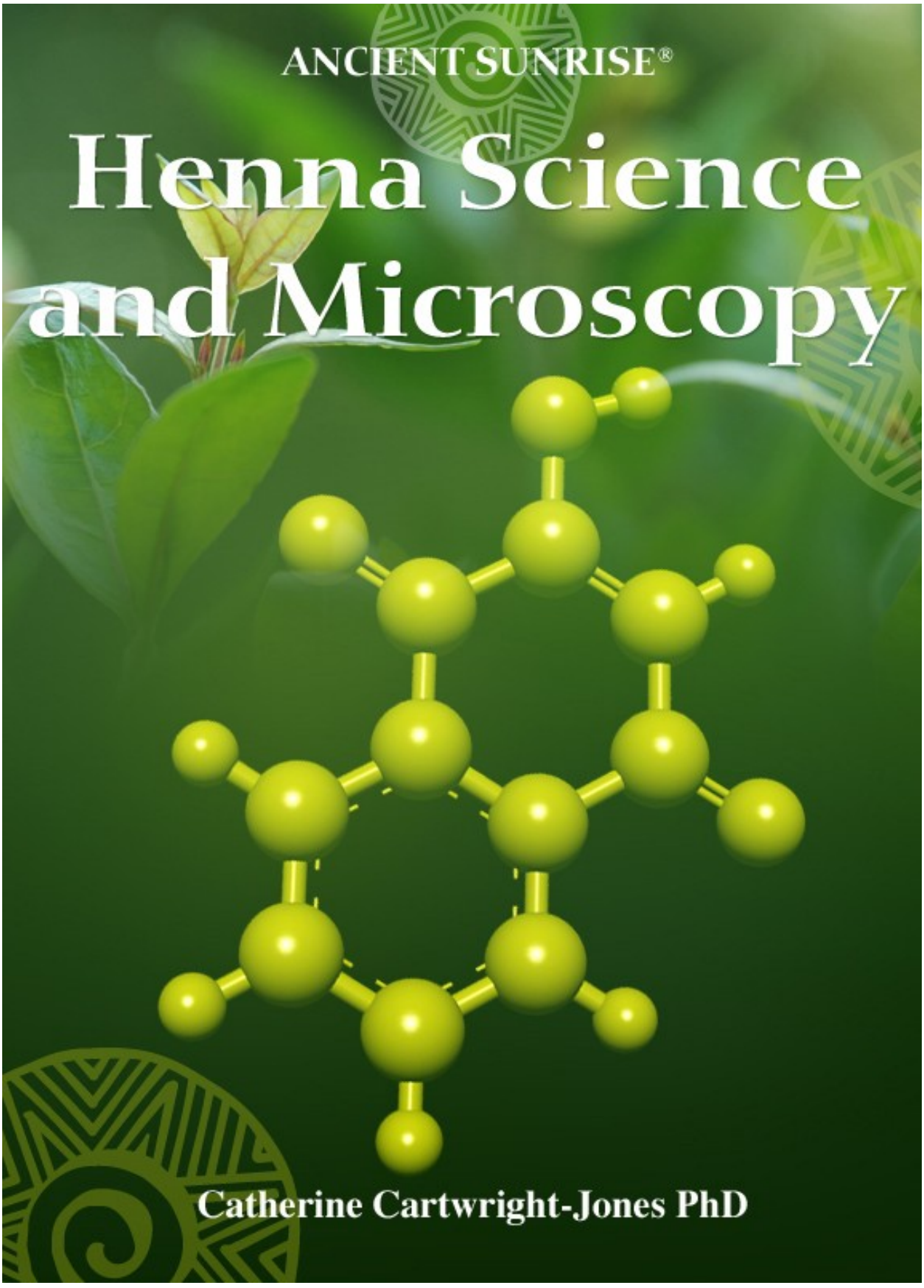

Ancient Sunrise® Henna for Hair Chapter 4: Henna Science and Microscopy

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Chapter 4: Henna Science and Microscopy

Part 1: The Botany of Henna

Species and Dispersion

Henna, *Lawsonia inermis* Fam. *Lythraceae* at dawn after a summer rainstorm[1]

Henna is *Lawsonia inermis* Fam. *Lythraceae*, a monotypic genus, the single example being *L. inermis*,[2] native to North and East Africa, introduced and cultivated in the Persian Gulf region, the Arabian Peninsula, the Levant, and South Asia.[3] It grows in semi-arid tropical zones, and tolerates dry soil and extended drought. It does not tolerate frost and thrives where temperatures are between 11C and 45C.

Henna is a biennial dicotyledonous herbaceous shrub-like desert tree. A henna tree will naturally grow six to twenty feet in height, though under cultivation, the tree is pruned once or twice a year to a short bush to produce more henna leaves per hectare. The leaves are smooth, opposite, sub-sessile, elliptically-shaped and broadly lanceolate, with depressed veins clearly visible on the dorsal surface.[4]

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[1] Henna grown by Catherine Cartwright-Jones Phd, photograph by Roy Jones

[2] Scientific classification: Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Myrtales; Family: Lythraceae; Genus: Lawsonia; Species: L. inermis;

[3] Semwal, R. B, D. K. Semwal, S. Combrinck, C., Cartwright-Jones and A. Viljoen. (2014) "Lawsonia inermis L. (henna): Ethnobotanical,phytochemical and pharmacological aspects." *Journal of Ethnopharmacology* 155(1): 80-103.

[4] Kumar, S., Singh, Y.V., Singh, M., (2005) "Agro-history, uses, ecology and distribution of Henna (Lawsonia inermis L. syn. Alba Lam)." *Henna: cultivation. Improvement and Trade*, 11–12.

Young henna leaves have sub-sessile opposite lanceolate leaves with depressed veins on dorsal surface. [5]

Merck's Report, Volume XXIX, 1920,[6] notes that nineteenth century western botanists erroneously claimed that the one species, L. *alba,* had two varieties, L. *inermis* (unarmed) and L. *spinosa* (with spines). This confusion arose from the fact that the young henna plants are devoid of spines while the older plants have branchlets that harden into spines: they were both the same plant at differing stages of maturity.

During the quaternary glaciation, henna would have had the best chance for survival in the semi-arid frost-free areas in Africa. The single genus implies a relatively recent genetic bottleneck from henna withdrawing to a relatively small survival area during the most recent glacial period, probably along 15° N, across what is presently the Sahel. During the Neolithic Subpluvial,[7] the henna growing range is likely to have expanded across the greening Sahara when it was a savannah, when Africa's seasonal monsoons shifted slightly north.[8] People living across that region probably first became familiar with henna during that period; henna was certainly used in Egypt, at the eastern edge of the region, to mask graying hair around 3000 BCE.

During the Holocene climate optimum, henna spread northward to the Mediterranean coast, probably spread by birds which consumed henna berries and excreted the seeds as they flew northward out of Africa along wadis (seasonally dry riverbeds) and oases. As the North African climate warmed and became arid, henna was reduced to the Saharan oases and wadis and was pushed to the perimeters of the region where it grows now: the Mediterranean rim of North Africa, the Atlantic coastal region of Africa, the Sahel, and the eastern coast of Africa. Since henna is a tree, producing flower and seed only after five years, and the tree's lifespan is about fifty years, a warm, dry climate must be sustained for decades without interruption for the species to survive. The proposal that henna is native to North African tropical semi-arid zones is supported by the relative genetic diversity of henna in African Oases and wadis.[9]

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[5] Henna grown by Catherine Cartwright-Jones, 1200 dpi scan

[6] Benjamin H. Hoffstein, (1920) "NOTES ON HENNA: Comprehensive Digest of History and Uses" *Merck's Report*, Volume XXIX, P. 140

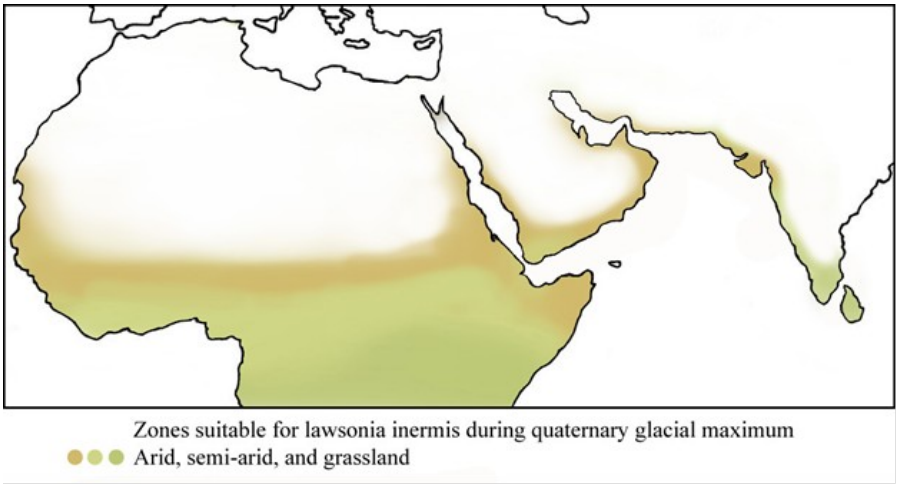
[7] 7500–7000 BCE to about 3500–3000 BCE

[8] Sereno et al. (2008) "Lakeside Cemeteries in the Sahara: 5000 Years of Holocene Population and Environmental Change." *PLoS ONE*; 3 (8)

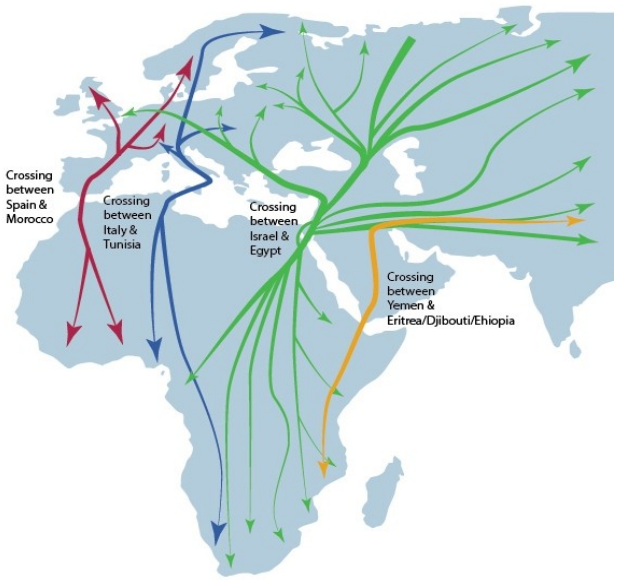
[9] Anissa Boubaya, Hédia Hannachi, Nidhal Marzougui, Tebra Triki, Ferdaws Guasmi, Ali Ferchichi (2013) "Genetic diversity assessment of Lawsonia inermis germplasm in Tunisian coastal oases by ISSR and RAPD markers" *Dendrobiology* 69, p. 31 - 39

The mosaic of frost-free semi-arid zones would have varied over many of years during the Last Glacial Maximum (LGM),[10] but the general area of potential survival was greatest across the width of Africa. This may account for *Lawsonia* being a monotypic genus: variants may have been lost during the LGM. Deglaciation began in the Northern Hemisphere at approximately 20 ka; and henna gradually moved northward and eastward during warming periods.

[11]



Area hospitable to henna during the Last Glacial Maximum (LGM) 26.5 ka BP



Bird migration routes suitable for transmitting henna seed from frost-free zones in Africa into north and eastern regions to the perimeter of frost-free regions[12]

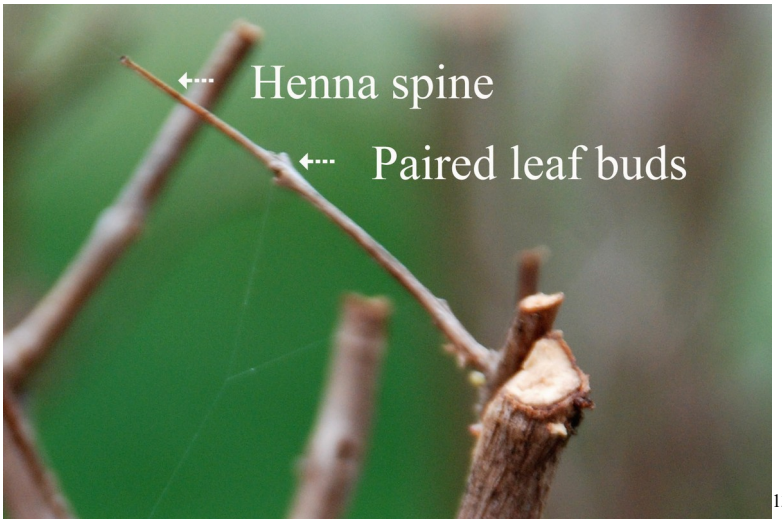
[10] deMenocal, Peter; Ortiz, Joseph; Guilderson, Tom; Adkins, Jess; Sarnthein, Michael; Baker, Linda; Yarusinsky, Martha (2000-01-01). "Abrupt onset and termination of the African Humid Period: rapid climate responses to gradual insolation forcing". *Quaternary Science Reviews*. 19 (1–5): 347–361

[11] Map by Catherine Cartwright-Jones PhD

[12] WysInfo Docuwebs (2014) "Birds Without Boundaries: Migratory, Nomadic & Other Wandering Birds" http://wysinfo.com/Migratory_Birds/Migratory_Birds_Without_Boundaries.htm accessed September 2018

There is evidence consistent with henna growing around the Mediterranean coast during the Bronze Age, particularly in texts and artifacts from the Levantine coast. The presence of henna was probably the result of bird-spread henna seed excreted during migrations from the Sahel along the Nile northward as the climate warmed. It is possible that around 5000 BCE, henna had found suitable habitat as far north as Anatolia; it was probably in the Cycladic islands, and certainly in Crete and the areas that are now the coastal plain of Syria, Jordan, Lebanon, and Palestine in the Bronze Age. Henna was used to mask gray hair in Egypt by 3000 BCE. During the Mid-Holocene Warm Period, henna gradually spread northward into Sicily and coastal Spain, as well as eastward into the Arabian Peninsula, later to the coastal regions of the Persian Gulf.

The growth of henna in South Asia occurred after the western areas became seasonally dry. During the glacial retreat, South Asia was more damp, cool, and forested that it is now. It is possible that henna was introduced by Muslim traders, as it was in Australia, the Philippines, and Indonesia. Henna is not native to any part of the western hemisphere. Henna was introduced to the Americas through bonded West Indian sugar plantation labor from India. Henna can be cultivated as an ornamental bush in Florida, or as a potted plant brought inside during cold weather in other areas of North America.



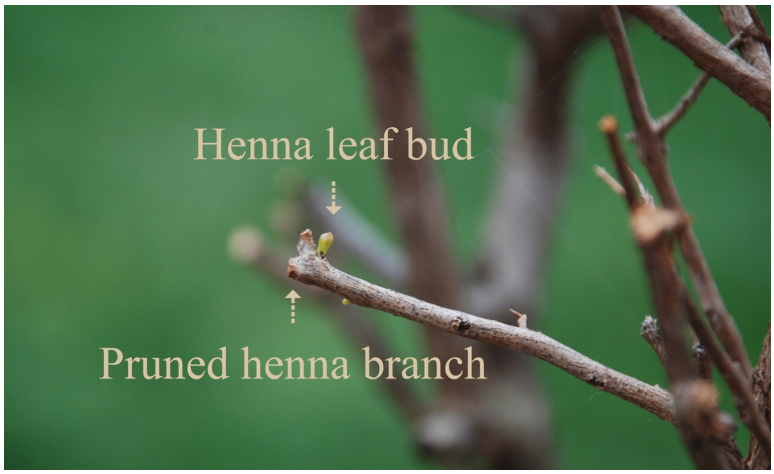
[13]

Henna can remain semi-dormant and leafless during extended drought. As the tree matures, branches develop spines on the opposing budding tips, an effective defense of leaf buds against hungry animals when the plant is dormant in a semi-arid zone ecosystem. A hungry browsing animal reaching out with its tongue for leaves would be deterred by the spine.

The Pali district in India grows henna as a commercial crop with 450 mm of rain per year and no additional irrigation. The pH of the deep, sandy soil ranges in this area from 7.7 to 9.0. The dry, sunny weather has relative humidity averages less than 50%, and 30 – 35 °C average daily temperatures during its period of active growth following the monsoon. This greater seasonal aridity, sun, and heat foster higher leaf dye content at harvest.[14] The October - November harvest

[13] Henna plant grown by Catherine Cartwright-Jones PhD, photograph by Roy Jones
[14] Rao, S. S.; Regar, P. L.; and Singh, Y. V. (2005) "Agrotechniques for Henna (*Lawsonia Inermis* L.) Cultivation" *Henna, Cultivation Improvement and Trade*. P. 25 Central Arid Zone Research Institute, Jodhpur, India.

following the withdrawal of the monsoon rains at the end of September has the highest dye content of the year according to samples tested.[15]



[16]

In wild growth, nibbling by animals such as gazelles is followed by greater density of the foliage which returns rapidly after the onset of rain. Heavy pruning in agricultural harvesting takes advantage of this characteristic: a hectare of pruned henna produces more leaves per season than unpruned henna.


[17]

Growth following rain in a warm season is rapid, with shoots growing more than one inch per day from pruned branches.

[15] Singh, M.; Jindal, S. K.; Kavia, B. L.; Chand, J.; and Chand, K.. (2005) "Traditional Methods of Cultivation and Processing Henna" P. 24 *Henna, Cultivation Improvement and Trade*. Central Arid Zone Research Institute, Jodhpur, India.
[16] Henna plants raised by Catherine Cartwright-Jones PhD, photography by Roy Jones
[17] Ibid

[18]

New henna growth after a summer rain

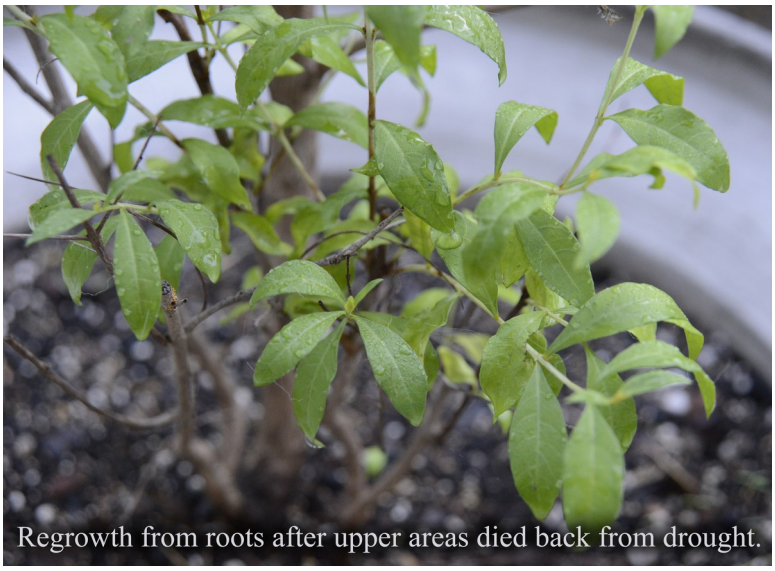
[19]

The vivid red in the new leaves diminishes as leaves mature and turn green.

Henna will survive seasonal droughts, and is valuable to farmers who need to have a reliable cash crop on marginally cultivatable areas with little or no irrigation.[20] Decreased water resource results in smaller leaves and a general reduction in leaf volume and stem length, though a henna crop may still be profitable after all other crops fail in a drought.[21] If the whole upper part of the henna tree is damaged by prolonged drought, there may be regrowth from roots under the soil surface as shown above. Henna bushes can be pruned for productive harvests from the third year to the twentieth year, though the plants can remain in the ground to hold the soil and have smaller harvests as long as fifty years. Most farmers in India who depend only on rainfall harvest

[18] Henna plants raised by Catherine Cartwright-Jones PhD, photography by Roy Jones
[19] Ibid
[20] Thakur, P. S. and R. Sood. (2005) "Drought tolerance of multipurpose agroforestry tree species during first and second summer drought." *Indian Journal of Plant Physiology* 10(11): 32-40.
[21] Enneb, H., A. Belkadhi, and A. Ferchichi1. (2015). "Changes in henna (Lawsonia inermis L.) morphological traits under different deficit irrigations in the southern of Tunisia." *Plant Science Today* 2: 2-6.

henna once a year following the monsoon. Farmers with irrigation and access to nitrogen supplements for their crops can harvest two or three times a year.



