

Physicochemical analysis of Agnihotra aerosols and their implications on human health and environment

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Agnihotra or Yagya or Yajna, a traditional Indian ritual is considered as a method of ancient non-invasive inhalation therapy as well as a convenient method for environmental remediation. Despite research advances in environmental science, this traditional system of bio-fumigation & medication and its effect on the environment has not been effectively studied and requires detailed scientific analysis. In this article, we have investigated the physicochemical properties of Agnihotra aerosol particles using state-of-the-art advanced instrumentation techniques and analyze their effect on human health and the environment. We have focused our study in three major domains, morphological and chemical composition analysis of particulate matter (PM), real-time concentration analysis of selected environmental gases and systematic study of various volatile organic compounds that evolved during Agnihotra. The EDS and XRF analysis reveal that no toxic elements were released during Agnihotra. The real-time monitoring of various gases using the multi-sensor gas analyzer instrument reveals relative reduction in concentration by 25%, 20.76%, 15.45%, 23.22% and 13.63% for CO, NO₂, SO₂, O₃ and H₂S respectively. Further, GC-MS analysis reveals that more than 42 bioactive compounds have been found in the Agnihotra fumes. These findings suggest that Agnihotra may influence air quality and release biologically active compounds; however the therapeutic potential via inhalation requires further *in vitro* and *in vivo* validation. This work provides a foundation for future systematic investigations into the scientific basis of Agnihotra.

Keywords: Agnihotra, Environment, Fumigation, Inhalation therapy, Particulate matter, VOC

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Since ancient times, among various civilizations, traditional delivery of herbal formulations through fumigation, steam and other inhalation routes has been considered as the best treatment modality for non-invasive delivery of therapeutic molecules¹⁻³. The ethnobotanical and ethnopharmacological information from more than 50 countries have shown the application of medicinal smoke by burning of herbal plants to alleviate the prognosis of various deadly diseases, including pulmonary, dermatological, gastrointestinal, genito-urinary, psychological, neurological, orthopedic and cardiac conditions⁴. According to Ayurveda (ancient Indian system for traditional medicine), the role of specialized inhalation strategies such as dhumapana (aerosol), nasya (nasal medications) and

dhupanartha (fumigation) have special emphasis on the administration of various herbal and non-herbal formulations⁵. The detailed beneficial effects of fumigation therapy for non-invasive systematic administration of herbal and non-herbal formulations can be found in ancient Ayurvedic texts like *Charaka Samhitha* and *Sushruta Samhitha*⁶.

Agnihotra is an ancient Indian ritual performed by offering clarified butter, hawan samagri (a mixture of specific woods, medicinal herbs and odorous substances) to the Holy fire in an inverted pyramid shaped copper pot (*Agnikunda*) to purify the environment, inhibition of diseases causing microbes and generation of volatile organic compounds with various therapeutic benefits^{7,8}. The concept of Agnihotra was first discussed in *Rig Veda* (considered as the oldest knowledge text present on earth) and has multifaceted domains from

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spiritual enlightenment, cosmic energy management through *Chakra* activation, bio-fumigation, environmental remediation, enhanced growth of plants and grains and specialized therapeutic outcomes^{7,9,10}. Among them, the role of Agnihotra in purifying the environment and as a holistic approach for traditional delivery of various herbal therapeutic moieties by inhalation through the nasal cavity is of prime importance^{11,12}.

In the present study, we have performed the Agnihotra as per the directions of ancient Vedic literature and procedure mentioned in *Rigvedaadibhasyabhumika* (written by Swami Dayanand Saraswati) and investigated the physico-chemical characteristics of Agnihotra aerosol particles¹². We envisioned the role of Agnihotra in systematic administration of herbal formulations by employing various lipid-based VOCs evolved during Agnihotra as a carrier for intranasal non-invasive drug

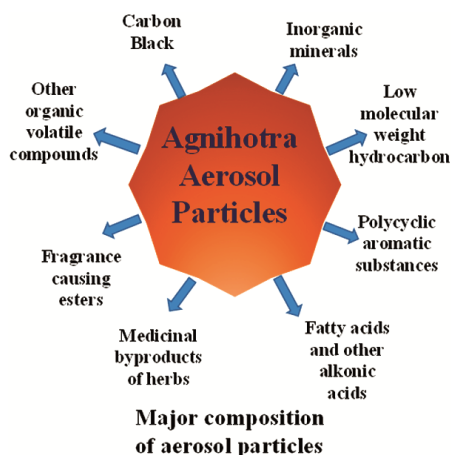


Fig. 1 — Major composition of Agnihotra aerosol system. It's a complex chemical composition consisting of particulate matter, inorganic minerals, hydrocarbons, aromatic compounds and various volatile organic compounds (VOCs)

delivery. The chemical identification of VOCs was carried out using Gas Chromatography - Mass Spectroscopy (GC-MS) analysis. On the other hand, in order to investigate the effect of Agnihotra fumes on the environment we have studied the real-time concentration of various environmental pollutant gases like CO, SO₂, NO₂, H₂S and O₃. Further, the elemental composition analysis of particulate matter (PM₅) that evolved during Agnihotra has also been studied at bulk as well as individual level for accessing the presence of toxic elements in Agnihotra aerosols. The present study aims to validate the ancient Vedic strategy of Agnihotra as a holistic approach for environmental remediation and providing enhanced therapeutic outcomes on modern scientific grounds.

