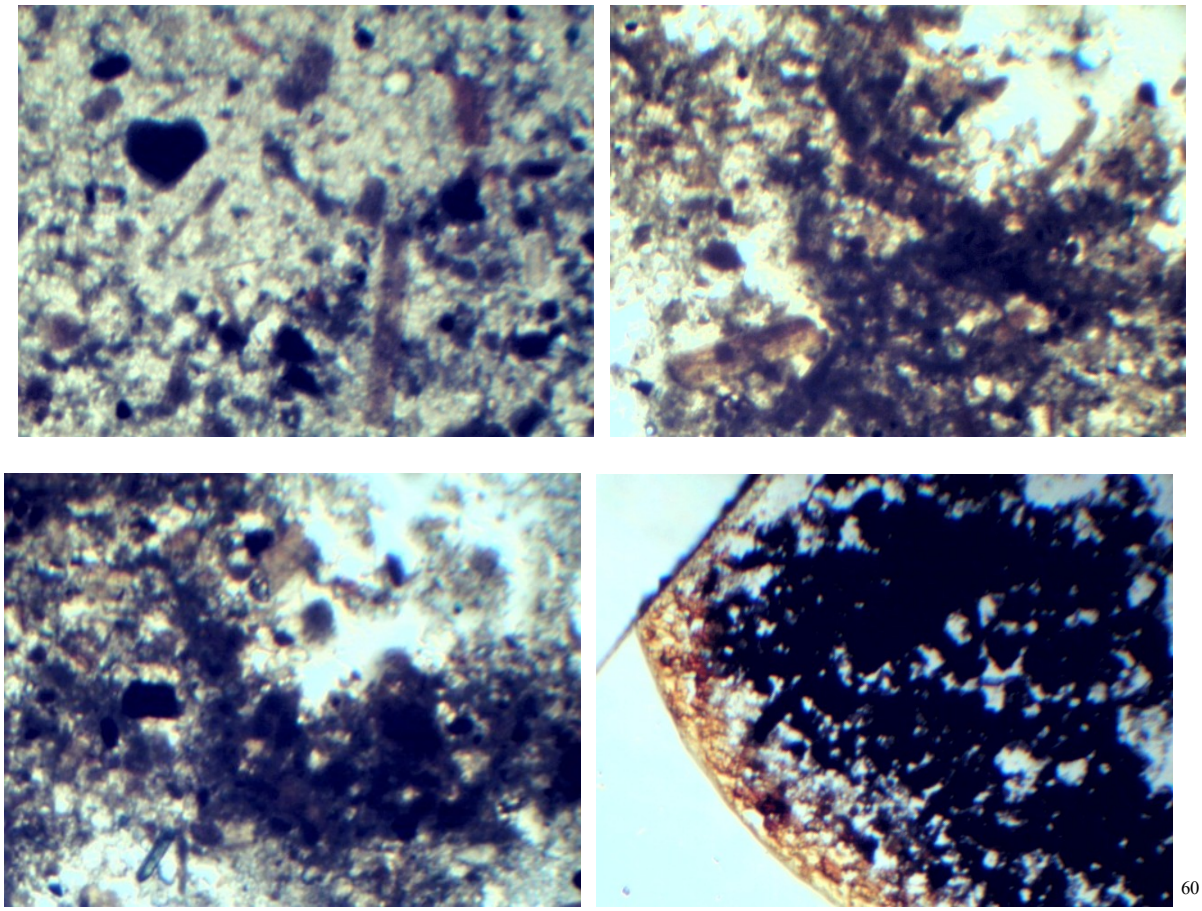
Below is a similar henna-clay product sold as henna hair dye, said to stain for four to eight weeks. The color of the powder is tannish-brown and smelled like damp earthenware clay.



The powder above contains plant particles up to 1 mm, and mineral material that is more finely milled. Red pigment spreads the margins of the cover slip. The contents are declared to be Henna (Lawsonia inermis), Red Clay, False Daisy (Eclipta alba), Myrobalan (Terminalia beffinica), Amla (Emblica Officinalis gaertn), Neem (Azadirachta indica).

Red color release from undeclared source

The following images are of a product labeled henna for hair dye with no ingredient declaration. The powder is olive green with no odor of pine or terpineol; the plastic sleeve shows red stain leaching through. It is advertised as dying hair within twenty minutes.