Regrowth from roots after upper areas died back from drought.

A henna plant may drop all of its leaves during extreme drought stress, but may have renewed leaf growth after the rains return. Even if the plant completely dies, the roots will stay tenaciously in the soil for years afterwards. Living, the henna acts as a hedgerow or windbreak preventing wind erosion and desertification.

[22]

The plant is deeply rooted and holds soil tenaciously against soil erosion.

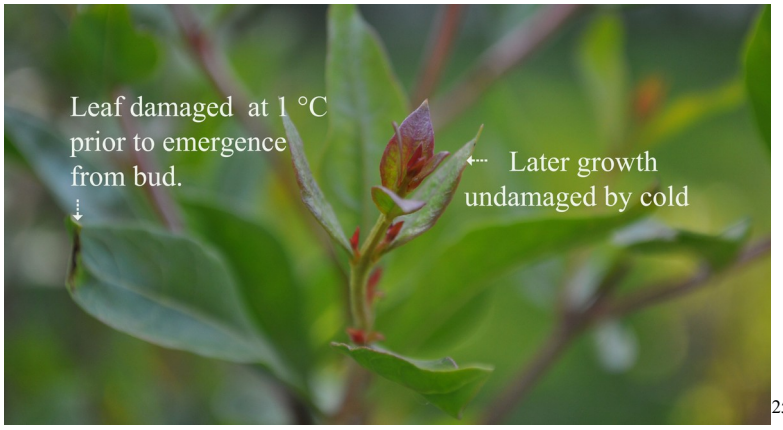
Farmers in the northern part Andhra Pradesh, Orissa and Chhattisgarh states in India planted henna to create living fences, and to protect their cultivated fruits and vegetables. Henna is also planted as a property boundary around their homesteads. As henna will remain in the ground for

[22] Henna plants raised by Catherine Cartwright-Jones PhD, photography by Roy Jones

fifty years, and is harvestable as a cash crop through that period, it is a sensible practice. Regular pruning of these henna fences maintains dense growth which helps to prevent browsing animals (both domestic and wild) from pillaging or trampling their crops.[23] The root structure of henna will stabilize the soil against wind erosion for years beyond the marketable crop life.

In Mali, farmers had used dead wood, often *zizyphus Mauritania*, to create fences but were encouraged to try living fencing to enclose and protect their crops by the International Centre for Research in Agroforestry. Henna was the most commonly chosen plant for farmers to create living fences as cultivation was simple and the plant was familiar. They would prune the branches, bring them to the house, strip the leaves from the branches to dry them in the sun, then crush and grind the leaves into powder which they could sell in the market or keep for family use. Young henna bushes were planted half a meter apart alongside the previous dead wood fences for protection during the first few years. When pruned, the increasingly dense of henna fence effectively deterred marauding animals from their garden during the dry season.[24] It should be noted that animals which did nibble on henna leaves had orange stains on their lips, resembling lipstick.

Henna during a period of climate change



[25]

During the Medieval Warm period, henna was commercially cultivated in southern Spain. In subsequent cooler centuries, henna could not survive snows that fell as far south as Andalusia. Though historical records indicated that henna grew and was used on Cyprus during the Bronze Age, when Victorians enumerated Cypriot flora, they found none and most dismissed the possibility that henna had ever grown there. Henna is damaged by near-freezing temperatures and will not survive being frozen; even barely visible emerging leaf buds are damaged by cold temperatures, and the damage will remain on the leaf tips as the leaf grows. Subsequent growth

[23] Choudhury, P.R.; Rai, P.; Patnaik, U.S.; and Sitaram, R. (2004) "Live fencing practices in the tribal dominated eastern ghats of India" *Agroforestry Systems* 63: 111–123
[24] Levasseur, V.; Djimdé, M.; and Olivier,A. (2003) "Live fences in Ségou, Mali: an evaluation by their early users" *Agroforestry Systems* 60: 131–136.
[25] Henna plants raised by Catherine Cartwright-Jones PhD, photography by Roy Jones

emergent during warmer nights does not show damage. Henna thrives in areas where the minimum temperature never falls below 11 °C.[26]

 [27]

Henna leaves exposed for four hours to -2 ° C withered and dropped; the plant did not recover.

At the time of this writing, climate change is beginning to have an impact on the cultivation of henna, though not necessarily adverse. Seasons of drought and heat produce higher lawsone content in henna crops; moist, fertile soils produce lower level lawsone content.[28] In my experience in HPLC testing henna crops for lawsone over the last ten years, El Niño–Southern Oscillation (ENSO events correlate with the highest lawsone content henna crops from the Punjab and Thar Desert, possibly correlating with the reduction in rainfall resulting from El Niño during the latter half of the monsoon season. La Niña crops have lower lawsone content, and Trans-Niño conditions yielded the lowest lawsone content henna crops.[29]

As climate changes progresses, the periods of drought are often longer and hotter, and torrential rains are more extreme and destructive. The 2010 flooding in Pakistan destroyed henna plantations by prolonged inundation; much had to be replanted. As climate warms, the boundary of Arctic Vortex becomes unstable, and unexpected flow of cold air can invade areas that normally support henna, such as the cold wave in Rajasthan during the month of January 2017, dropping temperatures to near freezing. May of the previous year had extremely high temperatures in the area, rising to 52 °C (125 °F). Henna trees, remaining in the ground for up to fifty years, will be vulnerable to increasingly extreme temperatures.

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[26] Kidanemariam, T. K., T. K. Tesema, K. H. Asressu and A. D. Boru. (2013) "Chemical investigation of Lawsonia inermis L. leaves from Afar region, Ethiopia." *Oriental Journal of Chemistry* 29:129–134.
[27] Plants and photograph by Catherine Cartwright-Jones PhD
[28] Yogisha, S., Samiulla, D.S., Prashanth, D.,Padmaja R. and Amit A. (2002) "Trypsin inhibitory activity of Lawsonia inermis." *Fitoterapia*. 73: 690–691
[29] Observation from HPLC lawsone tests 2008 - 2018 by Catherine Cartwright-Jones PhD. This does not include testing all henna crops from all plantations, but is an observation based on over batch tests.

Limitations on irrigation in Egypt due to climate change and variations in the amount of water flowing into the Nile from eastern African monsoonal rains may improve the outcome for farmers who choose henna crops; when soil is salinized from irrigation, henna can remain a cash crop in the Aswan and other irrigated areas when other crops are no longer remunerative.[30]

The western Sahel is becoming more supportive of henna during the present climate change and will continue for the probable future based on the warming of the southern Atlantic and Mediterranean.[31] Farmers in Mauritania, Mali, and Northern Nigeria grow henna for local use, but it has not supported by those governments as henna has been encouraged and supported in India. Greening northward into the Sahara is triggered by an increase in summer precipitation, amplified by a positive feedback between vegetation and precipitation. Depending on how fast carbon dioxide increases in the atmosphere, the greening into the Sahara may be rapid, up to one tenth of the desert region per decade, though not reaching half of the total area.[32] Henna could be part of the leading edge of the reclamation of the Sahara. Henna probably sheltered in the Sahel and Sahara three to thirty thousand years ago. Perhaps it will survive the climate change there again.

Propagation

[33]

Henna seeds

In the wild, henna is effectively propagated by birds as they eat henna berries and excrete seeds during their migrations. The percentage of seeds which grow into plants in the wild is not high, but the number of seeds dispersed through excretion is high. In cultivation, henna seeds hard seed coat must be soaked in water for eight to ten days with frequent change of water, and even then only about twenty percent germination can be expected. Soaking in a three percent salt water solution for one day improves germination to seventy percent. Optimal temperature for

[30] Jaimini, S. N.; Tikka, S. B. S; Prajapati, N. N.; and Vyas, S. P. (2005) "Present Status and Scope of Henna Cultivation in Gujarat" *Henna, Cultivation Improvement and Trade*. Central Arid Zone Research Institute, Jodhpur, India.

[31] Giannini, Alessandra, Michela Biasutti, and Michel M. Verstraete. (2008). "A Climate Model-Based Review of Drought in the Sahel: Desertification, the Re-Greening and Climate Change." *Global & Planetary Change* 64 (3/4): 119–28

[32] Martin Claussen, Victor Brovkin, Andrey Ganopolski, Claudia Kubatzki, and Vladimir Petoukhov. (2003) "Climate Change in Northern Africa: The Past Is Not the Future." *Climatic Change* 57 (1/2): 99–118.

[33] Henna seeds, 1200 dpi scan by Catherine Cartwright-Jones PhD

germination is 25 to 30 °C.[34] Conveniently for henna, a birds' digestive system and the water sources that are their rest stops during seasonal migration provide hospitable conditions for henna seeds to sprout and take root. Human cultivation from seed is dependent on finding a means of stabilizing moisture and temperature suitable for germination and growth for many weeks. Farmers in India may sow seeds in nursery beds in March, first soaking the seed in water, and watering daily for six months when they are 30 – 45 cm tall. They then can be planted in their permanent location towards the end of the monsoon in September or October. The crop yield from seed-started is low for the first two to three years, but can be harvested for decades.[35]

Henna cuttings may be rooted without hormone dips with a mean of nearly three quarters of cuttings succeeding. Cuttings in India are most likely to succeed in July with growth boosted by the onset of the monsoons.[36]

Pests and pesticide use

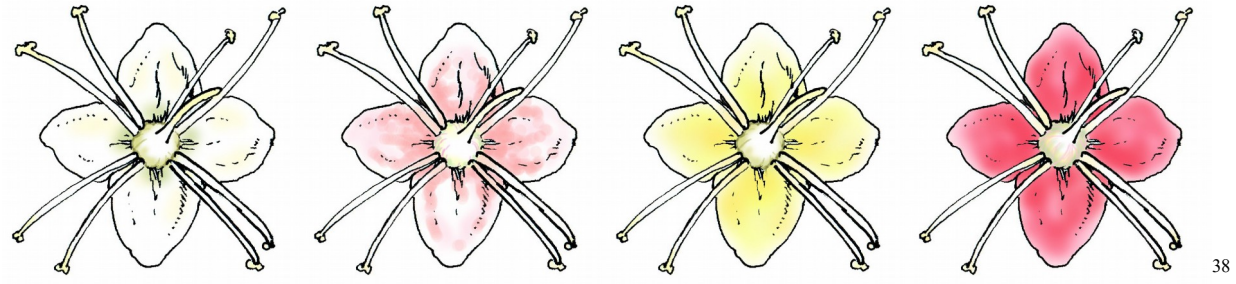
Ladybug eggs on a henna leaf.

Cultivated henna crops rarely are bothered by pests in their native semi-arid tropical climate zones. When newly planted, they may be attacked by termites, and semiloopers may attack adult plants.[37] In damp climates, aphids may overrun henna; ladybugs generally arrive to attack the aphids. I have run LUKE II pesticide tests on every shipment of Ancient Sunrise® henna since 2008, and the residue of pesticide occasionally applied to henna has been synthetic pyrethrin, harmless to humans. However, when henna is planted alongside of other crops, and those are sprayed, the tests reveal residue drifted on the wind from nearby crops. This is a concern when

[34] Rao, S. S.; Regar, P. L.; and Singh, Y. V. (2005). "Agrotechniques for Henna (*Lawsonia Inermis* L.) Cultivation" *Henna, Cultivation Improvement and Trade*. P. 25 Central Arid Zone Research Institute, Jodhpur, India.
[35] Singh, M.; Singh. K. and D. (2005) "Natural Variability, Propagation, Phenology and Reproductive Biology of Henna" *Henna, Cultivation Improvement and Trade*. P. 13 Central Arid Zone Research Institute, Jodhpur, India.
[36] Ibid, p. 15
[37] Narain, P.; Singh, M.; Roy, P. K.; Chand, K.; Sikh, J. and Y. W. (2005) "Production, Trade and Future Prospects of Henna" *Henna, Cultivation Improvement and Trade*. P. 3. Central Arid Zone Research Institute, Jodhpur, India.

henna is planted near cotton fields, where farmers are permitted to use organophosphates. One Luke II pesticide test showed residue from an organophosphate which, though declared illegal in most countries, still found its way into farmers' fields where it was probably sprayed on cotton fields where pests had become resistant to other sprays. When henna is growing near tomato fields, the pesticide drift is relatively benign. When henna is growing near a village or cluster of houses that were sprayed for mosquitoes, DDT may drift onto the crop. In the cases of pesticide drift discovered, the amount remaining on henna was too small to be a health risk, but it does contradict "Organic" claims. Henna is often raised as a hedge around other crops. If adjacent crops are sprayed, wind may carry pesticide onto henna, up to four hundred feet, depending on wind and the height of the sprayer.

Flowers and Seeds


[38]

Henna flowers grow in clusters similar to lilacs comes in several colors: white, pink, yellow, and rose.

Henna flowers comprise four sepals, a 2 mm calyx tube, with white or red stamens present as pairs on the perimeter of the calyx tube, and obvate, "crumbled" petals; The ovary is four-celled, with an erect style. The tree produces small cluster of flowers, then berries containing 32 to 49 angular seeds each.[39] The clusters resemble the flower clusters of lilacs, and are very fragrant. In India, the henna plant flowers form during the monsoon. Some farmers pluck the blossom clusters as they believe the berry formation lowers the leaf and lawsone volumes. Others sell the flowers to the perfume industry.

Flowers are small, white, numerous; in large pyramidal terminal cymes, fragrant, 1 cm across, with 4 petals crumpled in the bud. The calyx has a 2-mm tube and 3-mm spread lobes. The petals are orbicular to obovate, white or red; with eight stamens, inserted in pairs on the rim of the calyx tube. The ovary is 4 celled, style up to 5 mm long, erect. The fruits are small, brown, globose capsules 4-8 mm in diameter, many-seeded, opening irregularly, split into 4 sections, with apersistent style. The seeds are 3 mm across, angular, with thick seed coat.[40]

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[38] Drawings of henna flowers by Catherine Cartwright-Jones PhD

[39] "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects," Ruchi Badoni Semwala, Deepak Kumar Semwala, Sandra Combrinck, Catherine Cartwright-Jones, Alvaro Viljoen. Journal of Ethnopharmacology, June 2014

[40] World Agroforestry Center, (ICRAF) Agroforestry Database 4.0 2009

The henna cultivars most raised in the Pali district of India are yellow-flowering henna, the *desi* and the *muraliya* varieties. The *muraliya* type has a woody canopy with small greyish-green leaves, and the *desi* has a leafy canopy of larger leaves. The *desi* is preferred by producers because of the higher leaf potential.[41]

[42]

Formation of a henna berry from henna flowers

[43]

Initial blossoming of a cluster of henna flowers

[41] Rao, S. S.; Regar, P. L.; and Singh, Y. V. (2005). "Agrotechniques for Henna (*Lawsonia Inermis* L.) Cultivation" *Henna, Cultivation Improvement and Trade*. P. 25 Central Arid Zone Research Institute, Jodhpur, India.
[42] Illustrations by Catherine Cartwright-Jones PhD
[43] White flowering cluster of henna grown by Catherine Cartwright-Jones PhD

 [44]

Red henna flowers are more common in wild North African species of henna.[45]

 [46]

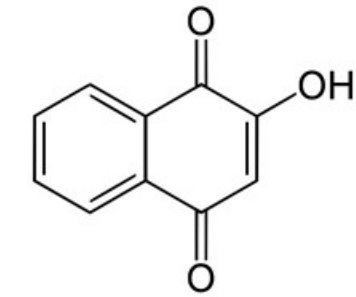
Flower oil contains alpha- and betaionone, the latter being the main component.

[44] Red flowering cluster of henna grown by Catherine Cartwright-Jones PhD
[45] Kidanemariam, T. K., T. K. Tesema, K. H. Asressu and A. D. Boru. (2013). Chemical investigation of Lawsonia inermis L. leaves from Afar region, Ethiopia. *Oriental Journal of Chemistry* 29: 129–134.
[46] White flowering cluster of henna grown by Catherine Cartwright-Jones PhD

Part 2: The Phytochemistry of Henna, Lawsonia inermis L. [Lythraceae]

Lawsone: 2-hydroxy-1,4- napthaquinone and its precursors

Some henna hair dye companies have claimed that all of the various colors of their henna hair dye come from henna and henna only; that red henna was from leaves, black henna was from henna roots, and brown henna was from henna bark. This was not, and cannot have been true.[47] Lawsone is the only dye molecule produced from henna in a significant amount and it is only produced from the leaves. There is no other dye in any other part of the henna plant that will dye hair, and henna stains keratin only in the range of copper to dark auburn. Though some exporters claim to be breeding blue, purple, pink, and other colors of henna, there is no evidence of such thus far.



Left: Lawsone: 2-hydroxy-1,4- napthaquinone as a 98% pure powdered chemical.[48]
Middle: Lawsone molecule
Right: henna leaves which contain the *precursors* to lawsone, 2-hydroxy-1,4- napthaquinone exist in henna leaves as hennosides, releasing about 1% lawsone.

In its pure state, lawsone is an orange molecule, with the specifications:[49] 2-Hydroxy-1,4-naphthoquinone, 98+%. MDL: MFCD00001678. EINECS: 201-496-3. Formula: C10H6O3. Formula Weight: 174.16 Melting point: ca 193° dec. Storage & Sensitivity: ambient temperatures. Solubility: Soluble in water: 2 g/L at 20°C. Lawsone is produced when released from the hennocide precursors in the henna leaf through testing and application.[50]

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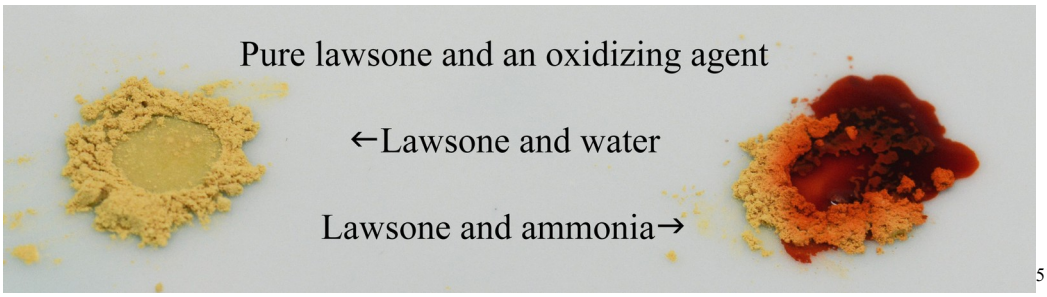
[47] This may have been a convenient lie to circumvent the FDA regulation that henna is the only plant unconditionally permitted to dye hair, or it may have been to keep competitors and consumers ignorant of what was actually in the box.

[48] Alfa Aesar: Applications for 2-Hydroxy-1,4-naphthoquinone is used for preparing decorative hair and skin dyes. 2-Hydroxy-1,4-naphthoquinone also demonstrates antimicrobial and antioxidant effects. It also suppresses the formation of hydrogen peroxide and superoxide radical anion by aldehyde oxidase-catalyzed reactions.

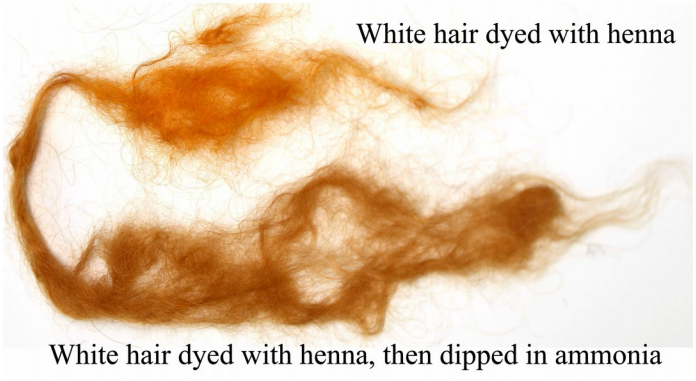
[49] Alfa Aesar, Thermo Fisher Scientific

[50] Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. "Henna through the Centuries: A quick HPTLC analysis proposal to check identity." *Revista Brasiliera de Farma Cognisia*. 24, 2



[51]

Lawsone is vulnerable to oxidation. Oxidation can come from exposure to air over time, or to strong oxidizing agents in a matter of minutes. People who use henna to dye their hair or to ornament their skin are familiar with the effect of an alkaline on henna stain. People who perspire profusely often find that their henna stain shifts from red-orange to brown or even black. People whose tap or well water is alkaline from mineral content find the bright henna tones in their hair darken over time. This can mitigated with antioxidants and may be reversed with Ancient Sunrise® Rainwash.[52]



An oxidant will push henna stain from reddish orange to a brown color.