Materials and Methods

Preparation of hawan samagri

The process of Agnihotra consists of offering a mixture of some herbal and medicinal plants/roots/extracts in an appropriate and fixed composition, commonly referred as 'Hawan samagri'. Controlled combustion of hawan samagri releases a variety of volatile organic aromatic compounds of high therapeutic efficacy with antibacterial, antimicrobial and antiviral properties. In order to prepare hawan samagri of high therapeutic efficacy with lesser amount of black carbon emission, appropriate and fixed composition of some special wood, aromatic substances and medicinal herbs and plant extracts were used as prescribed in ancient Vedic literature, detailed in (Supplementary Table S1).

Preparation of special hawan samagri with all constituents added in measured quantities as per described in Vedic literature is shown in (Fig. 2).



Fig. 2 — Preparation of special hawan samagri with all constituents added in measured quantities as per described in Vedic literature

Wood, medicinal plants (herbs) and healthy substance commonly used in yagya

Types of wood

Small sticks of wood commonly referred as 'samidhas' from medicinal plants like Pipala (*Ficus religiosa* Linn), Devdara (*Cedrus libani* L.), mango (*Mangifera indica* L.) and Dhakal Palasa (*Butea frondosa* Lam) were used as oblation in Agnihotra. It was ensured that wood should be dry and free from any kind of dust, dirt, insects and worms. During the Agnihotra ceremony, the samidhas were arranged in a specific manner so that controlled supply of air for continuous combustion of hawan samagri and other compounds should take place. Further, the appropriate and minimal amount of wooden sticks were used, requiring a continuous yellow colored bright flame rising from the fire pit.

Odors from substances

Some odorous plants/roots/powders were used as a part of hawan samagri for long-lasting antibacterial, antifungal fragrance. Appropriate amount of musk (*Abelmoschus moschatus*), saffron (*Crocus sativus*), rose petals (*Rosa centifolia*), white sandalwood powder (*Santalum album*), red sandalwood powder (*Pterocarpus santalinus*), agar (*Aquilaria agallocha*), tagar (*Ervatamia coronaria*), cardamom (*Elettaria cardamomum*), jayaphal (*Myristica fragrans*) and camphor were added to hawan samagri.

Substance with healthy constituents

Further, some substances (rich in essential fatty acids) like clarified butter (ghee), dried fruits, wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), til (*Sesamum indicum*), gram (*Cicer arietinum*) etc. were also added to hawan samagri for oblation in Agnihotra.

Herbs and medicinal plants/roots/extracts

For the preparation of hawan samagri about 70-80 types of herbs and medicinal plants/roots/ extracts were employed and crushed in a very fine powder without losing their natural origin characteristics. These herbs were added to hawan samagri in a fixed composition as prescribed in ancient Vedic literature. The complete list of herbs and medicinal plants used in the preparation of hawan samagri with their botanical names is shown in (Supplementary Table S1). Further, the herbs can be chemically categorized as terpenoids, alkaloids, aliphatic acids, steroids, phenolic compounds, phenylpropanes, lignins, aliphatic alcohols, aliphatic esters, aliphatic aldehydes and ketones, amino acids,

glycosides, flavonoids, carotenoids, carbohydrates, aromatic esters, anthocyanins etc.

Performance of Agnihotra

The Agnihotra ceremony is performed according to the guidelines/directions prescribed in ancient Vedas as well as documented methods/protocols by Arya Samaj philosopher Swami Dayanand Saraswati¹². Agnihotra was performed in the fire-pit (referred to as *agni-kunda*) of an inverted pyramid shape for meticulous combustion of hawan samagri and other constituents, controlled variation of temperature and optimal dissipation of energy. During the Agnihotra ceremony, a potential fumigation zone is developed inside the fire pit. The temperature gradient within this zone typically varies between 300°C and 650°C, depending on the specific part of the flame under consideration.

The experiment begins with invocation of prayers and ignition of fire with the help of pure ghee (clarified butter prepared from cow's milk) with chanting of some specific mantras or hymns (Sanskrit phrases). Further, small measured quantities (referred to as "ahutis") of hawan samagri and purified butter (ghee) were offered to the holy fire by each participant with rhythmic and systematic chanting of Vedic mantras. In the complete experiment, we offered 324 ahutis with chanting of the Gayatri Mantra and 21 ahutis of the Mayamritunjaya Mantra, along with some other Vedic mantras prescribed in ancient Vedas. The performance of Agnihotra as per the Vedic methodology in the present study is demonstrated in (Fig. 3). After completion of ahutis, a prayer (referred *yagya prathana*) followed by Shantipath was performed with deep feeling of gratitude towards Mother Nature and God. The entire Agnihotra ceremony, including chanting and the offering of oblations, was completed in approximately 180 min.