 [60]

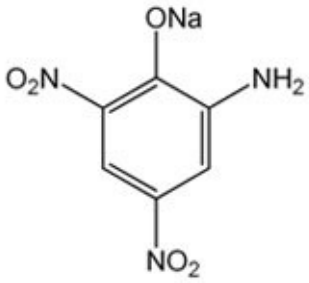
These sequential images show particles consistent with pulverized henna leaf and other plant material, with red-orange pigment emerging within five minutes.

The red-orange material in the 'henna hair dye' images above is water soluble. A drop of the wetted powder leaches out an orange ring on paper. Pure henna powder does not do this. The package claims the material will dye hair in twenty minutes, and that a forty minute application on skin will create a henna pattern. Pure henna takes more time than that. The dispersion in the wetted powder is slower than oxidative dye, so the red is probably not a para-phenylenediamine dye. Since there is no scent, it is probaby not terpineol, and probably not red clay. It is possible that the source of this color is sodium picramate.[61] Sodium picramate is added to hennas to create a cherry red color rather than orange-auburn that is usual for henna. This chemical is highly reactive in contact hair dye developer and sodium picramate is one of the reasons hair stylists disdain henna. When clients come in unhappy with their cherry red home-dyed-from-an-unknown henna product, and neither client nor stylist are aware that the product contained sodium picramate, the initial application of chemicals to correct the color can seriously damage the hair, in extreme instances, nearly incinerating it. In large quantities of pure industrial

60 Magnification 40x, plant particles up to 1.5 mm

61 $C_6H_4N_3NaO_5$

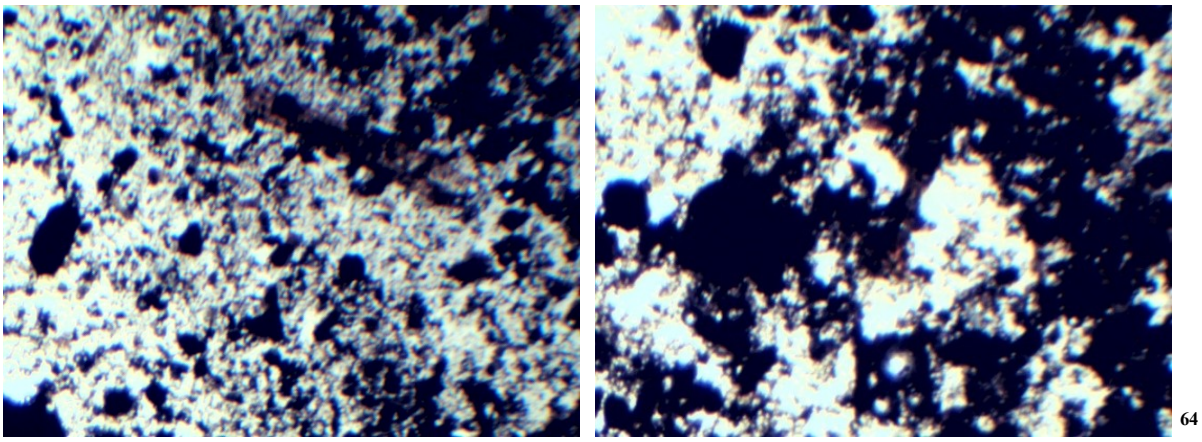
material, sodium picramate can react vigorously with reducing agents, including hydrides, sulfides and nitrides, causing a detonation and throwing fragments 1600 meters.[62]



Sodium Picramate

Hair dye powders marketed as 'henna' which contain little or no henna

A hundred years ago in Europe, the UK, and USA, there were various products marketed under the name 'henna' which contained no henna.[63] 'Henna' was a generic term for hair dye, implying harmlessness, whatever the contents might be. L'Oreal sold henna with undeclared metallic salts to create a range of 'henna colors,' and B. Paul marketed a similar product in the USA. There are still 'henna' products which seem to have very little to do with lawsonia inermis.