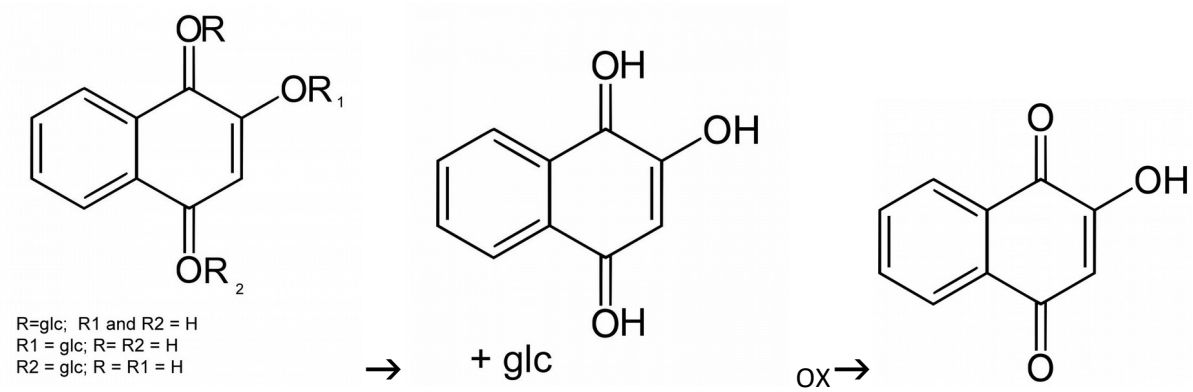
The formation of lawsone from precursors in the henna leaf by acidic hydrolysis

Lawsone is produced from hennocide precursors in the henna leaf. "Conversion of the three isomerglucosides (hennosides) into the unique aglycone by hydrolysis in mildly acid conditions. Further transformation of the aglycone leads to the more stable lawsone by easy oxidation."[53] As described by Gallo, Multari, et al, the sequence of henna dye release and binding is as follows:

> "Lawsone is not present as a free molecule in the leaves, but it is derived from its precursors, the hennosides, during henna preparation. Hennosides are three isomers

[51] 98% pure lawsone was measured in 2 samples. 4 drops of dilute lemon juice were applied to the sample on the left, and 4 drops of ammonia were applied to the sample on the right.
[52] Ancient Sunrise® Rainwash: http://www.mehandi.com/Ancient-Sunrise-Rainwash-Mineral-Treatment-p/rain_01.htm Citric Acid, Ascorbic Acid, Xanthan Gum
[53] Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. "Henna through the Centuries: A quick HPTLC analysis proposal to check identity." *Revista Brasiliera de Farma Cognisia*. 24, 2

derived from the tautomeric forms of the keto-enol interconversion of the naphtoquinone structure. In this case the second ring is thrice oxygenated, that can give rise to three possible hydroxyl groups and consequently change to the diketonic form. Each of the hydroxyls can be glucosidated, giving rise to the three isomers. The aglycone, derived from their hydrolysis, is further converted by oxidation into lawsone that is the dyeing active compound."[54]



Lawsone is produced from hennocide precursors in the henna leaf.

In less technical terms, lawsone's dye release from henna is facilitated when the powdered henna leaf material is mixed with a mildly acidic liquid; a pH 5.5 paste mix is ideal. In this mildly acidic pH paste, the lawsone molecule can be released and migrate from henna paste to stain keratin.[55] A Michael Addition facilitates a non-fading stable bond of the lawsone molecule with keratin. This red-orange stain can gradually oxidize to a brownish color when bound with keratin. In alkaline conditions, the stain can oxidize to black or greenish black.

The precursor is converted into the intermediate aglycone by hydrolysis in mildly acidic conditions. The aglycone intermediates will bind to keratin. Neither the precursor nor the final lawsone will bind as effectively to keratin as the aglycone intermediate.[56] In the mildly acidic henna paste at room temperature, the aglycone will become available after about an 8 hour soak, and remain at maximum in the paste for 12 – 24 hour hours, after which the percentage of the bindable aglycone form of the lawsone molecule will gradually diminish. This is termed 'demise' of the henna paste; at this point the henna paste produces diminishing stains. This transformation is gradual at room temperature. This sequence proceeds more quickly in warm conditions and slows under cold conditions.

The acidic paste maintains the hydrogen atoms on the corners of the aglycone, the intermediate form of the lawsone molecule. In acidic mixes of henna, the intermediate form of lawsone will migrate into the keratin in hair or skin and darken as it binds permanently with the keratin by a

[54] Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. "Henna through the Centuries: A quick HPTLC analysis proposal to check identity." *Revista Brasiliera de Farma Cognisia*. 24, 2

[55] Amro, I. H., James, K. C, and Turner, T. D.; 1993. "A Quantitative Study of Dyeing with Lawsone," *Journal of the Society of Cosmetic Chemistry*, **45**, 159 - 165

[56] Attempting to improve henna powder by adding lawsone powder will not improve the henna stain. Only the intermediate aglycone can effectively stain the hair.

Michael Addition.[57] If the henna powder is mixed only with water, the hydrogen atoms are not as well conserved. Henna mixed with water is more likely to fade from air because unbound lawsone will gradually wash out of hair. Henna mixed with a mildly acidic mix will leave a stain in hair that is not only permanent, but will gradually darken, and continue to darken for years.

The sequence of lawsone migrating from henna to bind with protein in the outer layer of skin or hair is as follows according to Dalglies, 1949.[58]

$$C_9H_5O_2C{=}O + NH_2\text{-keratin} \rightarrow C_9H_5O_2C{=}N\text{-keratin} + H_2O$$

All of the unstable aglycones in henna paste usually transform to the stable state of lawsone in about one week at room temperature in the presence of oxygen. In henna work, this is referred to as demise. This demised henna paste stains keratin a weak orange color which will not darken because it can no longer bind through Michael Addition.

A comparison test showing importance of the precursors to the stain

One might wonder why pure lawsone is not a popular hair dye product, given people's reluctance to spend the time and mess required to dye hair with henna. One might wonder why people do not simply paint their skin with lawsone to adorn their skin. The reason would be that pure lawsone does not contain the precursors, and without them, the binding to keratin is greatly decreased and stain results diminished.

[59]

This photograph compares naturally white hair dyed for 12 hours with 98% pure lawsone, and henna with 1.5% lawsone, both mixed in a mildly acidic medium, photograph taken three days after application and rinsing.

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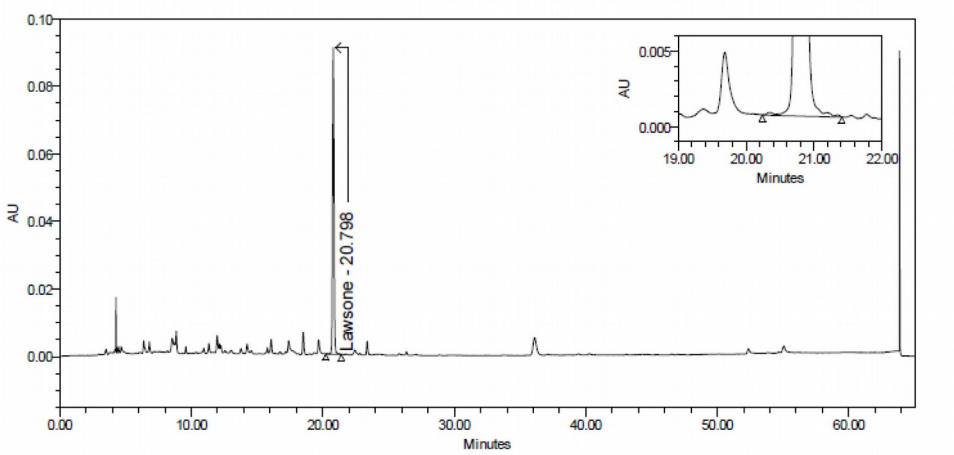
[57] Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014

[58] Dalglies, C.E., (1949) "Naphthoquinone antimalarials. Mannich bases derived from Lawsone. "*The Journal of the American Chemical Society*. 71, 1697-170

[59] 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 the lawsone and the henna mix for 12 hours, then rinsed.

In the preceeding image, the dye results of 98% pure lawsone is compared to the result of 1.5% lawsone henna paste on hair;[60] the difference is dramatic. The 1.5% lawsone from henna leaf precursors creates a vivid copper-red stain on white wool fiber whereas purified 98% lawsone which does not contain the precursors creates a weak apricot-orange stain. In addition, over time, the pure lawsone fades, and the henna stain darkens. This shows that presence of the lawsone precursor in henna is crucial for the dye uptake. A hair dye or skin dye made simply of pure lawsone would be ineffective and expensive,[61] as well as potentially hazardous. [62]

Measuring lawsone released from henna



HPLC test results of henna, 1.3 % lawsone[63]

HPLC (High Performance Liquid Chromatography) tests of powdered henna can be used to precisely calculate the amount of lawsone released from henna leaves. In HPLC tests done by Alkemist Laboratories, warm distilled water sonification[64] acts as a dye release so the released lawsone is calculated. The HPLC tests done on henna for Ancient Sunrise®, mehandi.com typically show 1.3% to 2.7% lawsone.[65] Poor quality henna may have 0.3% lawsone. A greater amount of lawsone measured in an HPLC test correlates positively to the saturation of color in hair: a higher lawsone content henna will give more vivid color on graying hair, and a lower lawsone content will deposit less color to hair.

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[60] Pure natural washed white mohair is used in this case

[61] A henna exporter offered to add pure lawsone powder to henna when I showed him the test results that his henna was only 0.07% lawsone. Adding pure lawsone to henna powder would make little, if any difference in the outcome of the color.

[62] Santa Cruz Biotechnology, Inc. Material Safety Data Sheet 2-Hydroxy-1,4-naphthoquinone sc-254254. http://datasheets.scbt.com/sc-254254.pdf

[63] HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC 2013

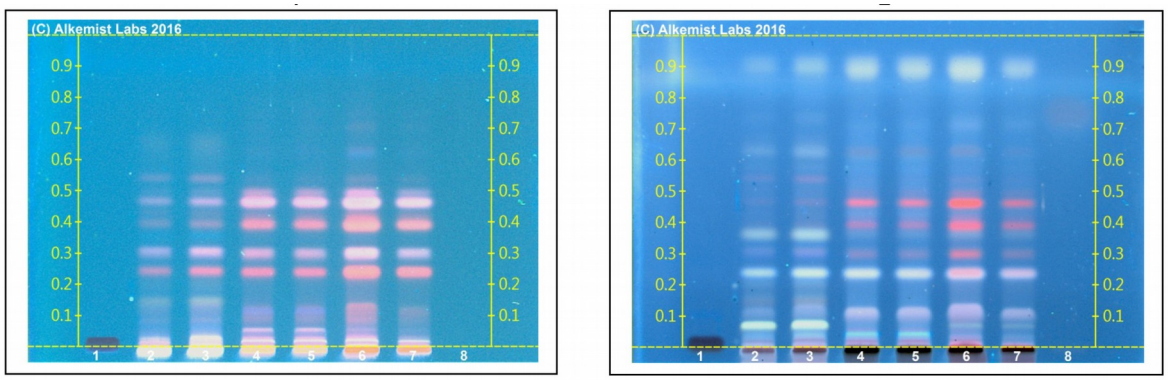
[64] Sample preparation: 450 mg henna powder into a 25 mL volumetric flask; added 20 mL water and sonicated for 30 minutes at 45 °C. Allowed to cool to room temperature and villed to volume with water and made a 5:50 dilution in water. Centrifuged for 10 minutes. Transferred to HPLC vial for analysis.

[65] HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC, 2008 - 2016

The fineness of sift does not seem to affect lawsone content. [66] There can be a negative correlation between the fineness of sift and lawsone content in the cases where the speed of the milling heats the henna; milling machinery that is slower, or which is artificially cooled appears to preserve lawsone content.

Lawsone content can also be extracted and measured from henna leaves with hexane, dichloromethane, and 95% ethanol, respectively, until exhaustion using soxhlet apparatus.[67] This test does not necessarily yield identical results to the water sonification test. The same testing procedure should be used on every batch of henna to ensure an accurate comparison between batches.

An HTPLC (High-performance thin-layer chromatography) test can be used to evaluate samples of alleged henna hair dye powders of unknown content, which may or may not contain henna and other adulterants. In an examination of eight commercial henna powders and two collected henna leaves, samples showed considerable variation in lawsone concentration ranging from 0.004 up to 0.608 wt%, indicating that some "henna hair dye" samples were almost devoid of lawsone,[68] and at best, these products meant as hair dye had very low lawsone content.



HTPLC test for lawsone[69]

An HTPLC test for lawsone does not measure the amount of lawsone as precisely as does an HPLC test but it does measure the presence or absence of lawsone. In the above HPTLC photo documentation,[70] Lanes 2 and 3, 6 and 7[71] are reference samples; lanes 4 and 5 are a sample of pure, unadulterated henna powder.

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[66] One henna exporter claimed that their henna is nearly 100% lawsone because it was triple sifted, claiming that because each time the henna was sifted, the lawsone content would be higher. This is not the case. The allegedly "near 100% lawsone henna was 1% lawsone.

[67] Charoensup, R., Duangyod, T., Palanuvej, T., and Ruangrungs, N. (2017) "Pharmacognostic Specifications and Lawsone Content of Lawsonia inermis Leaves" *Pharmacognosy Reseach* Jan-Mar; 9(1): 60–64.

[68] El-Shaer, N.S., Badr, J. M., Aboul-Ela, M. A., Gohar, Y. M. (2007) "Determination of lawsone in henna powders by high performance thin layer chromatography.".*Journal of Separation Science* 2007 Dec 30 (18): 3311-5.

[69] HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC 2013

[70] HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC

[71] Reference lanes for henna from The *Ayurvedic Pharmacopeia of India* Part I Volume II

Other commonly occurring compounds, contaminants, additives and adulterants in henna

Henna leaves contain chlorophyll, fiber, tannins, flavonoids, and the other phytochemicals that one might normally expect in the leaves of a small deciduous tree. Though there is no substantial amount of any dye produced by henna other than lawsone, there are other variable characteristics in henna that a person dyeing their hair or ornamenting their skin will be able to observe without a microscope or lab test.

The naphthoquinones in henna have received the greatest research attention, as the dye properties, antimicrobial, and strong antioxidant effects proceed from these. Henna contains a number of naphthoquinones including 2-methoxy-3-methyl- 1,4-naphthoquinone and lawsone (2-hydroxy-1,4-naphthoquinone). These henna naphthoquinones are derived from naphthalenes. A small number of naphthalenes, lawsoniaside (1,2,4-trihydroxynaphthalene-1,4-di-β-D-glucopyranoside) 1,2,4-trihydroxynaphthalene-1-O-β-D-glucopyranoside three methyl naphthalene carboxylates, lawsonaphthoate A-C, and 1,2-dihydroxy-4-Oglucosyloxynaphthalene have been isolated from henna stems and leaves.[72]

In addition to quinones and napthalenes, henna leaves contain flavonoids. These flavonoids present in henna leaves are acacetine, acacetin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, apigenin-7-O-β-D-glucopyranoside, apigenin-40-O-β-D-glucopyranoside, luteolin-30-O-β-Dglucopyranoside, apiin, cosmosiin, isoscutellarin, lawsochrysin, lawsochrysinin, lawsonaringenin, 30,40-dimethoxyflavone, 7-hydroxyflavone, 3,30,40,7-tetrahydroxyflavanone and rhoifolin.[73] These flavonoids may be responsible for the slight variation in henna stain results among cultivars.

 [74]

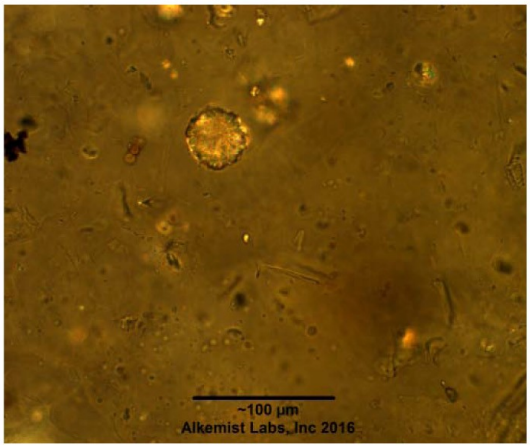
Mucilaginous “stringy” henna from Yemen

Henna leaves contain varying amounts of mucilage, a polar glycoprotein and an exopolysaccharide. Mucilage is produced to one extent or another by nearly all plants, and is abundant in okra, aloe vera, and flax seeds. For people who use henna, mucilage is what gives

[72] Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014, p 7.
[73] Ibid
[74] Photograph of “stringy” henna paste by Catherine Cartwright-Jones PhD

some henna the characteristic of gooiness which makes the paste as stringy as hot mozzarella cheese. In my experience, the cultivars of henna from Yemen have higher mucilage content than henna cultivars from Rajasthan, and henna cultivars from Pakistan have the lowest mucilage content. Mucilage has no effect on the dyeing action of henna in hair, but some people who use henna for body art find stringy, mucilaginous paste more difficult to manipulate. Henna paste with little mucilage is more prone to cracking and falling off the skin, so those who use henna for body art often add some form of sugar to the paste, monosaccharides being more conducive to smooth paste than polysaccharides.

Calcium oxalate is present in over a thousand[75] plants,[76] and is present at varying levels in henna.[77] In samples I have tested, higher levels of calcium oxalate occur in henna from Yemen and lower levels in henna from Rajasthan and Pakistan. Calcium oxalate does not affect henna as a hair dye. In body art, an acidic mix of henna with high levels of calcium oxalate becomes grainy 12 – 18 hours of initial mixing, and makes henna paste difficult to manipulate after that: the crystals dissolve in the acid and change the texture of the paste. Calcium oxalate does not penetrate the skin through scalp or body art, so external applications of henna with higher levels of the crystals will not increase the probability of or accumulation of kidney stones. Henna should not be ingested or used internally; needle-shaped calcium oxalate crystals can be very irritating to mucous membranes, causing numbness and burning sensation of the tongue, mouth, and lips and swelling of tongue or lips.



[78]

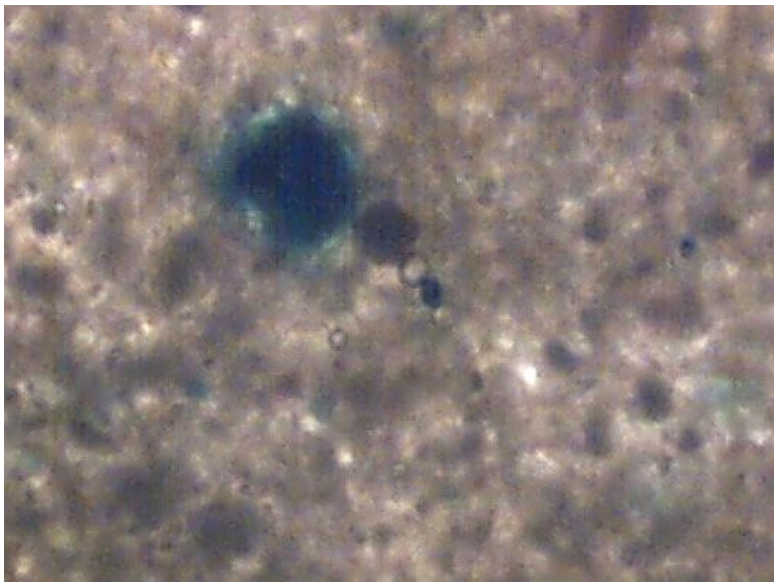
Microscopy of henna mesophyll showing fragments of calcium oxalate crystals, including a needle-like calcium oxalate crystal.

There is inevitably some amount of sand found in henna powder, though certainly not actually produced by the henna plant. Henna grows in semi-arid zones, and removing all of the sand can be nearly impossible, though green-dyed sand is an adulterant. This is known as "polishing" in the Indian henna industry. The presence of green dye is irrelevant to the stain. Henna with this

[75] Francesci, V.R.; Nakata (2005). "Calcium oxalate in plants: formation and function". *Annual Review of Plant Biology* (56): 41–71.
[76] Calcium oxalate exists in starfruit, rhubarb, beetroot, spinach and amaranth
[77] Microscopy: Alkemist Laboratories for TapDancing Lizard LLC 2012 - 18
[78] Microscopy: Alkemist Laboratories for TapDancing Lizard LLC 2016

green dye is often sold as being "fresher" and "higher quality", though the presence of green dye in henna indicates neither freshness nor quality.