Aerosol collection using PM₅ sampler

Ambient atmospheric particles of diameter < 5 µm (PM₅) were collected using Envirotech APM 801 low volume air sampler. Flow rates were kept at 1.5 L/min. For investigating the bulk chemical composition of particles using the XRF instrument, PM₅ particles were collected on pre-weighed quartz (diameter 37 mm) filters using a PM₅ sampler. For individual particle analysis, aerosols were collected on the conducting substrate. For specifying the exposure side of the substrate, it was marked with a



Fig. 3 — Performance of Agnihotra as per the Vedic methodology: providing oblation of hawan samagri and ghee ahutis and Yagya-ash after completion of Agnihotra

black spot on the corner and then placed on the quartz filter. After completion of Agnihotra, the exposed substrates were carefully collected and stored in small micro-biological specimen tubes with proper sample labeling. These specimen tubes were then stored in a desiccator until analysis.

Analysis of gases evolved during Agnihotra using gas sensing system

For real-time analysis of various gases present in the atmosphere, a gas sensing system developed by our team has been employed¹³ (as shown in Supplementary Fig. S1). The real-time concentration of various gases like carbon monoxide (CO), nitrogen dioxide (NO₂), sulphur dioxide (SO₂), ozone (O₃) and hydrogen sulphide (H₂S) were measured and recorded. In order to analyze the effect of Agnihotra on the environment, we have measured the amount of the aforementioned gases in three different time durations, *viz.*, before Agnihotra, during the performance of Agnihotra and after completion of the ceremony. The data from the gas sensing instrument was electronically converted and stored in a micro-SD card. Further, the collected

data was analyzed and compared with different time intervals.

Collection of Volatile Organic Compounds (VOCs)

Volatile organic compounds (VOC's) emitted during Agnihotra were collected using a special setup (supplementary information) consisting of an inverted funnel, a pipe connecting the funnel and vertical column, and a condensation assembly as shown in (Supplementary Fig. S2). The condenser was connected to a pump which was continuously supplying the cold water to the outer cylinder of the condenser. The conical flask filled with 100 mL of pure methanol was connected with a high pressure vacuum pump. The Agnihotra fumes were condensed using the condenser assembly and collected in the conical flask. All the collected extract has been stored in airtight vials at 4°C in a refrigerator and analyzed using Gas Chromatography- Mass Spectroscopy (GC-MS).

Physical characterization

Scanning Electron Microscopy (SEM)

The morphological parameters of individual particles were analyzed employing Scanning Electron

microscope (SEM, ZEISS EVO MA-10, Germany) instrument. Briefly, the particulate matter were collected on conducting substrate using PM₅ sampler and stored in air tight vessels till the final analysis.

High resolution Transmission Electron Microscopy (HRTEM)

For morphological analysis of particles, High Resolution Transmission Electron Microscopy (HRTEM) has been employed. Firstly, a copper grid was placed in the PM₅ sampler for collection of particles onto the surface of the Cu grid. After completion of the Agnihotra ceremony, Cu grid was collected carefully and kept in airtight sealed sample box until final analysis. The morphology of particles collected on the Cu grid were investigated using HRTEM (Technai G2 F30 STWIN) in conjunction with a field electron source working at the electron accelerating voltage of 300 KV.

Chemical characterization

X-Ray fluorescence spectroscopy

The elemental compositions of collected aerosol particles were investigated using Wavelength Dispersive X-Ray Fluorescence Spectroscopy (WDXRF) (Rigaku ZXS primus). Briefly, the particles were collected on the quartz filter and stored in an air tight vessel till the final analysis. Further, the samples were irradiated with X-Rays that leads to a transition of electrons from a lower energy orbital to a higher energy orbital. When the excited electrons come to the lower energy, they emit the characteristic X-ray. The characteristic X-rays work as a fingerprint for each element and are detected by the detector after passing through analyzing crystals.

Energy dispersive spectroscopy

Energy Dispersive Spectroscopy (EDS) was employed for estimating the elemental composition of the aerosol particles evolved during Agnihotra. This process provides data by generating and analyzing characteristics of X-rays produced upon irradiation of particles with high-energy accelerating electrons through their center. The data points were analyzed using software and standards. EDS (Oxford Link ISIS 300, England) was equipped with an SEM instrument for elemental composition analysis of individual particles. This instrument is capable of identifying the elements having the atomic numbers from beryllium (Be) to uranium (U).

Gas chromatography-Mass spectroscopy (GC-MS)

The Gas Chromatography - Mass Spectroscopy (GC-MS) technique was employed for the chemical

identification of Volatile Organic Compounds (VOC's) that evolved during the Agnihotra ceremony. Briefly, the VOCs were condensed and collected in methanol using the special condensation assembly. The alcoholic extract of collected VOCs was analyzed using GC-MS (Shimadzu QP-2010 Plus with thermal desorption system TD 20, Japan) instrument equipped with an elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30×0.25 µm ID×0.25 µm df). The electron ionization system was run in the electron impact mode with ionization energy of 80eV for GC-MS detection. Helium (99.99%) was used as carrier gas and the flow rate was maintained at 1mL/min. The injection volume was adjusted to 2 mL. The temperatures of the injector and the ion source were maintained at 250°C and 200°C, respectively. The oven temperature was programmed to maintain isothermal conditions for 4 min at 100°C, which was followed by an increase at a rate of 10°C/min to 300°C, ending with a 6- min isothermal at 300°C.