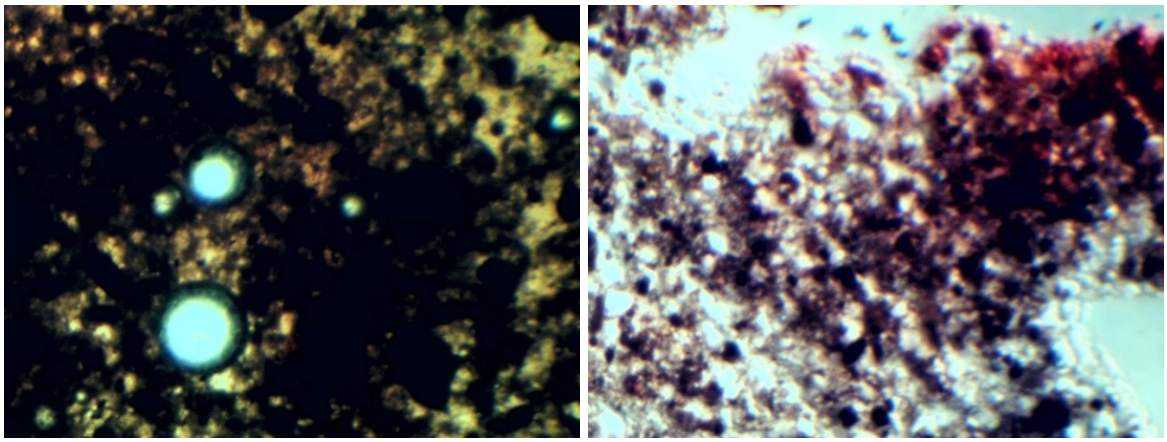
64

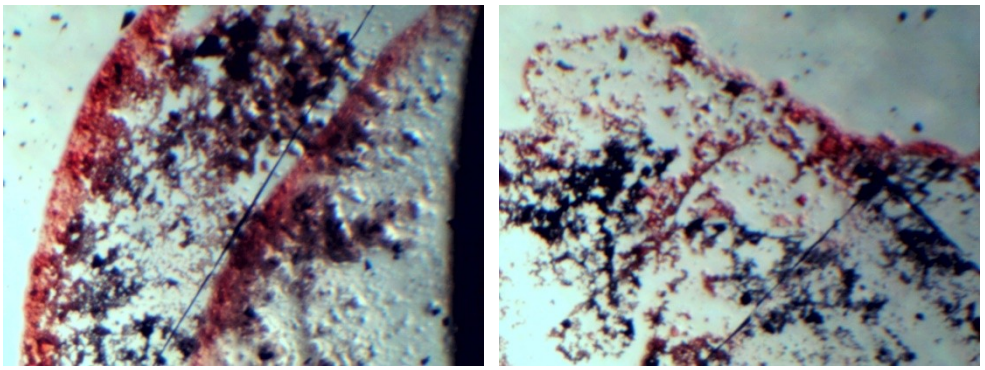
The above image is of a dark brown umber powder sold as black hair dye containing 'henna with natural herbs.' There are particles consistent with henna midrib and sand. The dark brown umber powder turns black when wetted and is difficult to disperse with water. There is no evidence of water soluble dye.

62 Compound Summary for CID 5362461, "Sodium Picramate." *National Center for Biotechnology Information PubChem Open Chemistry Database* https://pubchem.ncbi.nlm.nih.gov/compound/Sodium_picramate

63 Catherine Cartwright-Jones PhD. 2015. "Ancient Sunrise® Henna for Hair Chapter 3, part 2, Henna Mislabeling, Misinformation, and Disinformation" *TapDancing Lizard LLC.* http://www.tapdancinglizard.com/AS_henna_for_hair/chapters/chap3/henna_mislabeling_misinformation.pdf

64 Magnification 40x, plant particles up to 1.5 mm

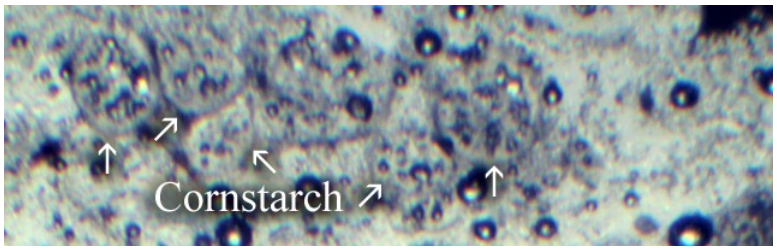




65

The images above are of a henna for hair product marketed as ‘henna and natural herbs,’ declaring lawsone (presumably henna) and mannite (possibly tamarisk) as the dye ingredients. The product is a brick-red dry powder that becomes vivid beet-red when wet.

The Nestle Le Mur product shown below was labeled ‘henna’ but contained more lactose and cornstarch than henna.