The addition of green dyed sand to henna was detailed in a paper published in India challenging the marketing claim that “greener henna is better henna.” The following microscopy is of henna powder with bottled lemon juice added, stirred, placed on a microscope slide, allowed 5 minutes to set, then photographed at 60 x through the microscope. The vivid green chunk shown in the center of the slide on this page illustrates the presence of an artificial green dye, a coal tar-derived dye, added to make the product more "eye-appealing." The Essential Oil Association of India investigated the green dye and published the statement,



Henna “polished” with green-dyed sand

> "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.
>
> "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. “[79]

[79] Chourasia, Sardar, Patil, Mathew, and Kanpur, (1989) "Study of Quality Characteristics of Henna", *Essential Oil Association of India*

Some minerals and metals naturally occur in henna, as are in every plant and animal on earth. Ancient Sunrise® henna contains only what occurs naturally in the plant leaves because it is plant leaves and nothing else. People who are concerned about "heavy metals" being in henna have likely heard rumors that began when compound hennas with added metals were misrepresented as unadulterated henna. The various additives and adulterants used by companies that sold 'henna hair dye' are covered in "Ancient Sunrise Henna for Hair Chapter 3: Compound Henna."[80]

The minerals and metals naturally occurring in henna are generally consistent with the soil and water they grow in and other trees and bushes: nitrogen, phosphorous, magnesium and potassium. Ancient Sunrise® is tested with a heavy metals panel to assure people of their safety.

Lead acetate progressive dyes are often confused with, and marketed as henna. This misinformation may be the reason some people believe henna contains lead. No pure henna contains lead and Ancient Sunrise® henna certainly does not. Varying amounts of lead occur in soil, and therefore also in plants which grow in soil.[81]

Ancient Sunrise® henna shipments have about level of lead as vegetables considered safe to eat, so the henna is harmless for applying to skin. The following are an example of a lead test performed by Silliker[82] on a batch of Ancient Sunrise® henna:

> ICP-MS Sample Prep Acid Digest - EPA 3050b 6/29/15 CHG
> Lead 0.29 ppm (w/w) EPA 3050/6020 USP730[83]

People are also concerned about rumors of other heavy metals in henna, probably from the same confusion with compound henna and progressive dyes. Ancient Sunrise® henna has full heavy metal testing panels by an independent third party certified laboratory. These tests show that the levels of heavy metals found are in the same range as with a healthy plant growing on an organic farm with no additional irrigation. Ancient Sunrise® henna has the same low, safe, but measurable levels of heavy metals that exist in water, fruit, and vegetables deemed safe for consumption.

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[80] Catherine Cartwright-Jones PhD (2018) Ancient Sunrise® Chapter 3 "Compound Henna" http://www.tapdancinglizard.com/AS_henna_for_hair/3_compound_henna.pdf

[81] Thomas , B., Roughan, J. A., Watters, E. D. (1972) "Lead and cadmium content of some vegetable foodstuffs" *Journal of the Science of Food and Agriculture*, Volume 23, Issue12, December 1972, Pages 1493-1498

> An analytical method and results are given for the determination of lead and cadmium in Brussels sprouts, apples, pears, cabbages, potatoes, onions, leeks, carrots, swedes, watercress, frozen vegetables, cucumber, celery, tomatoes, mushrooms and dried herbs. The lead content of the 231 samples was in the range 0.01 to 3.85 parts/million the mean being 0.05 parts/million; the range and mean of the cadmium content were 0.01 to 0.22 and 0.04 parts/million, respectively.

[82] SILLIKER, Inc. Southern California Laboratory Merieux NutriSciences (2015) report on JAR16615TD1_3:051915HRUT Client: TapDancing Lizard, LLC

[83] Thomas , B., Roughan, J. A., Watters, E. D. (1972) "Lead and cadmium content of some vegetable foodstuffs" *Journal of the Science of Food and Agriculture*, Volume 23, Issue12, December 1972, Pages 1493-1498

The following are an example of the results of a heavy metal panel performed by Silliker[84] on a batch of Ancient Sunrise® henna:

ICP MS Heavy Metals (4 analytes) EPA 3050/6020 USP730 8/3/17 CHG
ICPMS Prep Acid Digest -
Arsenic 0.45 ppm (w/w)
Cadmium 0.058 ppm (w/w)
Lead 1.48 ppm (w/w)
Mercury 0.029 ppm (w/w)

An arsenic level of 1 ppm is considered safe in drinking water by the EPA; Ancient Sunrise® henna has half that level, and it goes on skin rather than being consumed. Mercury content in fruits and vegetables considered safe for eating ranges from 0.1 ppm to 0.02 ppm,[85] Ancient Sunrise® henna is in that range. The average cadmium level in vegetables considered safe for consumption, 0.01 to 0.22 ppm with a mean of 0.04 ppm, is at about the same as the level in Ancient Sunrise® henna.[86] In comparison, one cigarette contains about 0.5 - 2 μg of cadmium and about 10% of the cadmium content is inhaled when the cigarette is smoked.[87]

Additional phytochemistry of henna

Plants are composed of elements, substances such as hydrogen, carbon, and nitrogen, and combinations of elements: molecules. The most common molecule in plants, and of course, henna, is dihydrogen monoxide, H_2O, more commonly known as water. The main chemical components of plants, including henna, are water, carbon compounds, soluble and polymeric sugars, organic acids, and mineral substances, all of which have rather long scientific names for scientific specificity. Phytochemicals are naturally-occurring parts of the henna plant, just as potato starch, ((C6H10O5)n, a polysaccharide (q.v.) comprising glucose monomers joined in α 1,4 linkages)[88] naturally occurs in the tubers of the potato plant, and is not an additive.

The phytochemistry of henna (the molecules which naturally occur in the henna plant) has been meticulously studied, isolating flavonoids, tannins, naphthalenes, xanthones, lignans, and other compounds. There are smaller amounts of terpenes. The complete details of the phytochemistry of henna and relevant research papers on the following list of phytochemicals isolated thus far (as of 2014) from the henna plant can be found in "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects."[89]

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[84] SILLIKER, Inc. Southern California Laboratory Merieux NutriSciences (2017) report on JAR19917TD2_1:070517hRUT Client: TapDancing Lizard, LLC

[85].Gerdes, R.A., Hardcastle, J..E., Stabenow, K..T. (1974) "Mercury content of fresh fruits and vegetables" *Chemosphere* Volume 3, Issue 1, February, Pages 13-18

[86] Thomas , B., Roughan, J. A., Watters, E. D. (1972) "Lead and cadmium content of some vegetable foodstuffs" *Journal of the Science of Food and Agriculture*, Volume 23, Issue12, December 1972, Pages 1493-1498

[87] World Health Organisation (WHO) (1992). Environmental Health Criteria 134 - Cadmium International Programme on Chemical Safety (IPCS) Monograph.

[88]IUPAC Name: 5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-2-[4,5-dihydroxy-2-(hydroxymethyl)-6-methyloxan-3-yl]oxyoxane-3,4-diol

Henna leaves and other parts of the plant also contain tannins; tannic acid is one of the main constituents of the plant.[90]

Alkylphenones have been isolated and identified in henna leaves: Lalioside (2,3,4,6-tetrahydroxyacetophenone-2-O-β-D-glucopyranoside), lawsoniaside A (1-butanoyl-3,5-dimethylphloroglucinyl-6-O-β-D-glucopyronoside), and 2,4,6-trihydroxyacetophenone-2-O-β-Dglucopyranoside.[91]

Lawsoniaside B (3-(4-O-α-D-glucopyranosyl-3,5-dimethoxy) phenyl-2E-propenol) and syringinosinol di-β-Dglucopyranoside have been isolated from henna leaves.[92] Other compounds have been isolated from stems and leaves: obtusafuran derivative, lawsonicin (2,3-dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-((3-hydroxypropyl)-3-methoxy)phenyl]-6-methoxy-1-benzofuran), p-coumaric acid and gallic acid.[93]

Xanthones were isolated in an analysis of sample material taken from the whole plant: of 1,3-dihydroxy-6,7-dimethoxyxanthone (also referred to as laxanthone-I) (47); 1-hydroxy-3,6-diacetoxy-7-methoxyxanthone (laxanthone-II) (48) and 1-hydroxy-3,7-dimethoxy-6-acetoxyxanthone (laxanthone-III).[94]

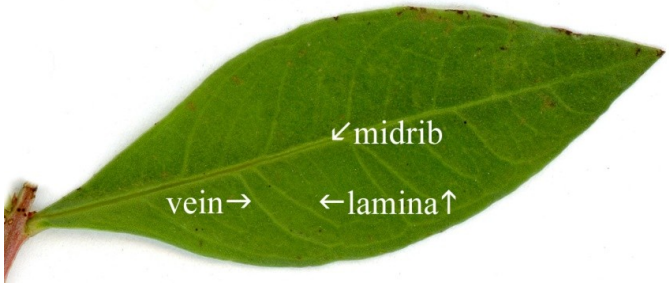
Henna flower essential oil contains α-ionone and β-ionone, 2-phenylethanol, and benzyl alcohol, together with other alcohols and aldehydes. Leaf volatiles include methyl-α-D-glucopyranoside, 2-hydroxy-1,4-naphthalenedione, ethyl hexadecanoate, (E)-methyl cinnamate, isocaryophyllene and methyl linolenate. Other terpenes isolated from henna that are included in henna leaf essential oil are 1,8-cineole, α-pinene and p-cymene bisabolene, eugenol, hexadecanoic acid, phytol, α-terpineol and etherphenylvinyl. Non-volatile terpenoids were also isolated from the leaves: Lupeol botulin, betulinic acid, 30-norlupan-3β-ol-20-one. [95]

[89] Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014
[90] Ibid p. 7
[91] Ibid p 9.
[92] Ibid p 9.
[93] Ibid p. 6 - 9
[94] Ibid p. 7
[95] Ibid p. 10

Chapter 4: Science and Microscopy: Microscopy of Henna

The microscopic structures of a henna leaf

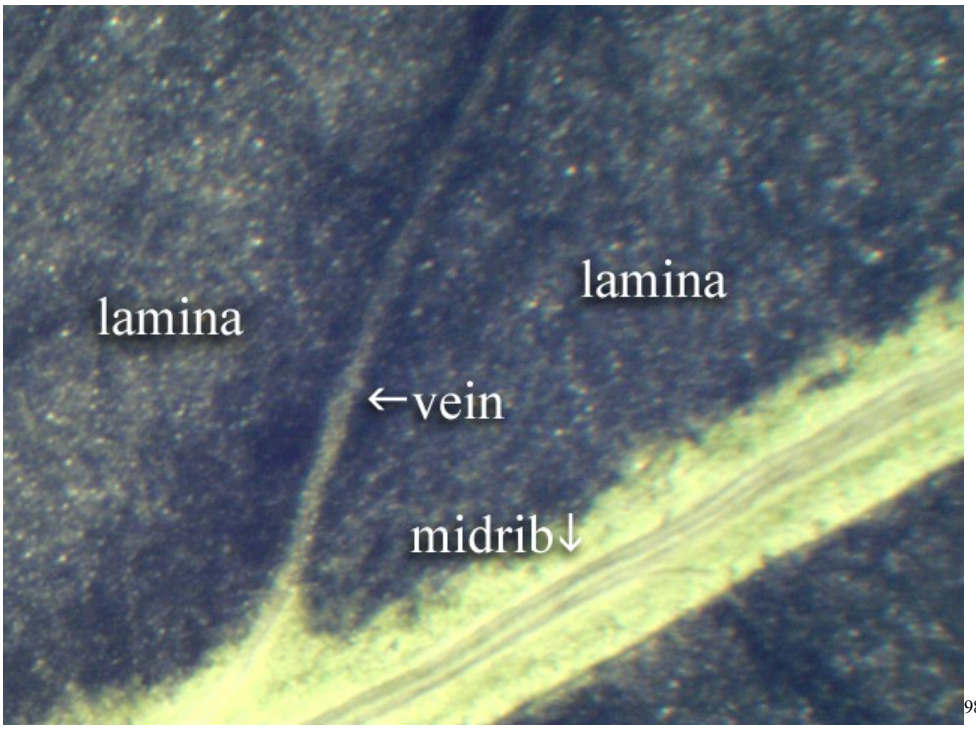
This is a quantitative microscopy survey of different brands of henna hair dye products. Henna products in the marketplace frequently contain unlisted additives and adulterants, and the milling and sifting varies with particles from 0.2 mm to over 3 mm. Consumers become frustrated by henna products' 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.'



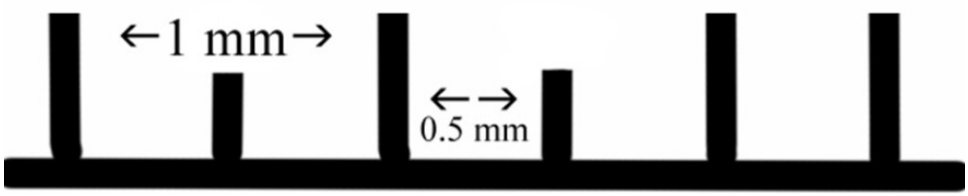
The above image is are macroscopic view of the ventral side of a 38 mm long henna leaf, 1.5 inches, with the midrib, vein, and lamina indicated.[96]

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.[97]

[96] Henna plants raised by Catherine Cartwright-Jones PhD
[97] Epson Perfection V600 Photo scanner 1200 DPI



[98]



[99]

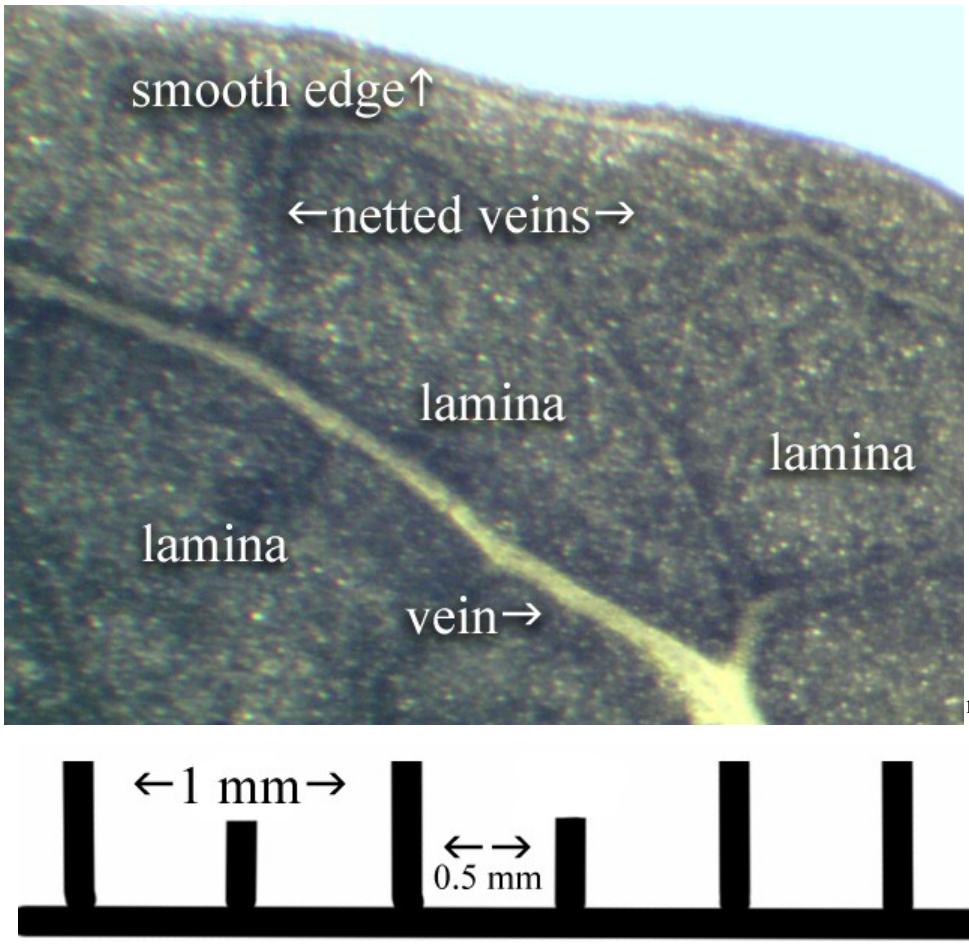
The above is a microscopic view a henna leaf section at 40x magnification with the midrib, vein, and lamina indicated. The section of this leaf is about 3 mm, 0.1 inches

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.

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

[99] 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

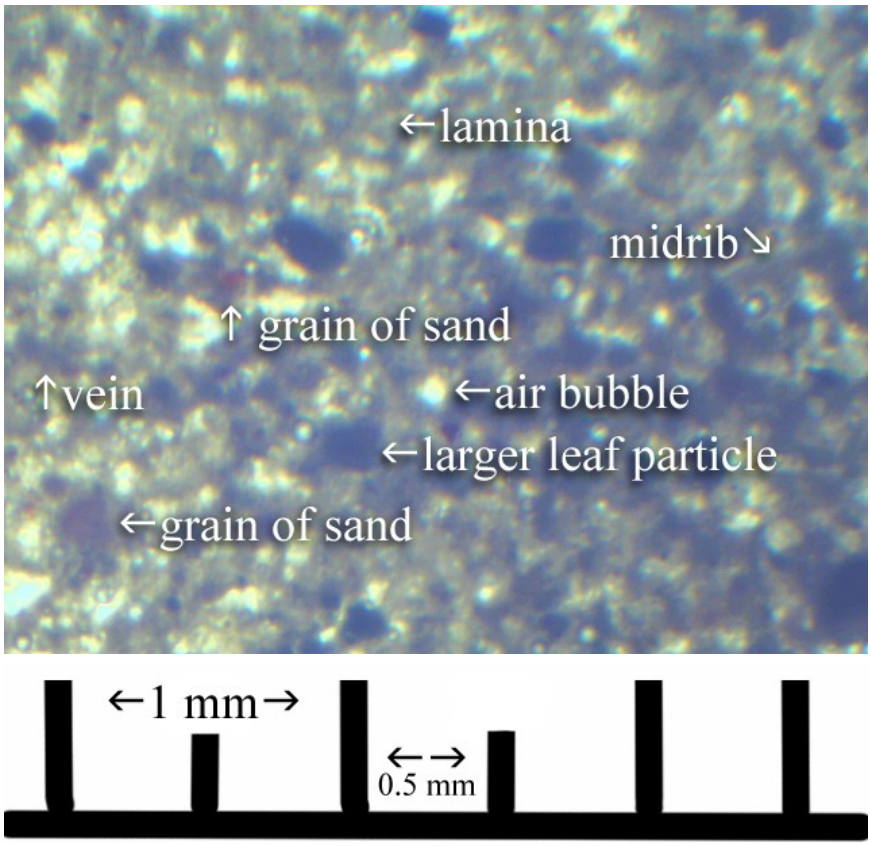
The same henna leaf structures exist in henna powder as the whole leaf: midrib, veins, netted veins, and lamina, though they are fragmented and randomized by milling and sifting. Midrib particles are often the largest particles in henna powder, because they are structurally strong and resist crushing 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.[101]

[100] 40x magnification

[101] 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.