Results

Elemental composition of Agnihotra aerosol particles using X-ray Florescence

The elemental composition of Agnihotra aerosol particles collected on the quartz filter using PM₅ sampler were investigated using XRF technique. The result reveals the presence of C, Al, Si, Mg, K, B, Cl, Fe, Ca, Cr and N elements as constituents of the aerosol particles (Fig. 4). Carbon was observed in the most prominent amount in the aerosol particles. Further, the observed carbon could either be of any form, viz. Black carbon (BC), Organic Carbon (OC) and Brown Carbon (BrC).

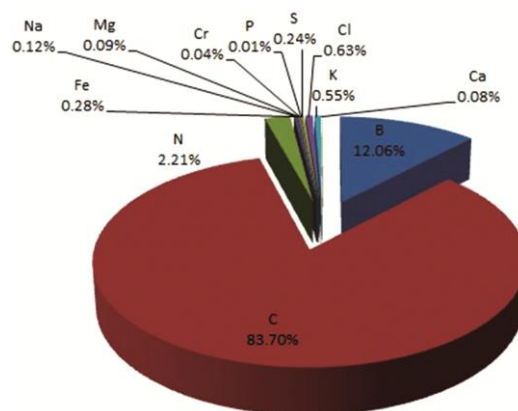


Fig. 4 — X-Ray fluorescence analysis of Agnihotra aerosol particles collected using PM₅ sampler

Individual particle analysis using SEM-EDS

The morphology of the aerosol particles or particulate matter (collected by PM₅ sampler) emitted during Agnihotra was investigated using SEM. SEM monographs of Agnihotra aerosol particles are shown in (Fig. 5). The particles were observed to be irregular in shape, size and morphology. Further, the external appearance of some particles indicates the presence of carbon fractals on the outer surface of PM. The elemental compositions of individual Agnihotra aerosol particles were investigated employing EDS technique.

EDS analysis reveals the particles are rich in C, Si, Al, Fe, Mg, K and Cl elements (Fig. 5). The C observed in the analysis could be of any type, like: Organic Carbon (OC), Brown Carbon (BrC) and Black Carbon (BC) (Fig. 5 a-c). Further, the presence of clearly visible black carbon fractals was also observed in the analysis of Agnihotra aerosol particles¹⁴ (Fig. 5 d).

Morphological analysis using HRTEM

The morphology of Agnihotra aerosol particles was also investigated using HRTEM (Fig. 6). The surface

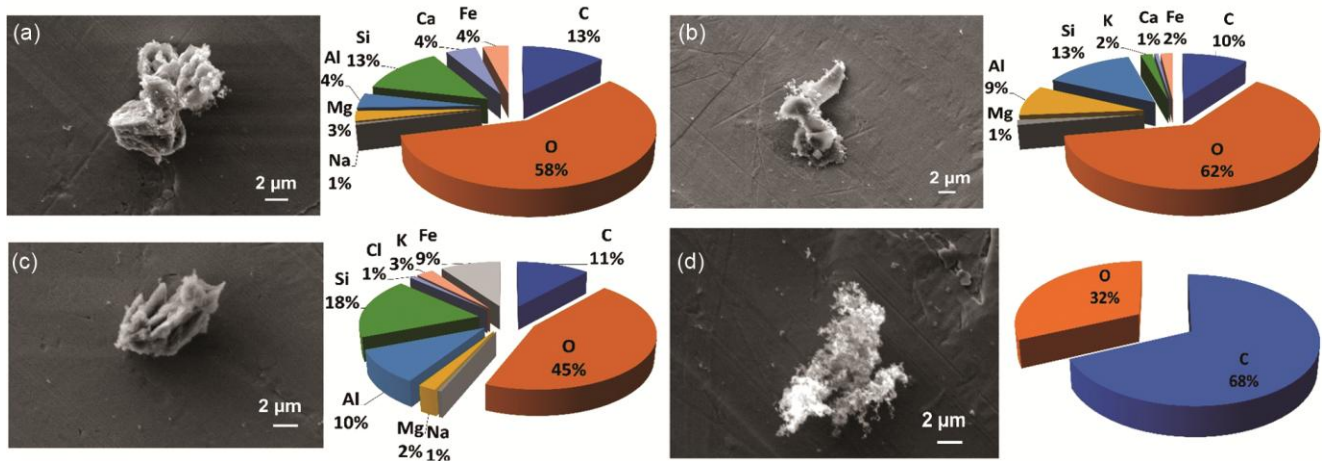


Fig. 5 — Morphological and elemental analysis of individual particles collected by PM₅ sampler employing Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS), respectively