66

Cornstarch particles after Egyptian Natural Henna Conditioner” is wetted.

The ingredient declaration of “Nestle Egyptian Natural Henna Conditioner” above and below, claimed “Lactose, Corn Starch, Hydroxypropyl Methyl Cellulose, Quaternium-5, and Henna.”

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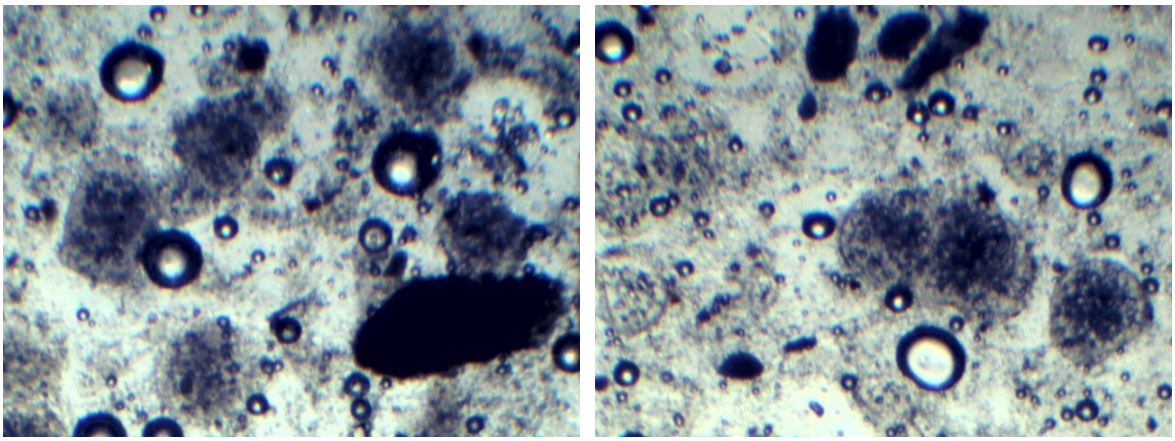
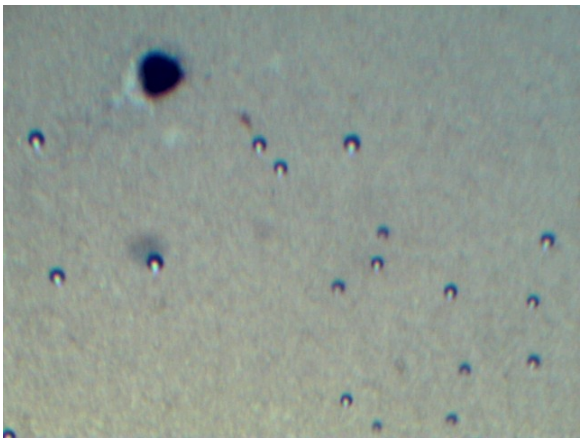
65 Magnification 40x, largest plant particles are 0.6 mm.
66 Magnification 40x, largest plant particles are 1.5 mm.

 [67]

The above image shows a few plant particles consistent with henna mixed with a greater amount of cornstarch particles. This product was sold in the mid to late 20th century in the USA, alongside two other Nestle Le Mur henna products, one of which was henna, and a 'black henna' product that contained cornstarch and black iron oxide.