[102]

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[103]

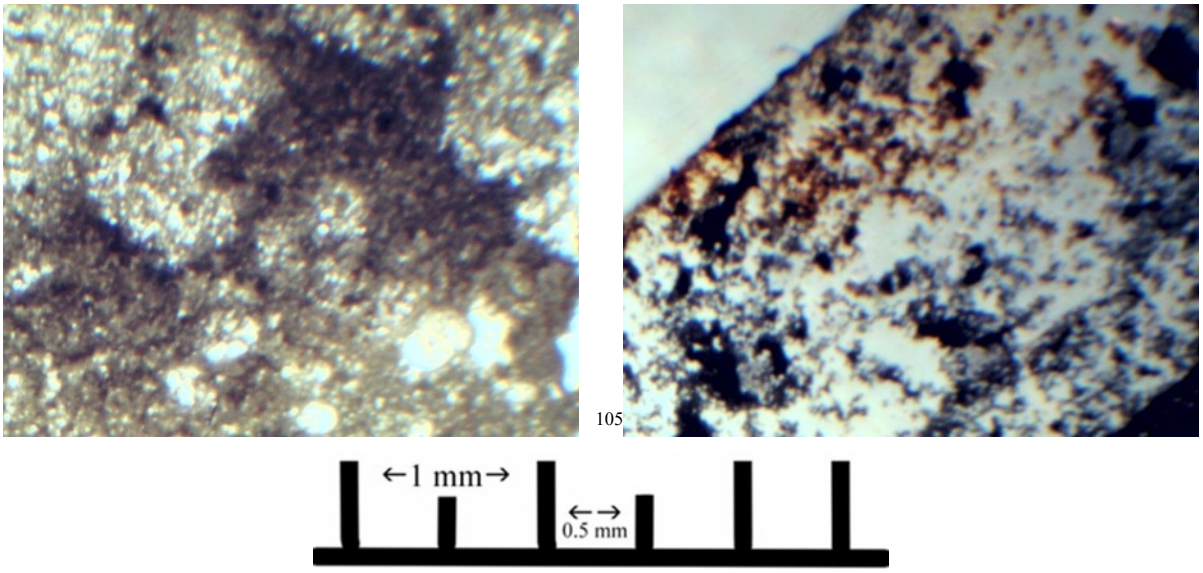
The white orbs in the above image are air bubbles. Air bubbles are not an intrinsic part of henna powder, but when henna is mixed with water on a slide and the slip cover applied, there will be air bubbles. These are irrelevant and may be ignored.[104]

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

[102] Henna leaf dried, crushed and scanned at 1200 DPI by author
[103] Ancient Sunrise® Rajasthani Twilight Henna from mehandi.com 40x magnification, certified to be pure henna by Alkemist Laboratories
[104] 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



105

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.

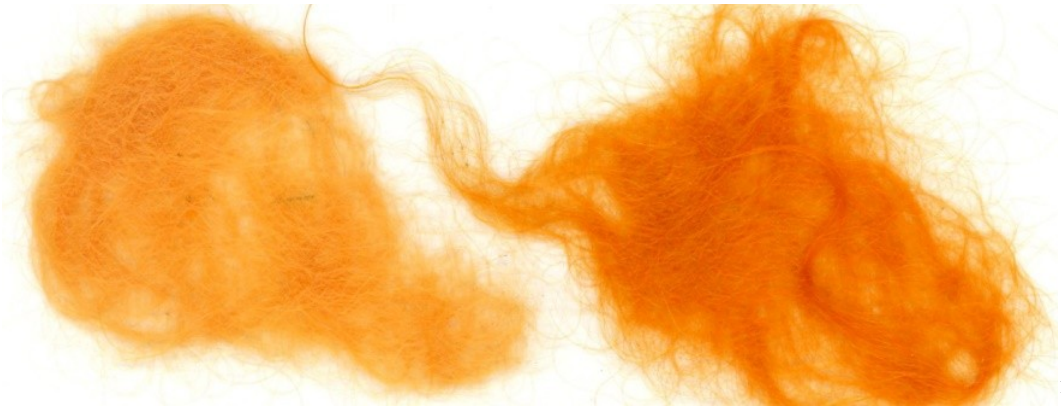
Lawsone cannot be directly observed in a henna leaf or henna leaf powder. Lawsone (2-hydroxy-1,4-naphthoquinone) is in a glycosidic bound in a henna leaf, which is cleaved by acidic hydrolysis of the glycosidic hennosids and autooxidation of aglucons.[106] It cannot be directly observed under a microscope 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

[105] Alfa Aesar, Thermo Fisher Scientific

[106] 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.

 [107]

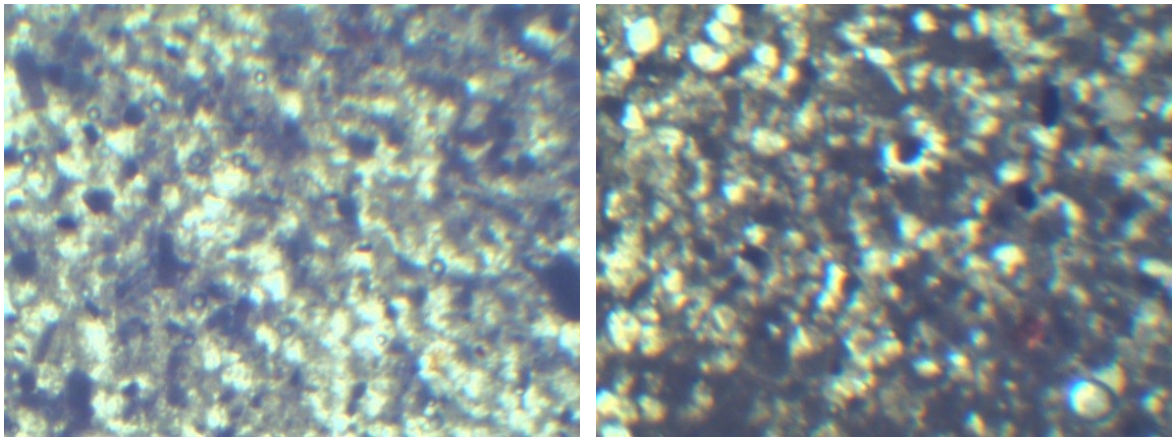
Left: White hair dyed for 12 hours with 98% pure lawsone
Right: henna with 1.5% lawsone.
Application done October 30, 2018, scanned November 2, 2018

The scan above compares naturally white hair dyed for 12 hours with 98% pure lawsone,[108] 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

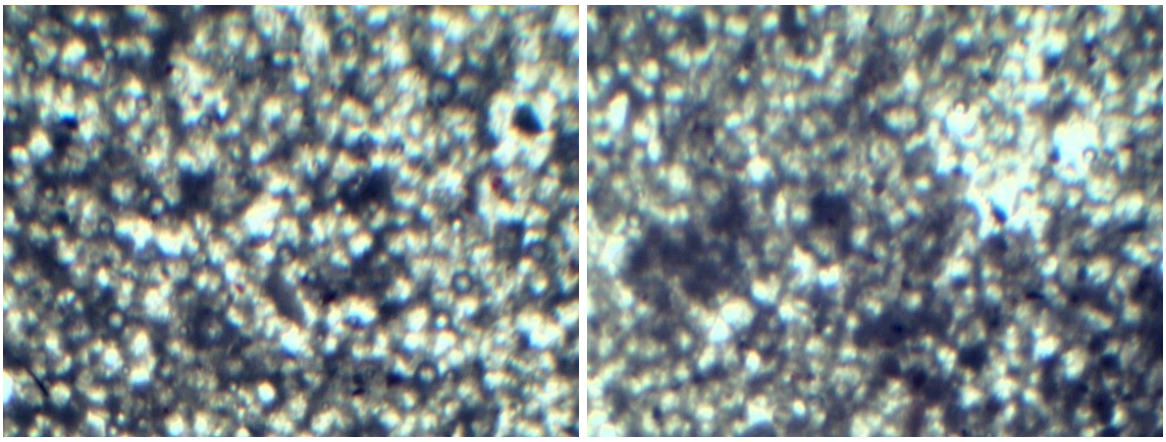
[107] Pure lawsone was mixed with lemon juice and water, the henna was mixed with lemon juice. The hair is natural white mohair, unbleached and undyed. The hair was left to soak in both the lawsone and the henna mix for 12 hours and then rinsed. The results were scanned at 300 dpi against a white background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

[108] 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.[109]

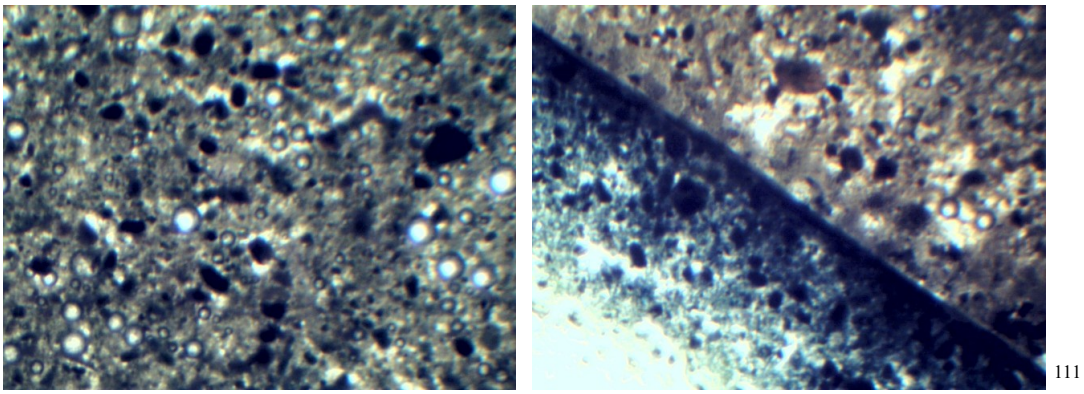
To evaluate henna products for hair under a microscope, it is necessary to have a known, verified example for comparison. The above Ancient Sunrise® hennas have been certified to be dried, powdered henna leaves of lawsonia inermis. Both contain a few particles of wind-blown sand. The henna at the left 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.[110]
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.

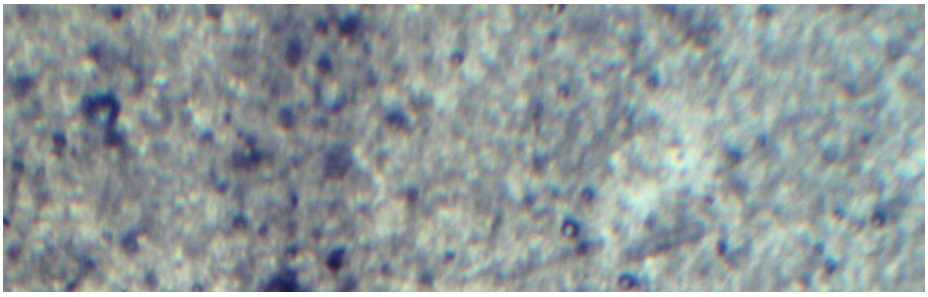
[109] Left: Ancient Sunrise® Rajasthani Monsoon Henna from mehandi.com Right: Ancient Sunrise® Rajasthani Jasmine Henna. Both at 40x magnification and certified to be pure henna by Alkemist Laboratories.
[110] Left: Ancient Sunrise® Zekhara Cassia obovata from mehandi.com. Right: Ancient Sunrise® Rajasthani 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.

 [111]

Left: Ancient Sunrise® Indigofera tinctoria, partially fermented, dried, milled and sifted for hair dye[112]
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.



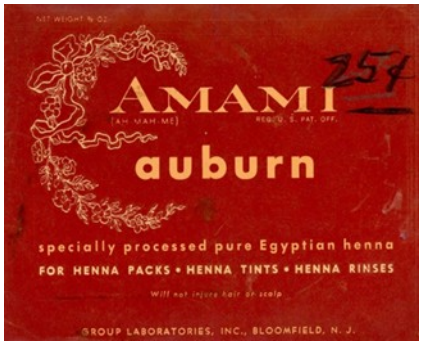
Ancient Sunrise® Amla powder (emblica officinalis)[113] fruit dried, milled, and sifted. The largest particles are 0.1mm, image at 40 x. Amla fruit is dried, milled, and sifted. Amla is in many mixed henna hair dye products from India as the acid for dye release.

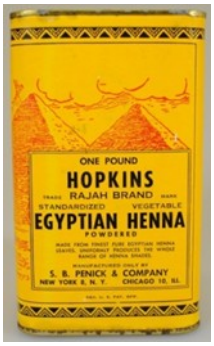
[111] Magnification 40x, largest plant particles are 0.3 mm.
[112] Left: Ancient Sunrise® Zekhara Indigo from mehandi.com at 40x magnification, certified to be pure fermented Indigofera tinctoria by Alkemist Laboratories.
[113] Ancient Sunrise® Amla (emblica officinalis) from mehandi.com at 40x magnification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

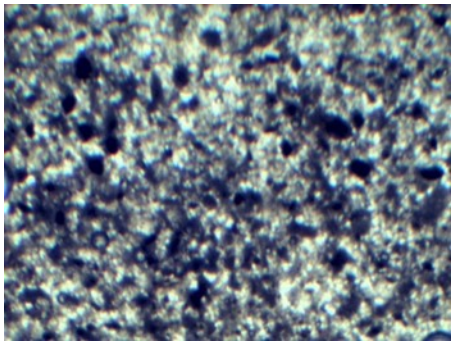
Identifying the contents of packages of henna sold as hair dye

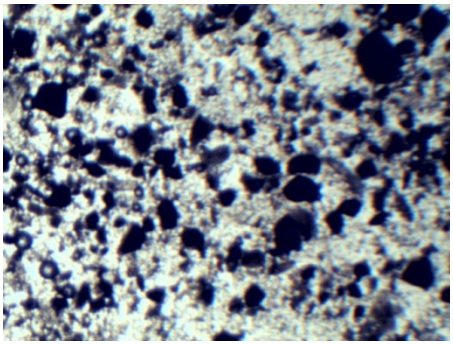
Henna in the USA in the Mid-20th century

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.







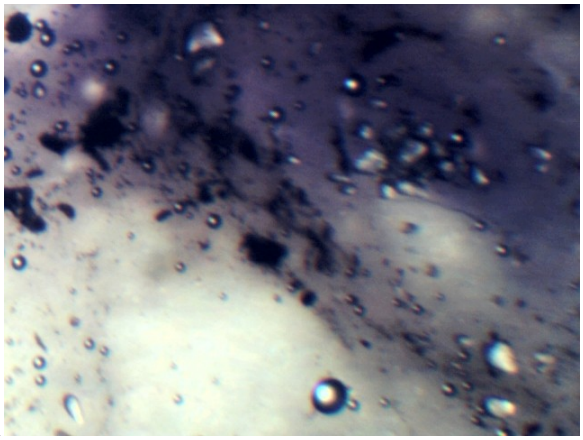


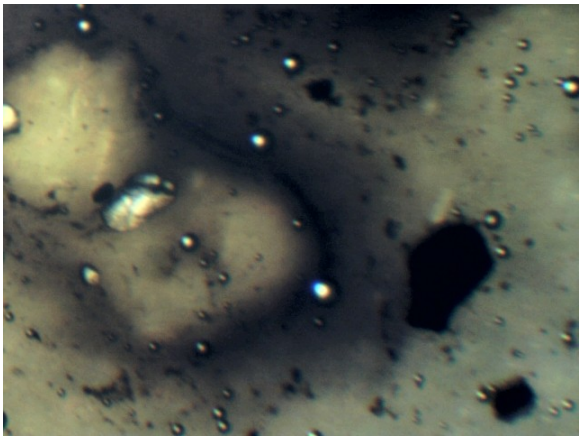
Henna for hair products from Egypt available 1930 – 1950 in the USA,[114] henna produced in Egypt. All particles are consistent with henna. Products are declared to be henna.

[114] 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.[115] 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.


1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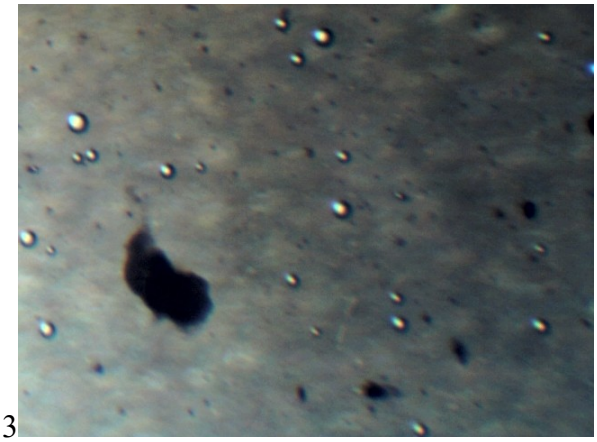
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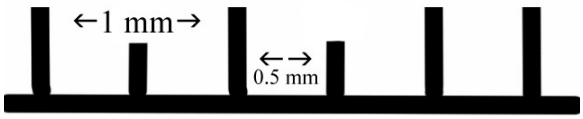
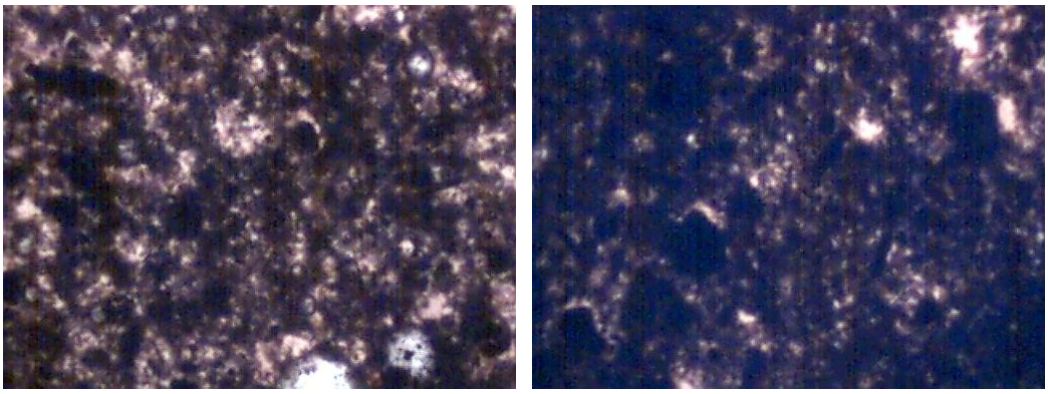


Sequential images of para-phenylenediamine dispersing in water over a period of one minute from a PPD/henna product, images are magnified 40x.

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

[115] 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.[116] 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.[117]



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.

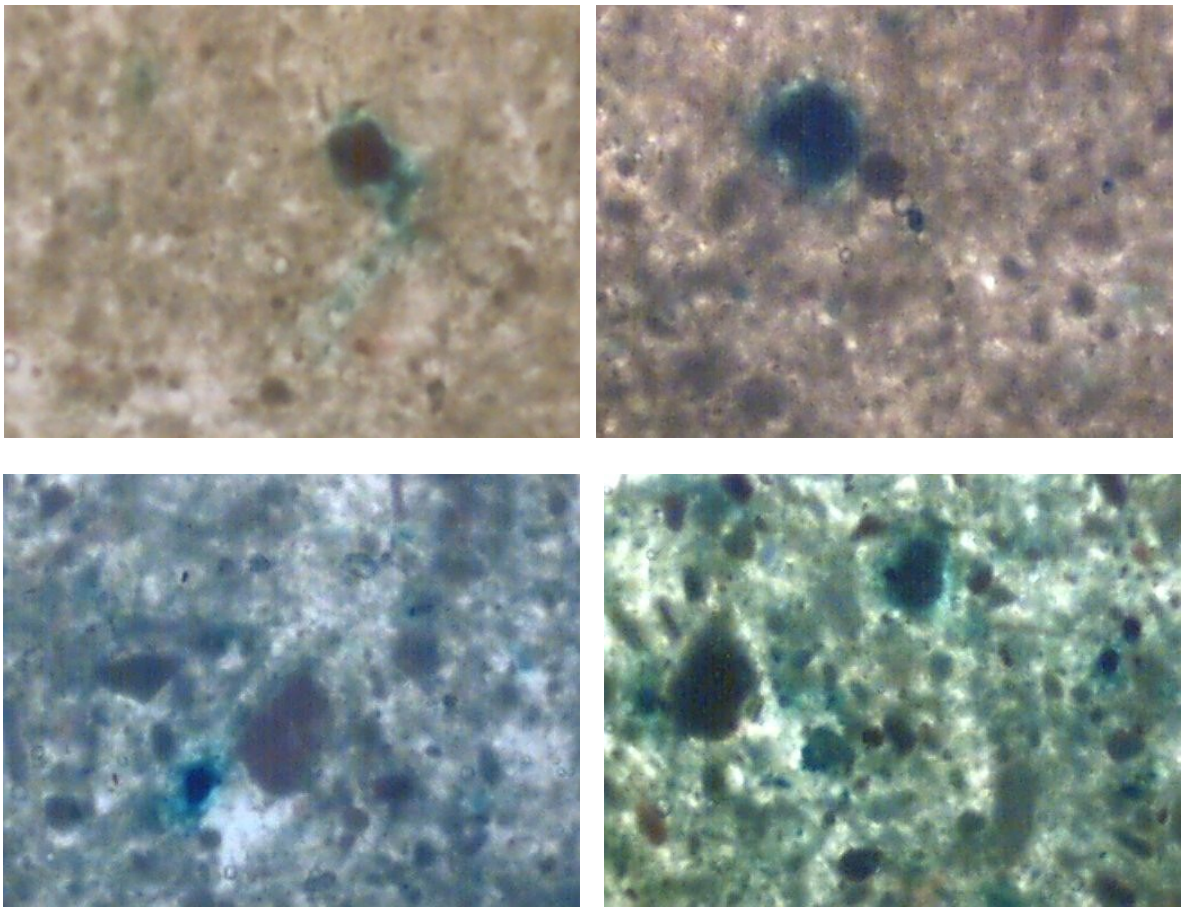
"Polished" henna with green sand

Most henna samples originating in India and available in the US markets in 2003 contained sand dyed azo green.[118] 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.
"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

[116] 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.
[117] 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
[118] Chourasia, Sardar, Patil, Mathew,Kanpur, (1989) "Study of Quality Characteristics of Henna", *Essential Oil Association of India.*

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."