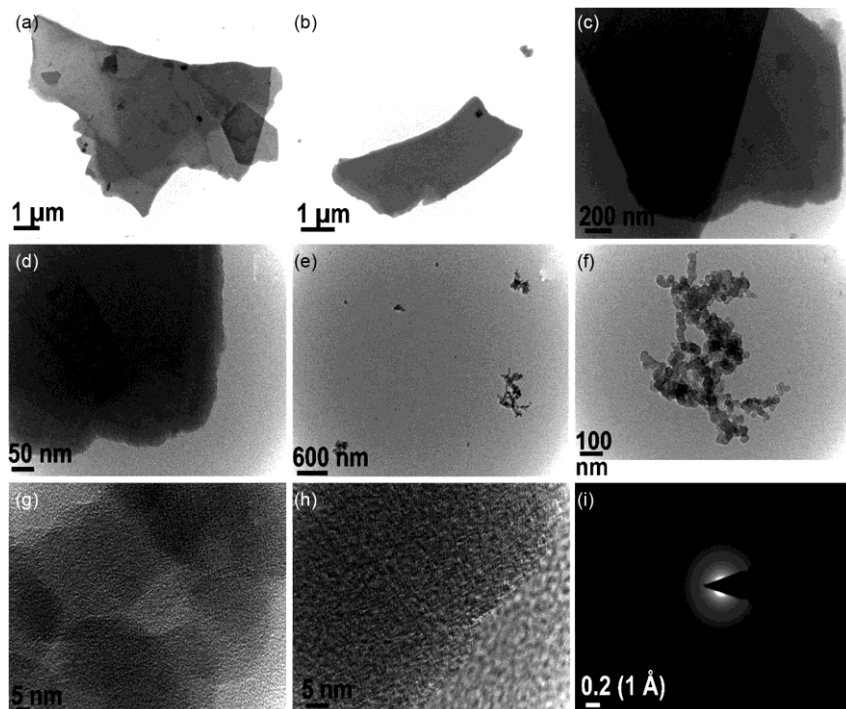


Fig. 6 — Morphological analysis of aerosol particles collected on Copper (Cu) grid employing High Resolution Transmission Electron Microscopy (HRTEM)

characteristics and morphology of observed particles reveal the presence of carbon black fractals mixed with minerals, soot, metal, organic and fly ash particles. More specifically, the particles observed in (Fig. 6 a-d) have shown characteristics morphology of black carbon fractals rich in minerals and fly ash particles. Further, the appearance of aggregation of small particles in (Fig. 6 e & Fig. 6 f) indicates the presence of aromatic soot particles¹⁵. Specifically, the small aromatic soot particles of around 20-40 nm in diameter appeared to aggregate in (Fig. 6 g & Fig. 6 h) respectively. The observed morphological results are in good corroboration with SEM analysis of Agnihotra aerosol particles. Further, in (Fig. 6 i), the selected area electron diffraction (SAED) pattern of observed Agnihotra aerosol particles confirms the presence of amorphous carbon.

Analysis of gases evolved during Agnihotra using gas sensing system

Agnihotra is the process of controlled combustion of medicinal plants which contributes some gases to the environment. As combustion of biomass mainly contributes towards air pollution by enhancing the concentration of various greenhouse gases in the environment, thus, in order to differentiate the burning of raw wood and Agnihotra, we have investigated and monitored the real-time concentration of some hazardous gases like carbon monoxide (CO), nitrogen dioxide (NO₂), sulphur dioxide (SO₂), ozone (O₃) and hydrogen sulphide (H₂S) in three-time intervals viz before Agnihotra, during Agnihotra and after Agnihotra. Further, in order to exclude the possible influence of ventilation, dilution, and ambient atmospheric conditions, the entire experiment was carried out inside the closed Yagya Shala (Arya Samaj Mandir, West Patel Nagar, New Delhi).

The relative concentration of gases that evolved has been analyzed employing a state-of-the-art gas analyzer instrument which is indigenously developed by our team at CSIR-NPL. The relative concentration of CO, NO₂, SO₂, O₃ and H₂S gases at different time intervals is shown in (Fig. 7). The real-time concentration has been measured by monitoring the concentration of gases over a one-minute time interval. Further, the data was plotted by taking an average mean of 10 consecutive values for each interval *i.e.*, before, during and after Agnihotra. As the concentrations of gases are in parts per million (ppm) and parts per billion (ppb), so for the relative comparison, the concentrations for each gas were

plotted in log₁₀ units. Further, all experimental data from the gas analysis were subjected to statistical evaluation, and the variation was consistently found to be within 5% in each subset. The results showed a