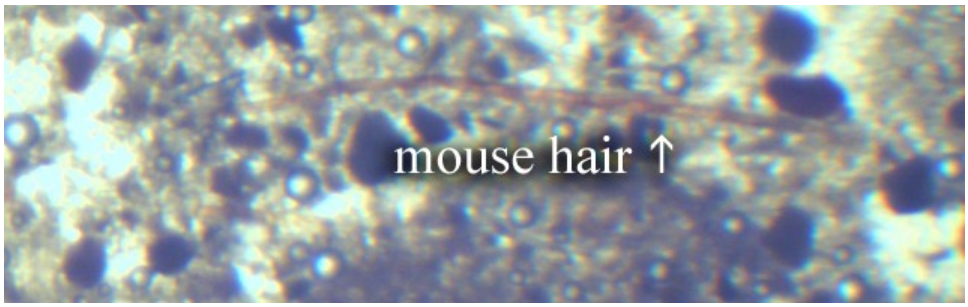
Ingredients: Aqua, Propanediol, Cetearyl Alcohol, Cetrimonium Chloride, Alcohol, Dimethyl Palmitamine, Benzyl Alcohol, Dehydroacetic Acid, Sorbitol, Orbignya Oleifera Seed Oil, Tocopherol, Hydroxyethylcellulose, HC Blue N° 2, Sodium Phytate, Achillea Millefolium Extract (Achillea), Aloe Barbadensis Leaf Extract (Aloe Vera), Arnica Montana Flower Extract (Arnica), Bertholletia Excelsa Seed Extract (Brazil Nut), Corylus Avellana Seed Extract (Hazelnut), Daucus Carota Sativa Root Extract (Carrot), Euterpe Oleracea Fruit Extract (Açai), Genipa Americana Fruit Extract (Genipapo), Lawsonia Inermis Leaf Extract (Henna), Malpighia Glabra Fruit Extract (Acerola), Malva Sylvestris Leaf Extract (Malva), Paullinia Cupana Seed Extract (Guaraná), Phyllanthus Emblica Extract (Amla), Zizyphus Joazeiro Bark Extract (Juá), Chamomilla Recutita Flower Extract (Chamomile), Aminomethyl Propanol, Disperse Black 9, Disperse Violet 1, HC Yellow N° 4, 4-Amino-3-Nitrophenol.

The above product, marketed as "Henna Cream" does not appear to have any actual henna plant fragments in it, though 'henna extract' is claimed in the declaration. Any color in this mixture would be from the synthetic colors, HC blue Nº 2, disperse black 9, disperse violet 1, HC yellow Nº 4, not from henna or henna extract.

Unintended objects in henna

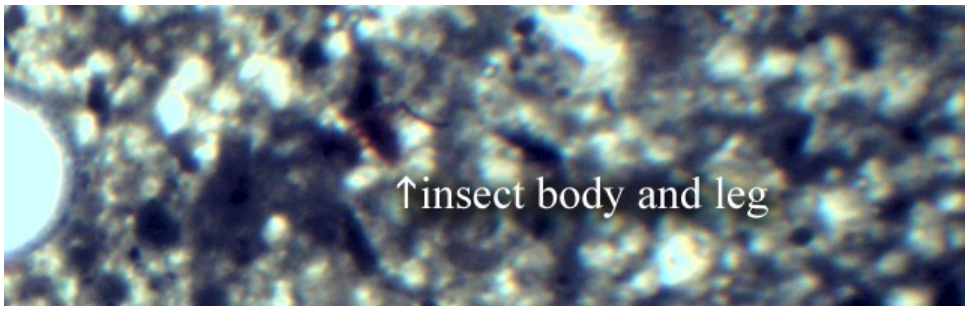
The occasional presence of unintended objects in henna is inevitable; henna is neither sterile nor a synthetic chemical product. Henna is an agricultural product world, and some things wander in. The following images are a reminder of why you should not eat henna, not drink henna tea, not put it on abraded skin, and not try to use henna as eyeliner.

67 Magnification 40x, largest plant particles are 1.5 mm.



68

Things accidentally fall into the henna production line in the factories.



69

Henna is only to be sold in the US as a hair dye according to USFDA regulations,[70] and is required to be leaf and petiole plant material from Lawsonia alba, (Lawsonia inermis L.) and is to be free from admixture with material from any other species of plant. Virtually all henna for hair products on the market in the USA are mixtures of henna and other species: Ancient Sunrise® henna is not mixed with any other plant species. As previous chapters of "Ancient Sunrise® Henna for Hair" show, the mixtures of henna and indigo powder together do not give optimal results because they require different pH, and different preparation times for dye release. Most of the other plants, artificial dyes, and minerals added to henna mixtures give temporary color at best.

Ancient Sunrise® henna for hair. We prove the purity.

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68 Magnification 40x, largest plant particles are 0.5 mm.
69 Magnification 40x, largest plant particles are 1.mm.
70 [Code of Federal Regulations] [Title 21, Volume 1] [Revised as of April 1, 2018] [CITE: 21CFR73.2190] TITLE 21-FOOD AND DRUGS CHAPTER I-FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN, SERVICES, SUBCHAPTER A—GENERAL PART 73 - LISTING OF COLOR ADDITIVES EXEMPT FROM CERTIFICATION, Subpart C—Cosmetics, Sec. 73.2190 Henna.

Ancient Sunrise® Henna for Hair Chapter 4: Science and Microscopy

Part 3 : The Microscopy of Henna

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