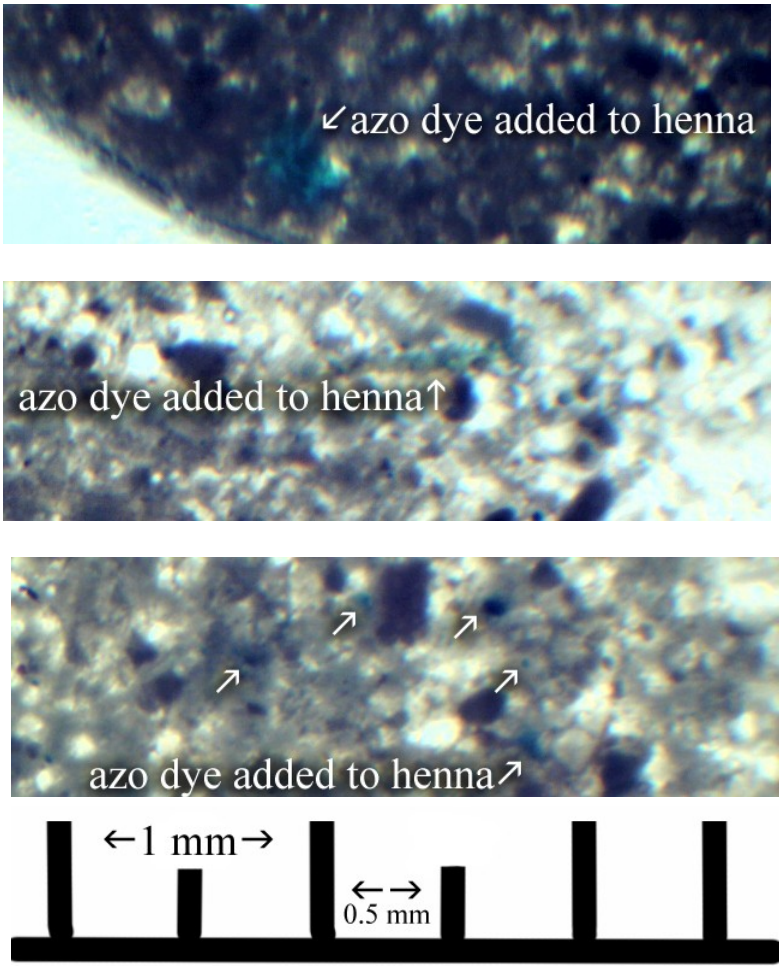


Azo green dyed sand in henna products in collected in 2001- 2, dispersing green dye after the paste was wetted and imaged at 60x.

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.[119] 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 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

[119] 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.

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[120]

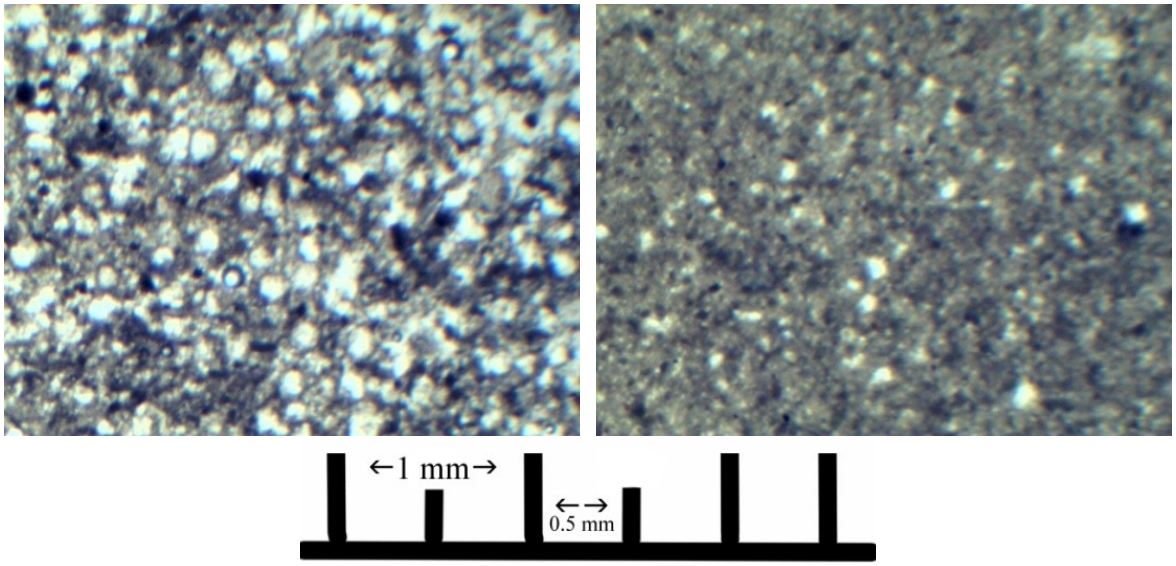
Milling and sifting henna

Henna arrives at henna processing factories[121] 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 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

[120] Henna products sourced from Middle Eastern and South Asian markets, Amazon.com and Ebay.com in 2013 and 2018.
[121] In 2005, there were 25 major henna processing factories in Sojat.

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.[122]

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..[123] The particle sizes[124] 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.

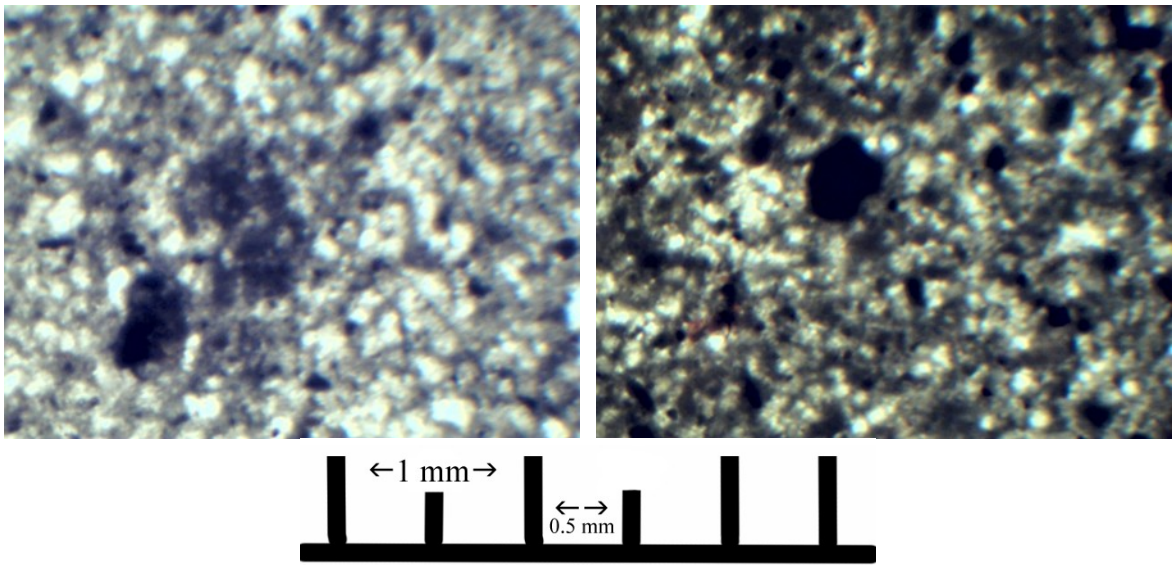
Henna powders put through a sequentially finer meshes are designated by the marketing term 'triple sifted.' Each sift separates out more waste. There is also more labor required so factories charge more for finer sifts. Exporters often quadruple the price of the finest sift and market it to artists in 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

[122] 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

[123] Sieve size confirmed in testing by Alkemist Pharmaceuticals.

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

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.[125] 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.[126]



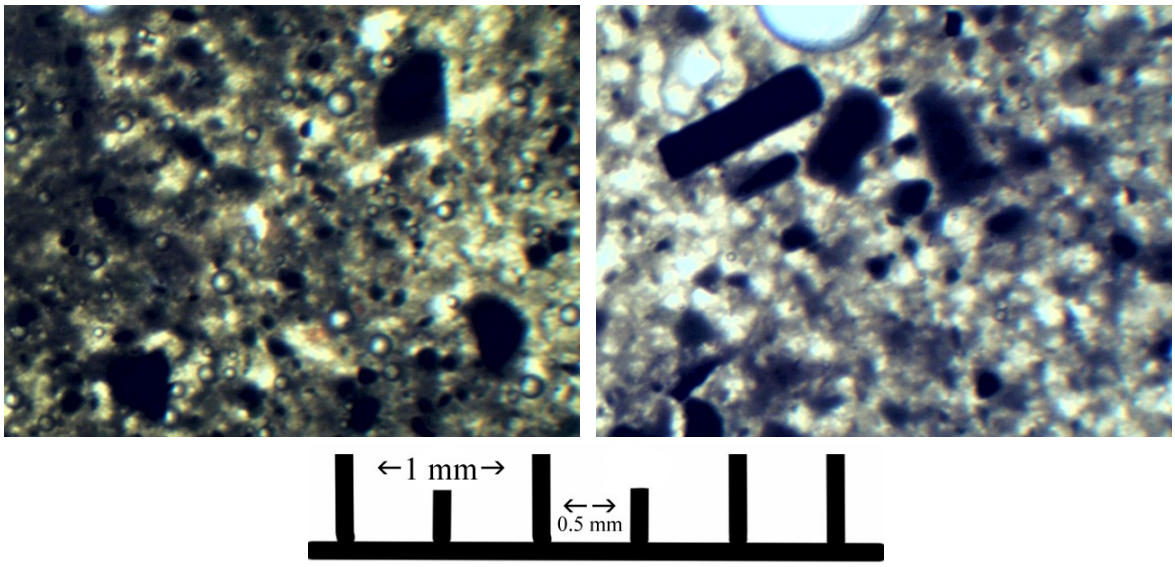
The above hennas have particles up to 0.5 mm that would pass through a #30 mesh, would clog an artist's jac bottle unless strained through a stocking, and would take some persistence to rinse from hair.

[125] 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)

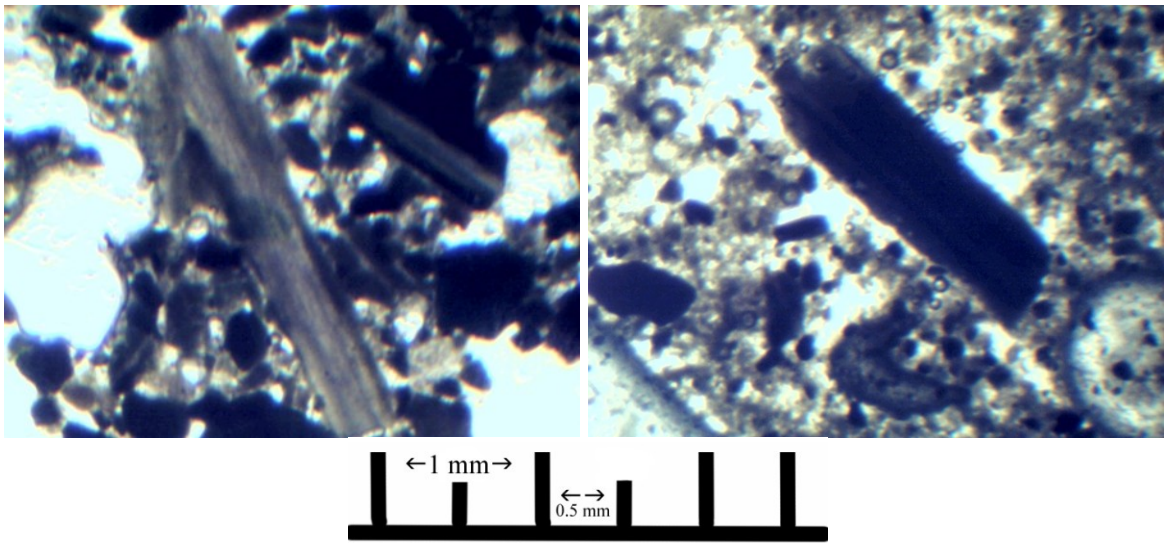
[126] Roy, P. K.. Singh. M., Tewari, P. (2005) "Composition of Henna Powder, Quality Paramters and Changing Trend in its Usage" *Henna Cultivation, Improvement and Trade*, Central Arid Zone Research Institute, Jodhpur, India. p. 39, table 1

Pali district June harvest average henna lawsone content: 2.38%

Pali district October harvest average henna lawsone content: 2.82%



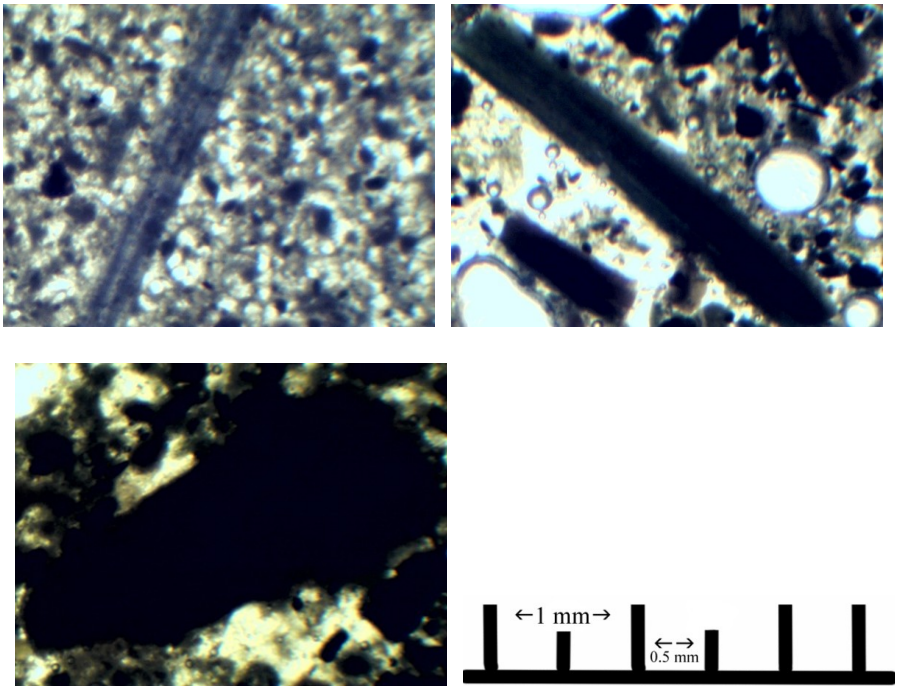
The above hennas have particles up to 0.7 mm which would pass through a #20 mesh. These cannot be used with a jac bottle for henna body art, would cause clogs in a polypropylene cone, and would be difficult to rinse from hair.



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.[127] The sensitizations caused by this misunderstanding of henna products gave rise to the current epidemic of para-phenylenediamine sensitization.[128] 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 for customer acceptance and products with their "Does she...or doesn't she?" slogan.[129] Within six years of that ad campaign, 70% of all adult women were coloring their hair at home with

[127] 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

[128] 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

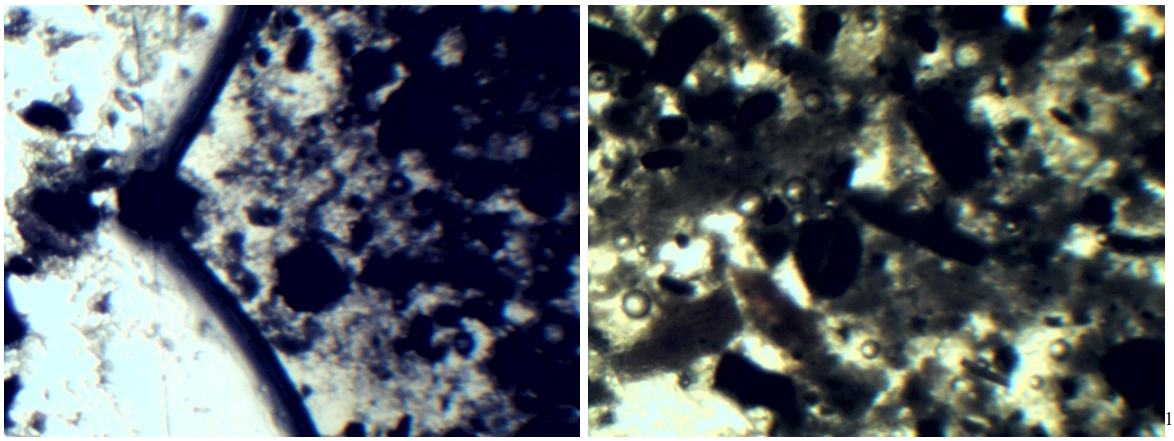
[129] In 2 004, Clairol sold $1.6 billion in hair dye products.

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." [130]

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."[131]


[132]

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.

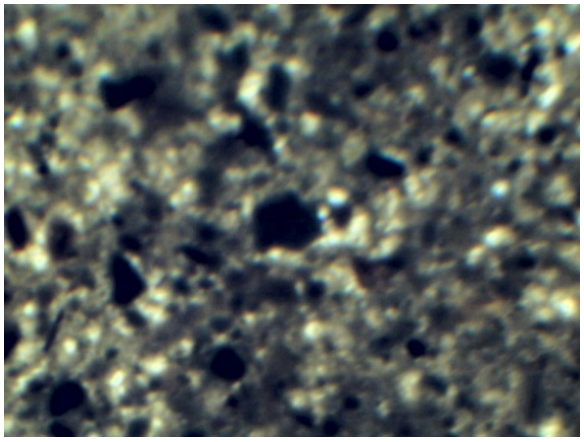
[130] Taylorjan, A. (1977) "All About: Henna, for Hair With a Shine" *The New York Times* January 19, 1977, Page 52 https://www.nytimes.com/1977/01/19/archives/all-about-henna-for-hair-with-a-shine.html
[131] Avigal Henna ingredient declaration.
[132] 40x magnification. Plant particles up to 0.8 mm

Henna hair dye ingredient declarations in the current marketplace

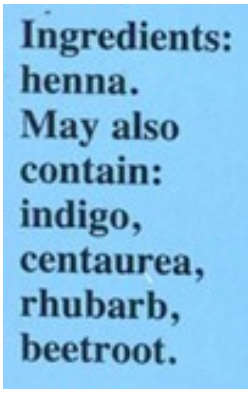
Since 2003, henna hair dye companies selling in North America have been under pressure by the FDA and consumers to properly declare ingredients. The FDA requirements for labeling are:

> *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.

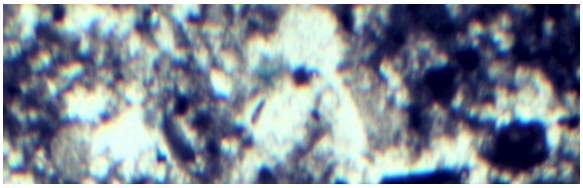
Though it is not possible to confirm the identity of botanicals in a henna product simply by viewing the powder at 40x, one can at least see if the declaration is consistent or inconsistent with the visible material. More precise confirmations would require complex lab testing.



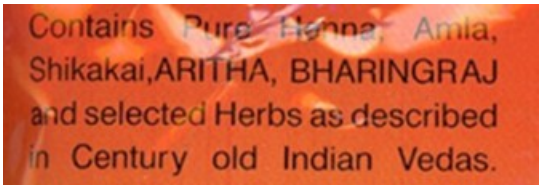
[133]



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.