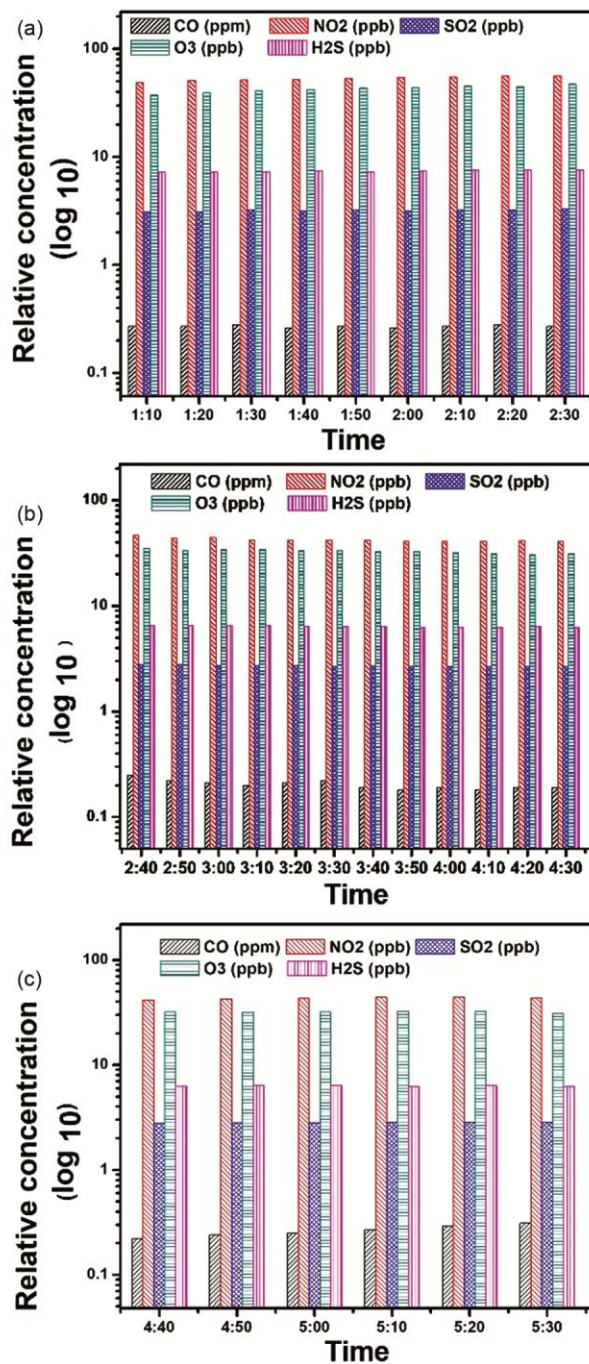


Fig. 7 — Relative concentration of CO, NO₂, SO₂, O₃ and H₂S during different time intervals (time in hrs represented as a local time (LT)): (a) before Agnihotra; (b) during Agnihotra and (c) after Agnihotra. The concentration of each gas was monitored by a one-minute time interval and an average of 10 consecutive readings were taken for a single data point

good correlation between the concentration of gases at different time intervals and indicates the repeatability and reliability of the instrument.

Further, the mean average concentrations of CO, NO₂, SO₂, O₃ and H₂S gases were plotted and compared with each time interval i.e. before, during and after Agnihotra. A significant reduction in the relative concentration of gases monitored during Agnihotra as compared to before was observed (Fig. 8). Statistically, relative reductions in concentration by 25%, 20.76%, 15.45%, 23.22% and 13.63% for CO, NO₂, SO₂, O₃ and H₂S respectively were observed during Agnihotra. On the other hand, it was found that the relative concentrations of gases were slightly increased one hour after the completion of the Agnihotra ceremony. Mathematically, an increase in concentrations by a factor of 30% for CO, 2.4% of NO₂ and 4.35% for SO₂ were observed. Interestingly, a slight decrease in the concentrations for O₃ (2.48%) and H₂S (1.29%) gases were also observed, indicating the relatively longer chemical interaction/effect of Agnihotra on these gases.

Analysis of volatile organic compounds (VOCs) emitted during Agnihotra using Gas Chromatography- Mass spectroscopy (GC-MS)

Agnihotra fumes or other medicated fumes (fumes of herbs/medicinal plants) have been used as inhalation therapy treatment in Vedic, Iranian, Chinese, African, European and other civilizations since ages⁴. Controlled combustion of hawan samagri and other constituents generates VOCs into the environment. These VOCs are responsible for the induction of various therapeutic effects like antifungal, antibacterial and antimicrobial action in the surroundings as a result of Agnihotra^{7,8}. Therefore, in order to access the chemical characteristics and qualitative analysis of VOCs liberated during Agnihotra, we have collected the fumes using a special condensation setup (details in methodology section). The fumes generated during Agnihotra were condensed and dissolved in methanol: hexane (80:20) solvent. Further, the solvent extract was analyzed using Gas Chromatography- Mass spectroscopy technique for qualitative analysis of VOCs present in Agnihotra fumes. From numerous peaks obtained in GC-MS chromatogram, some peaks of relatively high intensity were distinguished and analyzed using GC-MS solutions software (Shimadzu). We have found 41 most prominent peaks and analyzed with maximum hits using GCMS solutions software (Shimadzu)

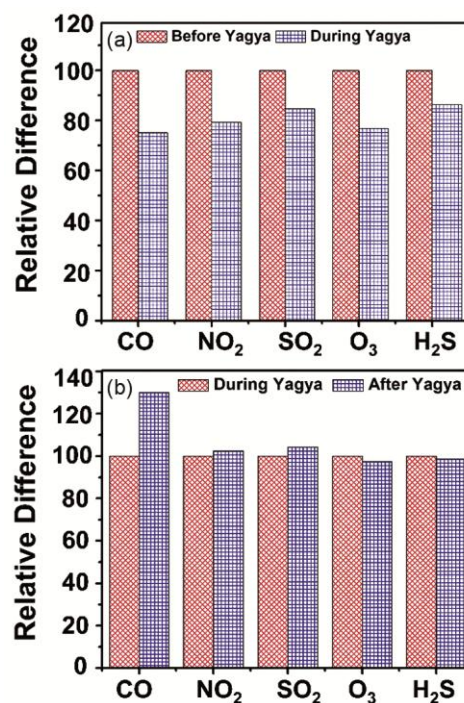


Fig. 8 — The relative difference in the concentration of CO, NO₂, SO₂, O₃ and H₂S: (a) Concentration of the gases during Agnihotra relative to the respective average concentration before Agnihotra and (b) Concentration of the gases after Agnihotra relative to the average during Agnihotra

software. (Supplementary Table S2), comprising the analyzed peak with relative area and height percentage with their corresponding compounds with maximum hits obtained using the NIST and Shimadzu library.