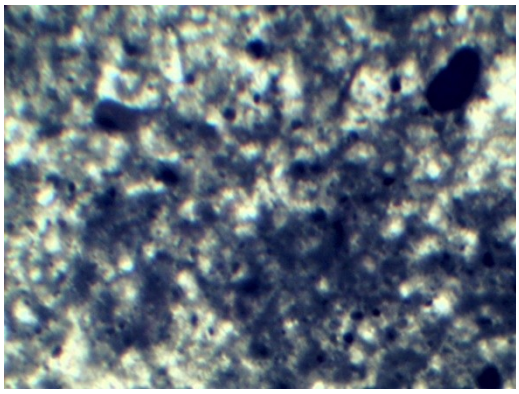


[134]



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.

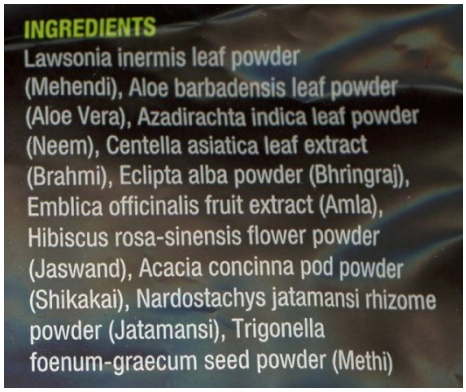
[133] 40x magnification. Plant particles up to 0.5 mm
[134] 40x magnification. Plant particles up to 0.6 mm



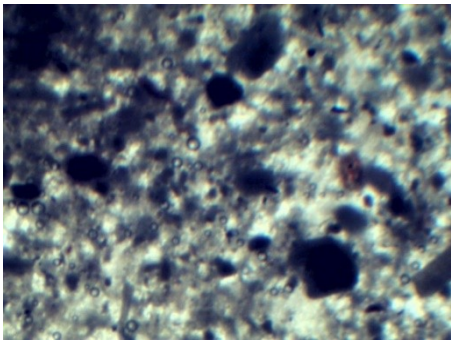
[135]

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.

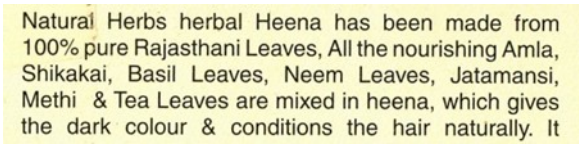
[136]



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

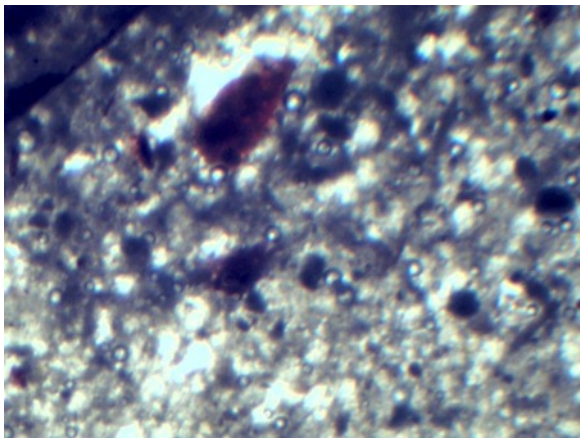


[137]



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.

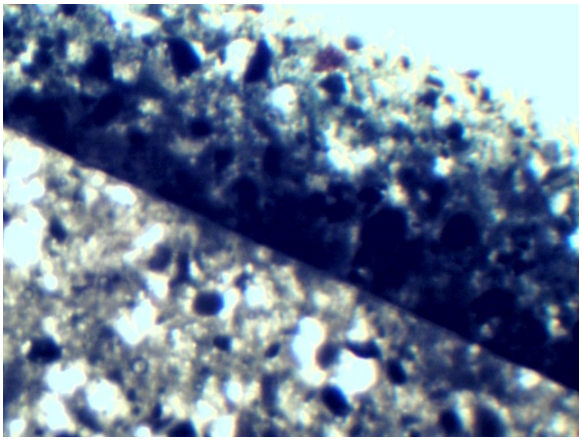
[135] 40x magnification. Plant particles up to 0.5 mm
[136] 40x magnification. Plant particles up to 1.5 mm
[137] 40x magnification. Plant particles up to 0.8 mm



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

138

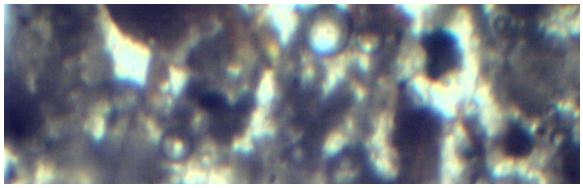
The above henna powder has particles consistent with henna and other declared plant material. The large red particle is consistent with acacia concinna (shikakai).

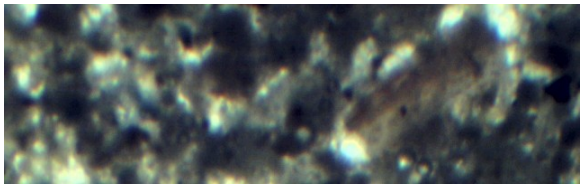


Ingredients: Indigofera tinctoria, Lawsonia Inermis, Emblica officinalis , Eclipta alba, Azadirachta indica, Bacopa monnieri, Vetiveria zizanioides.

139

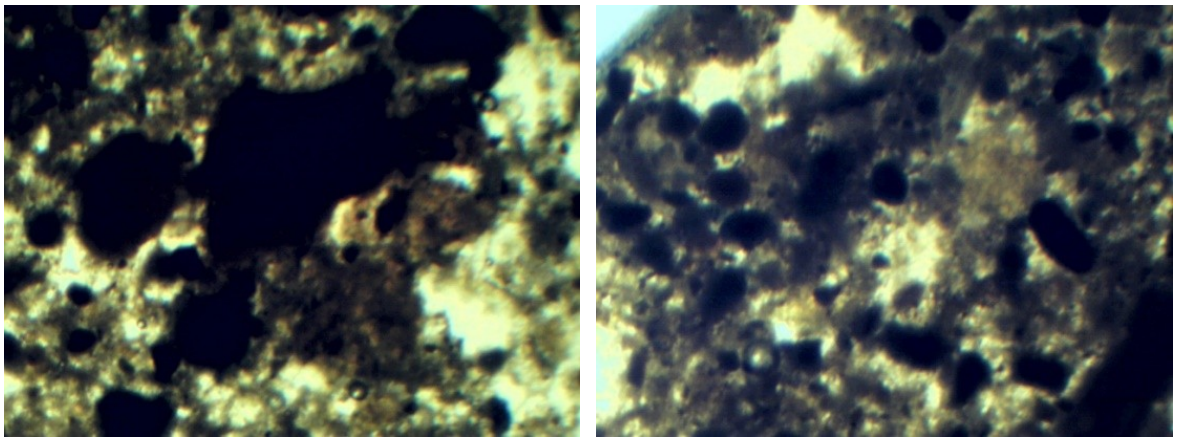
The above 'black' henna powder contains particles consistent with indigo, henna, and other declared plant material. At the edge of the slip cover, indoxyls oxidize to blue indigo within four minutes of being wetted and coming into contact with air.





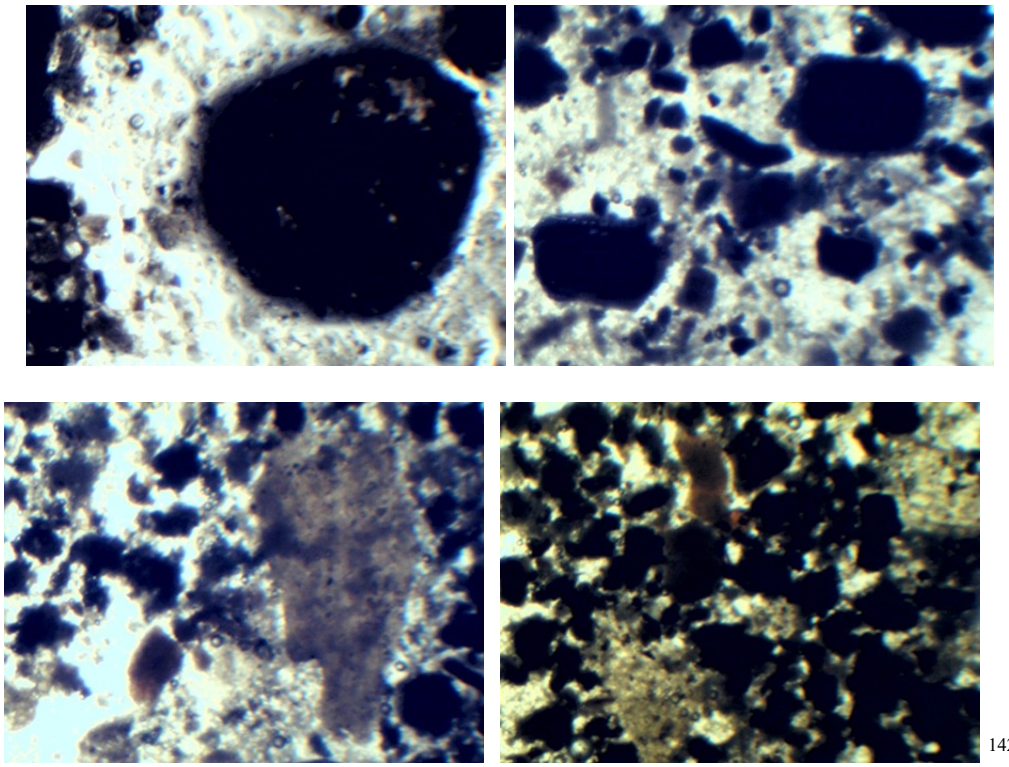
The above light green powder, marketed as 'Marigold Blond Henna' is declared to be cassia obovata (neutral henna) (sic), marigold flowers, and chamomile.[140] Plant particles consistent with the declaration are visible.

[138] 40x magnification. Plant particles up to 1 mm
[139] 40x magnification, plant particles up to 3 mm
[140] 40x magnification, plant particles up to 1.5 mm



[141]

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.



[142]

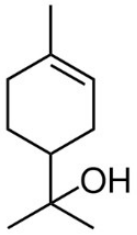
The above henna powder product contained two packets: one of henna (upper two images above) and one of herbs (lower two images above). There was no ingredient declaration. There are particles consistent with henna and other plant material as well as large particles of sand.

[141] Magnification 40x, plant particles up to 1.5 mm
[142] 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,[143] is the terpineol alpha-isomer, a white crystalline powder.[144] 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.[145]



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[146] 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.[147]

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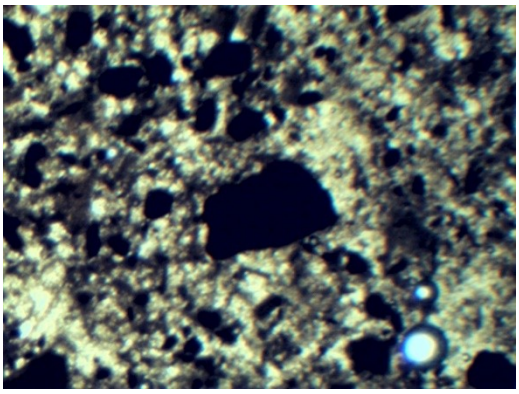
[143] Since I have not found this in any ingredient declaration, only in discussions with henna exporters, this is my best etimate.

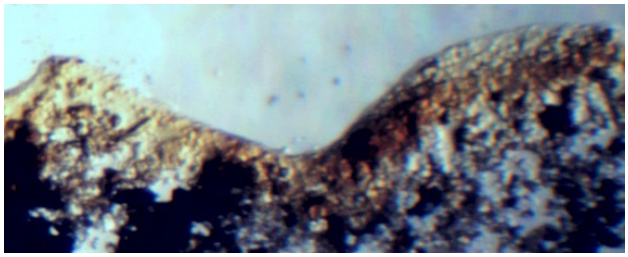
[144] Alpha-TERPINEOL; Terpineol. *Pub Chem Open Industry Database*. National Center for Biotechnology Information https://pubchem.ncbi.nlm.nih.gov/compound/alpha-TERPINEOL

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

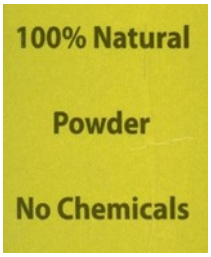
[146] The term 'terps' as used in henna body art was invented by myself, Catherine Cartwright-Jones PhD in 2000, because I did not want to type 'essential oils with high monoterpene alcohol content' hundreds of times on the hennapage.com forum and in websites and research papers. This term abbreviated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

[147] 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.

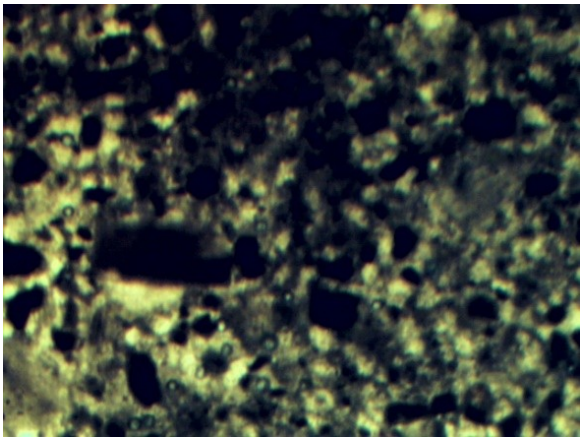


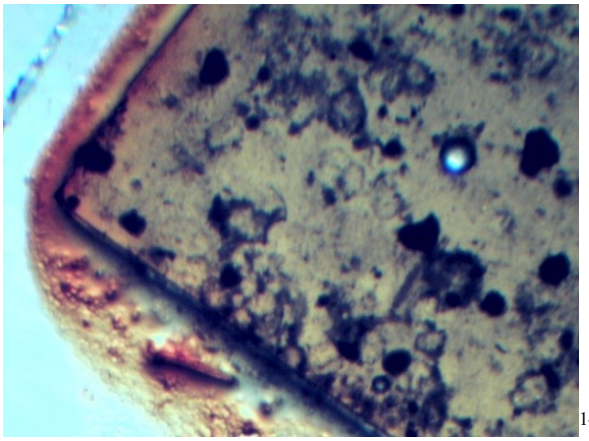


[148]



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.

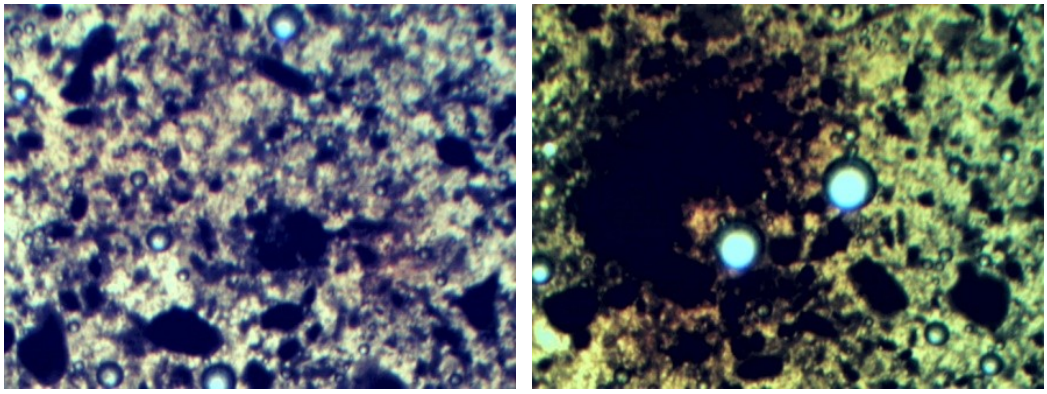




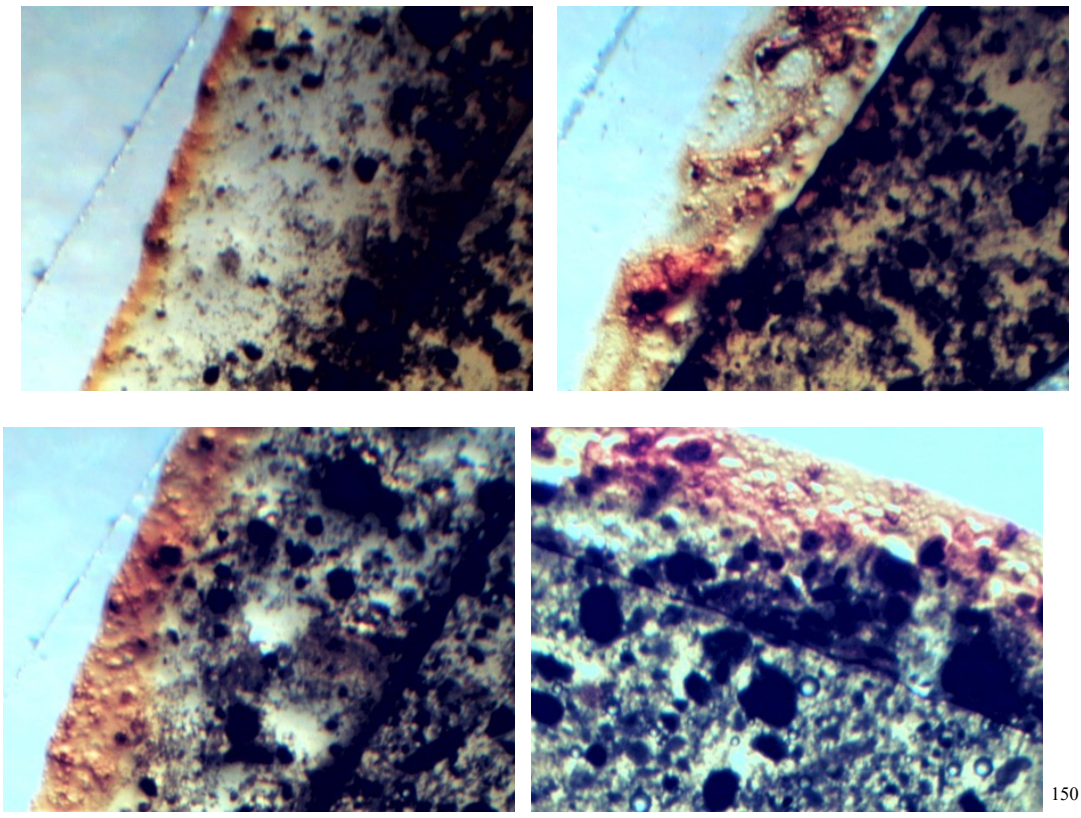
[149]

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.

[148] 40x magnification. Plant particles up to 0.9 mm
[149] 40x magnification. Plant particles up to 0.6 mm

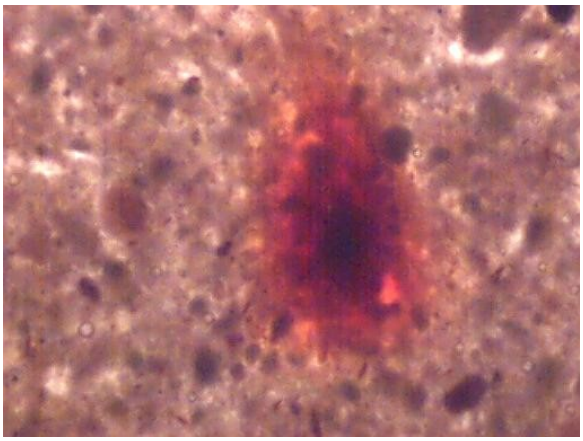
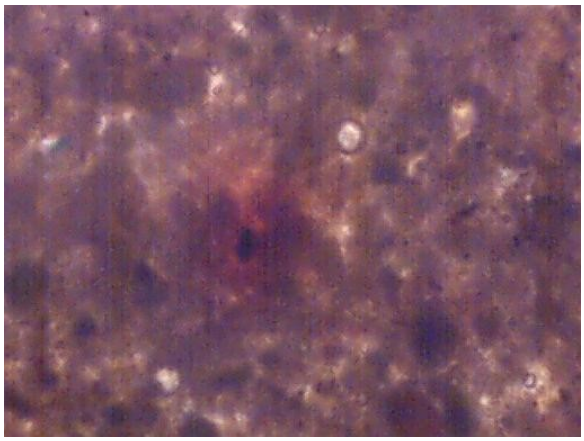


The above images are of a powder that strongly smells of terpineol releasing orange color during the first minute after adding water, with the orange reaction spreading towards the margins of the microscope slide.