Discussion

One of the oldest written evidence about the performance of Agnihotra (or hawan or Yagya) for attaining spiritual enlightenment, purification of body, mind and surrounding environment can be found in *Yajurveda* (considered as the oldest religious text and scriptures from the Vedic period, Chapter 1: Shloka 2)¹⁶. According to ancient Vedic philosophy, Agnihotra is considered as a process of bio-fumigation as well as inhalation therapy through generation of medicinal phytochemicals for treatment of various diseases, killing airborne microorganisms and purification of environment^{7,11}. A number of scientific documented evidence has been advocating the bio-fumigation potential of Agnihotra over chemical fumigation techniques for ecofriendly and safer disinfection of air by reducing the population of aeromicroflora (disease causing bacteria and viruses) in the surroundings^{10,16}. On the other hand, Agnihotra

provides an alternate medium to deliver various herbal and medicinal extracts through intranasal inhalation drug delivery route¹⁷. Interestingly, recent *in-silico* findings indicate the potential of Indian herbal steam inhalation therapy employing medicinal fumes of *Vitex negundo* L., *Justicia adhatoda* L. and *Eucalyptus globules Labill* plants/herbs in combating SARS-CoV-2¹⁸.

The Agnihotra ceremony consists of systematic and controlled combustion of medicinal herbs/extracts along with essential oils/clarified butter milk etc. in an inverted copper pot. During the combustion process, the temperature of the pot varies from 300-1200°C (from the bottom of the pot to above the flames) and creates optimal conditions for fumigation, termed as “potential fumigation zone” (PFZ)^{7,12}. Most of the hydrocarbon undergoes partial oxidation and gets converted to other functionalized derivatives of high therapeutic efficacy. Further, the temperature variation (*i.e.*, 300-1200°C) during combustion of herbal/medicinal plants causes the liberation of various functionalized VOCs in the atmosphere *viz.*, aromatic compounds of fatty acids in the PFZ region and derivatives of esters and organic acids through the bottom of the pyramid-shaped pot (relatively colder region)⁷.

The VOCs obtained through GC-MS chromatogram were studied thoroughly. The fundamental characteristics/information like molecular weight, molecular formula, chemical structure (drawn using ChemDraw software), physical properties like color, state and odor with their chief biological applications of observed VOCs through GC-MS have been summarized in (Supplementary Table S3). Among them, some compounds have been reported to exhibit antiviral, antibacterial, antifungal and some may prove to be useful in cardiac treatment^{18,19}. Specifically, Cyclouron are among the herbicidal urea family which are absorbed by the plant roots and act as herbicides by demethylation of nitrogen atom²⁰. Cinnamic acid and its derivatives are well known for their extraordinary anti-inflammatory, anti-tumor and free radical scavenging properties²¹. These derivatives have been employed for treatment of various bacterial infections, diabetes, malaria, tuberculosis, neurological disorders and some types of cancers²². Naturally occurring cinnamic acid and its derivatives have been used as traditional medicines in India, Thai, Chinese and other cultures since ancient times²³.

Further investigation of the GC-MS chromatogram reveals the presence of various fatty acids, esters of

fatty acids, essential oils of herbal formulation etc. liberated from the fumes of Agnihotra. Chiefly, derivatives of lauryl alcohol, isopropyl myristate, myristic acid, substituted long chain fatty acids and their esters, evolved during Agnihotra. Recent investigations by prominent groups reveal the application of lauryl alcohols and its functional derivatives as novel drug delivery nanoplatfoms owing to their topical and targeted penetration directly to skin dermatophytes²⁴. Further, Isopropyl myristate, an ester of isopropyl alcohol and myristic acid, has shown excellent transdermal efficacy for the treatment of skin syndromes, fungal infections, removal of head lice and has been extensively used in a number of commercial personal care products²⁵. Further, myristic acid has also exhibits skin penetration and transdermal properties as well as has shown potential to treat patients with African Trypanosomes (sleeping sickness, disease caused by infection with the parasite *Trypanosoma brucei*)²⁶. Presence of compounds like lauryl alcohol, isopropyl myristate and myristic acid in the medicated fumes of Agnihotra indicate the potential topical transdermal action of Agnihotra for treatment of fungal infections. However, in order to investigate the true potential of these compounds, further *in-vitro* and *in-vivo* investigations are required. Further, a few long- chain fatty acids like palmitic acid, oleic acid, linolenic acid, margaric acid, palmitelaidic acid, linolenelaidic acid, stearic acid and arachidic acids have also been found in the methanol extract of Agnihotra fumes. These fatty acids and their derivatives have shown excellent anti-viral, anti-fungal, anti-microbial, anti-bacterial, anti-inflammatory and anti-cancer response in a number of studies^{19,27,28}. Most of them are on the Generally Recognized As Safe (GRAS) list of the United States Food and Drug Administration (FDA)²⁹.