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.

[150] 40x magnification. Plant particles up to 0.8 mm



[151]

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

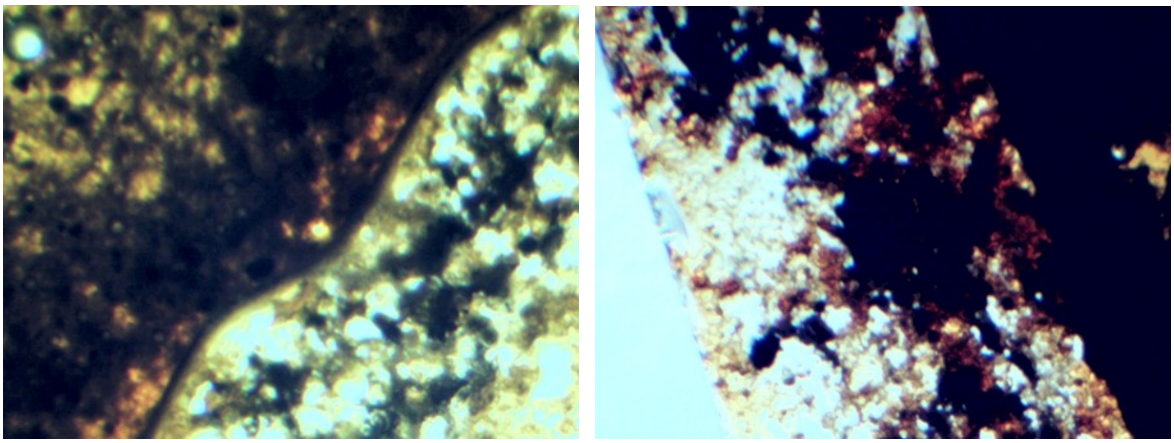
Red, yellow, white, blue, and black clays have been pulverized and used as cosmetic paints by nearly every human group, and were also used by Neanderthals. These clays are fairly harmless on skin except for those which contain lead. Some henna products contain red clay[152] to make hair appear fuller 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;[153] 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.[154] 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.

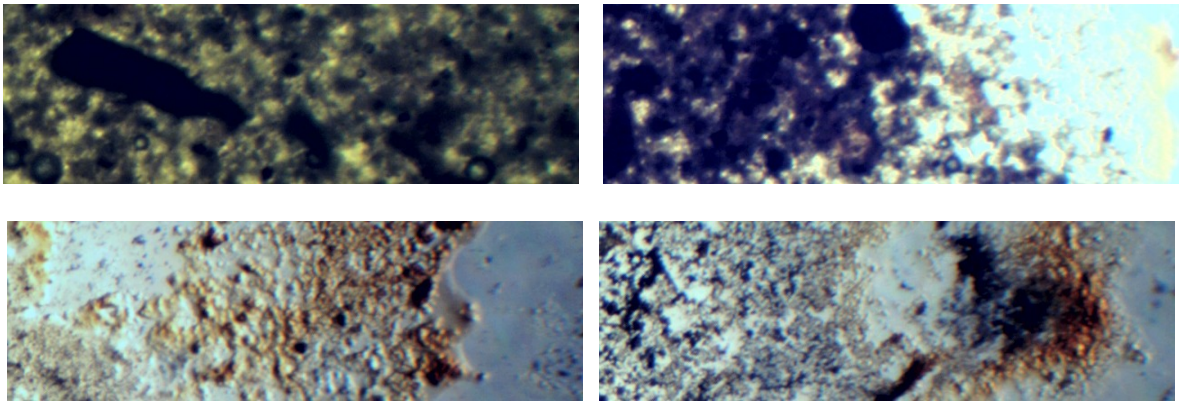
The following henna hair dye product claims 3% red clay in the ingredient declaration; the color of the powder is yellowish olive, but turns red as soon as water is added

[151] 'Instant henna' product acquired in 2000. 60x magnification. Plant particles up to 0.2 mm
[152] 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.
[153] $Al_2Si_2O_5(OH)_4$.
[154] Long term long term inhalation of clay dust can cause silicosis, or "potter's rot."



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.

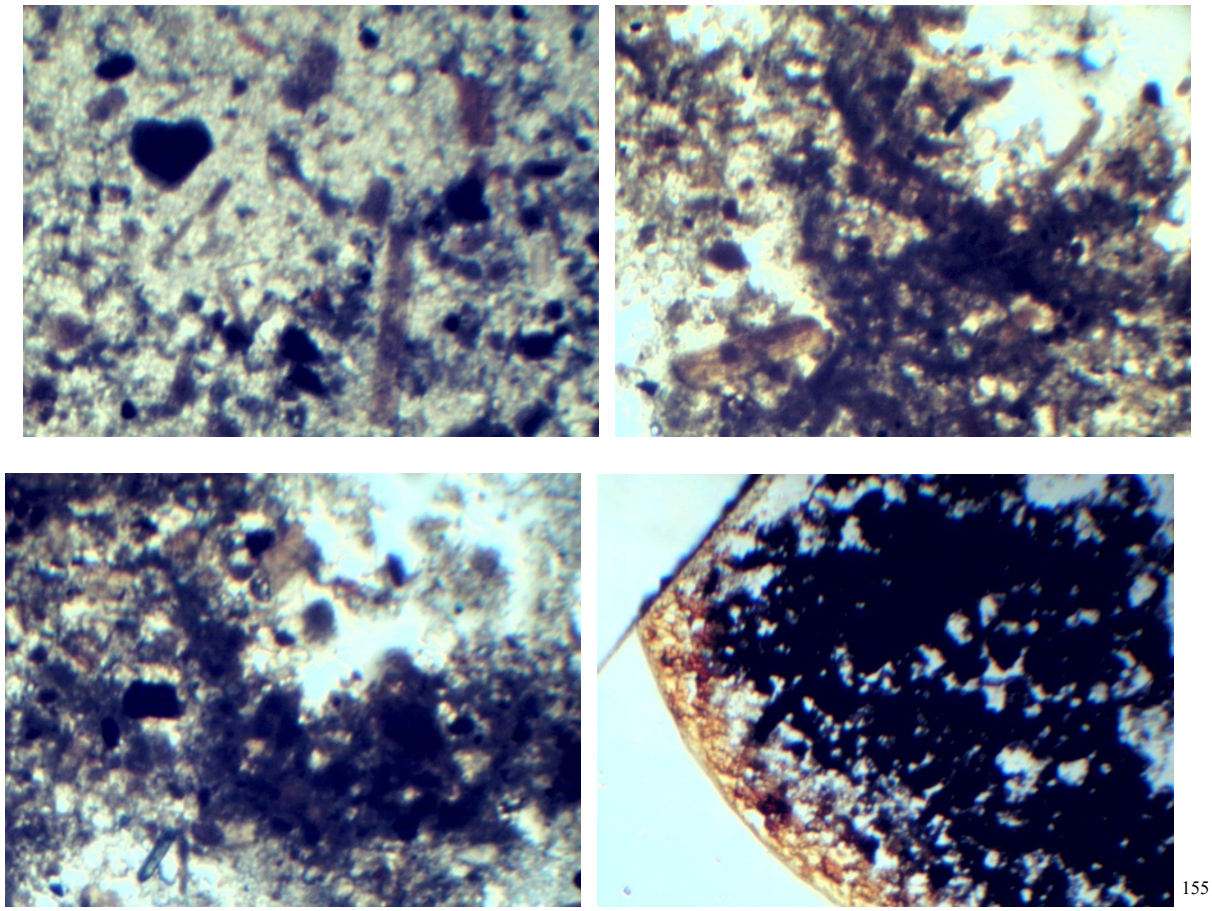
Below is a similar henna-clay product sold as henna hair dye, said to stain for four to eight weeks. The color of the powder is tannish-brown and smelled like damp earthenware clay.



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 an 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.

 [155]

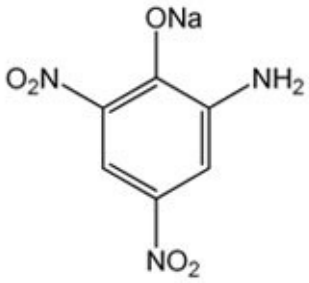
These sequential images show particles consistent with pulverized henna leaf and other plant material, with red-orange pigment emerging within five minutes.

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.[156] 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

[155] Magnification 40x, plant particles up to 1.5 mm

[156] $C_6H_4N_3NaO_5$

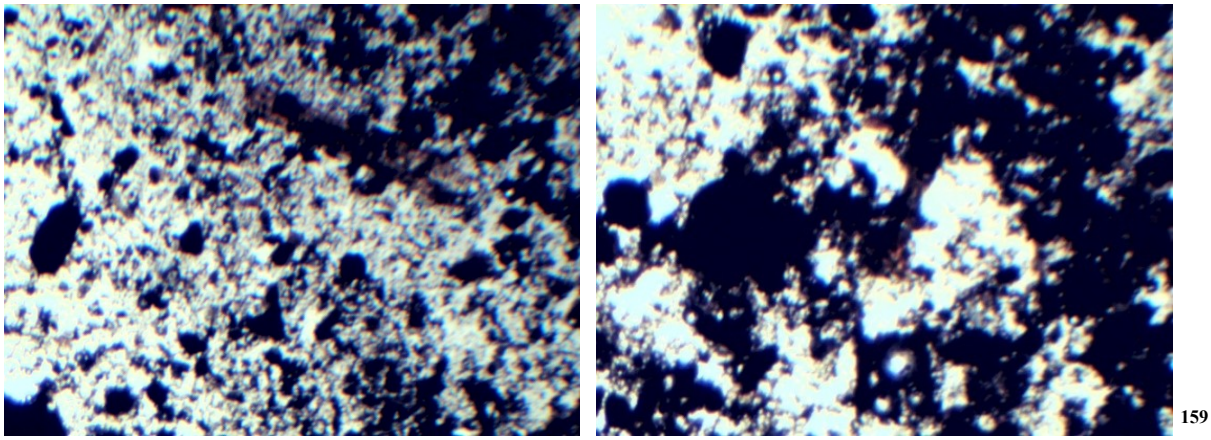
material, sodium picramate can react vigorously with reducing agents, including hydrides, sulfides and nitrides, causing a detonation and throwing fragments 1600 meters.[157]



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.[158] '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.



159

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.

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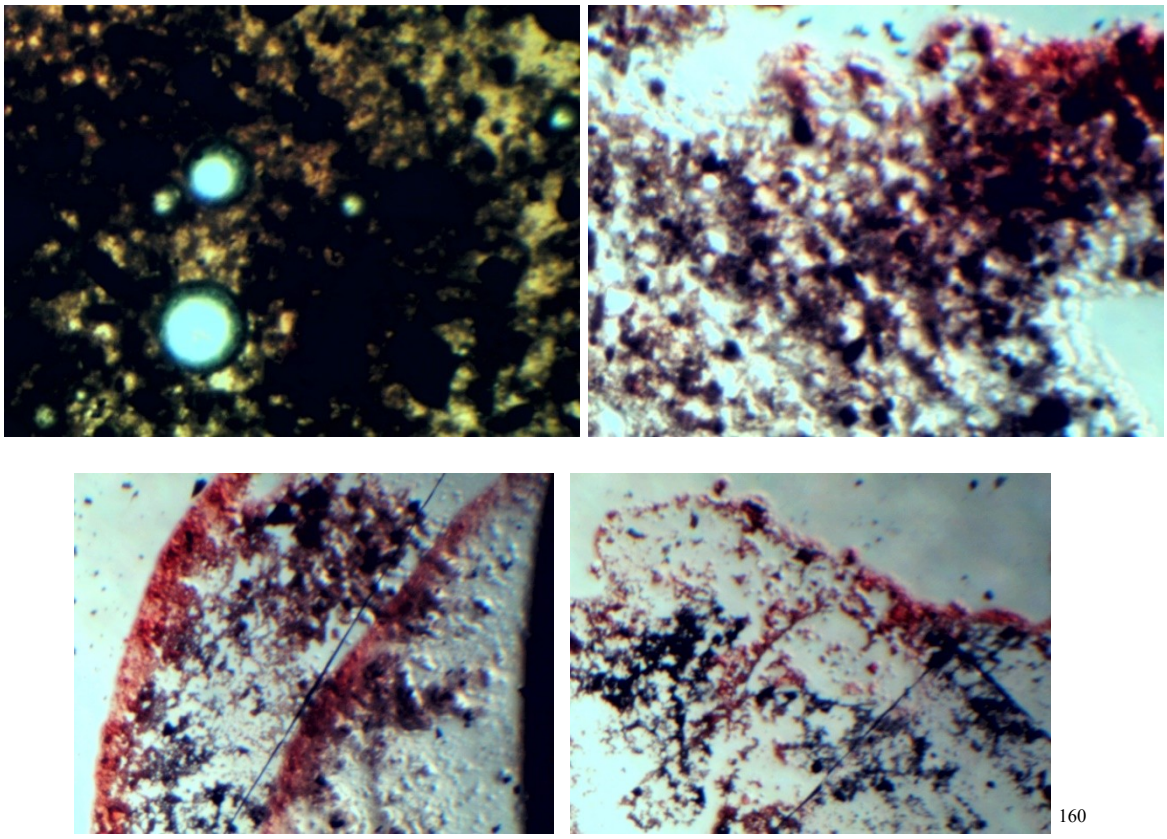
[157] Compound Summary for CID 5362461, "Sodium Picramate." *National Center for Biotechnology Information PubChem Open Chemistry Database* https://pubchem.ncbi.nlm.nih.gov/compound/Sodium_picramate

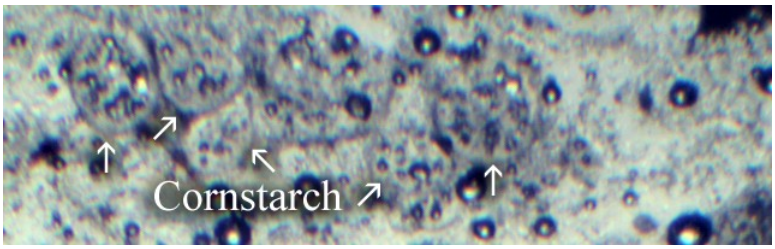
[158] Catherine Cartwright-Jones PhD. 2015. "Ancient Sunrise® Henna for Hair Chapter 3, part 2, Henna Mislabeling, Misinformation, and Disinformation" *TapDancing Lizard LLC.* http://www.tapdancinglizard.com/AS_henna_for_hair/chapters/chap3/henna_mislabeling_misinformation.pdf

[159] Magnification 40x, plant particles up to 1.5 mm

 [160]

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 'natural henna' but contained more lactose and cornstarch than henna.

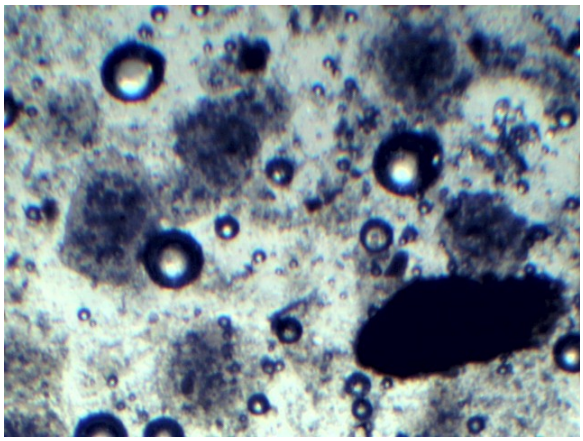
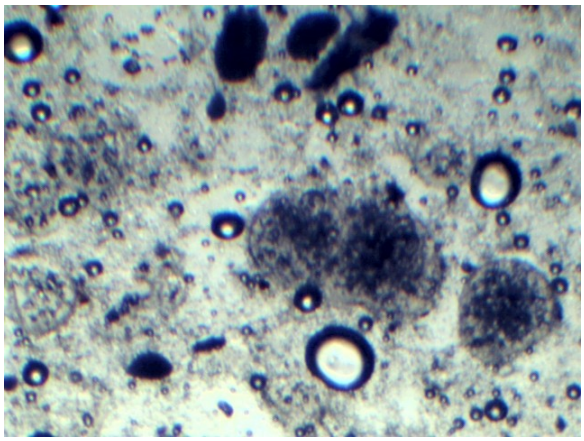


[161]

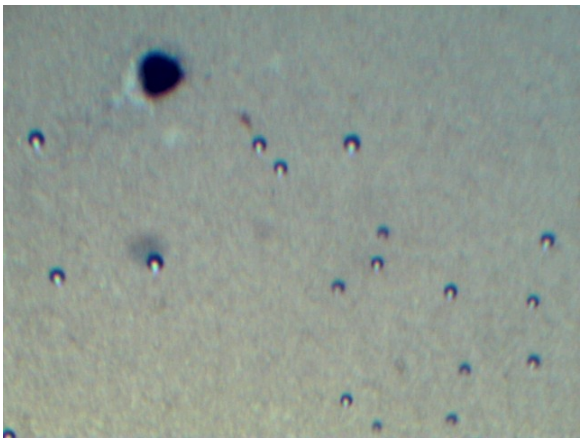
Cornstarch particles after Egyptian Natural Henna Conditioner" is wetted.

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

[160] Magnification 40x, largest plant particles are 0.6 mm.
[161] Magnification 40x, largest plant particles are 1.5 mm.



[162]

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.



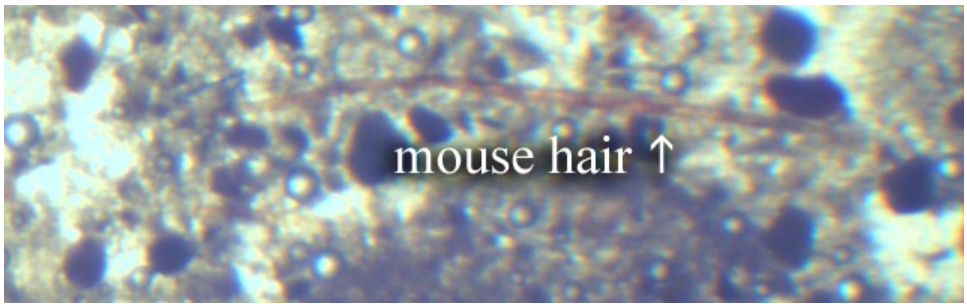
Ingredients: Aqua, Propanediol, Cetearyl Alcohol, Cetrimonium Chloride, Alcohol, Dimethyl Palmitamine, Benzyl Alcohol, Dehydroacetic Acid, Sorbitol, Orbignya Oleifera Seed Oil, Tocopherol, Hydroxyethylcellulose, HC Blue N° 2, Sodium Phytate, Achillea Millefolium Extract (Achillea), Aloe Barbadensis Leaf 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

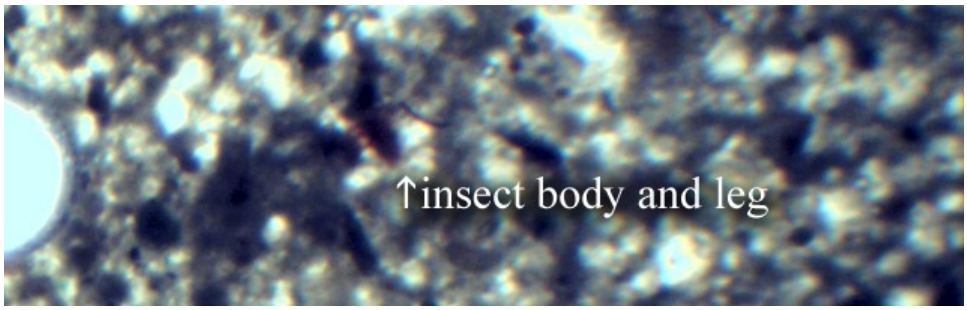
The occasional presence of unintended objects in henna is inevitable; henna is neither sterile nor a synthetic chemical product. Henna is an agricultural product world, and some things wander in. The following images are a reminder of why you should not eat henna, not drink henna tea, not put it on abraded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

[162] Magnification 40x, largest plant particles are 1.5 mm.



[163]

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



[164]

Henna is only to be sold in the US as a hair dye according to USFDA regulations,[165] 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.

[163] Magnification 40x, largest plant particles are 0.5 mm.
[164] Magnification 40x, largest plant particles are 1.mm.
[165] [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.