Recent reports suggest the role of fatty acids as a nasal invasion therapy treatment with enhanced therapeutic efficacy. The single-blinded randomized clinical control trials by Attia *et al.*³⁰, found the beneficial effect of nasal administration of omega -3 fatty acids on delaying the incidence of recurrence of nasal polyposis (a form of chronic inflammatory process causing allergy, asthma infection and cystic fibrosis). Another study by Khunt *et al.*³¹, suggested the application of moderate to long chain fatty acids as permeation enhancers for nasal administration of donepezil hydrochloride to the central nervous system by avoiding the complications of blood brain barrier

(BBB). On the other hand, fatty acids can induce antibacterial action mainly by disrupting the bacterial cell membrane and alteration of crucial bacterial cellular processes like the disruption of electron transport chain, uncoupling oxidative phosphorylation and inhibiting the activity of bacterial enzymes²⁸. As from the literature, it is evident that these compounds exhibit diverse therapeutic properties individually. However, in the context of Agnihotra, a systematic and rigorous investigation is essentially required employing specialized *in vitro* and *in vivo* experimental approaches to elucidate the healing potential and bioactivity of these compounds released during the process.

Further, the presence of aroma producing compounds like galaxolide (a polycyclic musk, extensively used in household air fragrances, air fresheners etc.) in the collected extract of Agnihotra fumes is responsible for the long lasting clean and sweet aroma of Agnihotra medicated fumes³². The presence of phytosterol class compounds like stigmasta-5,22-dien-3-ol, acetate and stigmasta-3,5-diene in Agnihotra fumes is responsible for the anti-microbial, analgesic, anti-inflammatory, antioxidant and anti-nociceptive properties^{33,34}. Further, the presence of a few esters of fatty acids like linolelaidic acid ethyl ester, glycidyl palmitate, butyl-linolenate etc. are the chief constituents, have shown antimicrobial and antifungal activity in a number of studies and may indicate the possible antimicrobial action of Agnihotra. Pachori *et al.*¹⁰, reported a significant decreases in the concentration of aeromicroflora (including the growth of bacteria, fungi and Actinomycetes) and suggests Agnihotra as an efficient method of bio-fumigation and as an environmentally friendly alternative for chemical fumigation. However, they could not address the characteristic components responsible for this bio-fumigation behavior. From the present findings, it becomes evident that Agnihotra fumes consist of VOC's that can show bioactivity with no observed environmental and animal toxicity. Further, the associated therapeutic and environmental outcomes could be modulated by modifications in the constituents of hawan samagri offered as oblations.

The associated effects of Agnihotra on the environment have also been investigated by various groups in a few domains. Abhang *et al.*¹¹, have reported the effect of Agnihotra on reduction of NOx and SOx levels in the environment. According to

recent figures from NASA, the global average surface temperature rose 0.6 to 0.9 degrees Celsius (1.1 to 1.6° F) between 1906 and 2005, and the rate of temperature increase has nearly doubled in the last 50 years ("GISTEMP Team, 2021: GISS Surface Temperature Analysis (GISTEMP)³⁵. Despite being a complex phenomenon, the primary factors associated with the unprecedented rise in global surface temperature are uncontrolled elevation in concentration of various greenhouse gases like carbon monoxide (CO), nitrogen dioxide (NO₂), sulphur dioxide (SO₂), ozone (O₃) and hydrogen sulphide (H₂S). Thus, extending the previous investigation by various research groups, we have encompassed the real time analyses of CO, NO₂, SO₂, O₃ and H₂S gases and compared their concentrations before, during and after the performance of Agnihotra. The significant reduction in the concentration of these gases during Agnihotra can be associated with various photochemical reactions, possibly converting the toxic gases to other forms³⁶⁻³⁸. Our present findings have indicated the atmospheric remediation behavior of Agnihotra.

In a nutshell, Agnihotra is a process for spreading benevolence in all forms of Mother Nature without any race, religion, caste and creed. It will generate positive energy as well as prevent environmental pollution. Most importantly, it will enhance one's life with the positive energy of spirituality, which is above all materialistic luxuries. The performance and execution of the Agnihotra ceremony is pure science that includes the systematic procedure and methodology for offering herbs to fire as oblations. Due to lack of systematic documentation, this ancient Vedic science is still unable to find its actual worth. We aim to design specific experiments in the future to investigate the bio-activity of the Agnihotra ritual in a more scientific manner.

Conclusion

The present study provides a fundamental basis for understanding the physicochemical characteristics of Agnihotra aerosol particles, real-time gas analysis, and the evolution of VOC's during Agnihotra. Interestingly, XRF-based elemental analysis reveals no toxic element evolved during Agnihotra. Further, the relative reduction in concentration by 25%, 20.76%, 15.45%, 23.22% and 13.63 % for CO, NO₂, SO₂, O₃ and H₂S respectively, indicate the environmental remediation behavior of Agnihotra. The GC-MS based

analysis of Agnihotra fumes reveals the presence of 42 VOCs. These VOCs have shown to exhibit bio-active properties in various well-documented studies. In order to analyze the therapeutic potential of Agnihotra aerosol particles and fumes, specific *in vitro* and *in vivo* experiments need to be conducted. We aim to design detailed experiments in the future to bridge the gap between this ancient traditional ritual and modern scientific understanding.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at [https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25\(2\)\(2026\)148-159_SupplData.pdf](https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25(2)(2026)148-159_SupplData.pdf)

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Conflict of Interest

The authors declare “no conflict of interest”.

Author Contributions

BDA, VG, SKM and SPS have conceptualized the entire idea and methodology of this experiment. BDA and SPS have written the manuscript, VG, AS and SKM have contributed in relevant discussions.

Ethical Approval

The present study did not involve any direct human or animal experimentation, and therefore no institutional ethical approval was required.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request

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