Journal of Saudi Chemical Society (2016) **20**, 465–473

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Chemical composition and potential health risks of raw Arabian incense (Bakhour)

Yehya Elsayed *, Sarah Dalibalta, Ioline Gomes, Nagelle Fernandes, Fareedah Alqtaishat

Department of Biology, Chemistry & Environmental Sciences, American University of Sharjah, P.O. Box 26666, Sharjah, United Arab Emirates

Received 19 August 2014; revised 9 October 2014; accepted 15 October 2014
Available online 7 November 2014

KEYWORDS

Bakhour;
Arabian incense;
Chemical analysis;
Hazardous materials

Abstract Burning Arabian incense (Bakhour) is a common indoor practice in the Middle East and the Arabian Gulf region. However, the chemical composition of this substance has never been studied. Three different Bakhour brands were selected for this study. A complete chemical profile for the raw samples was determined using carbon, hydrogen, and nitrogen elemental analysis, inductively coupled plasma optical emission spectroscopy, scanning electron microscopy coupled with energy dispersive X-ray spectroscopy and gas chromatography mass spectrometry techniques. A wide range of elements and compounds were identified, many of which are hazardous to health. Nitrogen was found in all samples which should raise concerns due to the known health implications of amines, nitrogen oxides and nitrites. In addition toxic metals such as cobalt, copper, iron, nickel, lead, and zinc were also determined in all samples. The amounts of these metals are equivalent to those in raw tobacco, where they are known to pose health risks. Three types of solvents (acetone, dichloromethane and toluene) were used for the extraction of organic compounds. Carcinogens, toxins and irritants were found along others of different health implications. Isolation of these compounds provides preliminary evidence on the harmful consequences of being exposed to Bakhour.

1. Introduction

Bakhour, traditional Arabian incense, is commonly used in the Middle East and North Africa to perfume houses, shops, clothing, and in various ceremonies and worship homes [1,2]. Bakhour is traditionally burned to purify the surroundings from evil spirits, create a pleasant atmosphere or eliminate undesirable odors in an indoor environment [3–8]. Bakhour is therefore one of the most common sources of indoor smoke in the Arabian Gulf region to which individuals are exposed to

* Corresponding author. Tel.: +971 6 5152576; fax: +971 6 515 2450.
E-mail address: yelsayed@aus.edu (Y. Elsayed).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2014.10.005
1319-6103

on a weekly basis in most homes [3,4]. Bakhour can vary in composition, but it usually contains a wide variety of natural ingredients such as agarwood (oud), woodchips, musk and sandalwood that are soaked in scented oils [1,2]. The ingredients are mixed and formed into small bricks which are burned in charcoal, gas or electric burners to produce the scented smoke [9].

A few studies on Bakhour suggest that the smoke produced from burning the raw material is a potential health hazard [3,4,10–15]. An indoor air pollution study conducted in the United Arab Emirates (UAE) in 2012 on 628 homes of Emirati nationals found that the burning of Bakhour on a daily basis is associated with increased headaches, difficulty in concentration and forgetfulness [3]. A community survey conducted in Oman revealed that Bakhour burning triggered wheezing among asthmatic children [10]. Clinical studies have also reported noticeable cell inflammatory responses upon the exposure of individuals to components in Bakhour smoke, such as particulate matters smaller than 2.5 μm ($PM_{2.5}$), carbon monoxide (CO), sulfur dioxide (SO_2), oxides of nitrogen (NO_x), formaldehyde (HCHO), and carbonyls [3,10]. Research on rats and mice indicated that continuous exposure to Bakhour smoke results in reduced lung weight, necrosis and degradation of epithelial bronchioles, and ultra-structural pulmonary changes known to impair respiratory efficiency [12–14]. Although there is accumulating evidences to suggest the potential harm that may be associated with burning Bakhour, very little is known about the chemical composition of the raw material [3,4,10–15]. For instance, the *Aquilaria malaccensis* plant's woodchips that are processed to make Bakhour were found to sequester high amounts of metals such as cadmium (Cd), nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), and lead (Pb) from soils [16]. The presence of such metals and their salts in the raw Bakhour could pose a serious health risk. Cadmium is a poisonous metal that can severely affect the lungs, kidneys, and bones [17]. Cadmium oxide (by-product of pyrolysis and combustion) is a carcinogen and can also cause tracheobronchitis and pulmonary edema [17]. Lead is toxic and known to cause heart and kidney failure in humans [17]. In addition, nickel (Ni)-containing compounds are known to cause asthma as well as to be a "Group 1" carcinogen [17].

Few studies on incense products postulated a possible relationship between the chemistry of the raw material and the composition of the emitted smoke [17–19]. A study by Hsueh et al. correlated the particulate emission in the smoke generated from burning Asian incense to the metallic elements in the raw incense [17]. They revealed the presence of the Na, Ca, Mg, Al, and K elemental species in both the aerosol and the ash of the Asian incense [17]. The presence of particulate matters that are smaller than 2.5 μm ($PM_{2.5}$) raises concerns as air pollutants and as a threat to human respiratory health [17]. A hazard assessment study on smoke produced from burning Bakhour in the UAE estimated the concentration of particulate matters (PM) to be 1.34–1.36 mg/m^3 with an emission rate of 5.9 mg/min, which is higher than the emission rate from cigarettes (0.7–0.9 mg/min) [11]. In another study, the relationship between smoke, ash formation and metallic content in incenses similar to Bakhour was evaluated [18]. The study concluded that the presence of higher metallic content in the raw incense materials results in higher burning rates associated with a reduction in the level of suspended particulate emissions by about 40% [18]. The authors of the study hypothesized that a temporary protective shield of melted inorganic salt may be formed, surrounding the burning section of the incense, insulating it from the cool air [18].

Although there is no specific research conducted to quantitatively correlate the levels of metals in raw Bakhour to their levels in smoke and how that might impact respiratory health, some studies were done on tobacco. Tobacco has been reported to contain 440–1150 mg Fe/kg of tobacco. However, only 0.1% of this iron enters the mainstream smoke [19]. The human respiratory tract is exposed to 40–100 mg PM from smoking one cigarette [19]. Research conducted on male rats exposed to cigarette smoke for 6 h per day for three days consecutively showed an increased accumulation of iron in the lower respiratory tract due to inhalation of PM [19]. Furthermore, the study showed that the particles in the cigarette smoke alter iron homeostasis, which may contribute to other diseases, due to elevations in catalytically active iron promoting oxidative stress that triggers a chain of biochemical events leading to inflammation [19]. Due to the PM lodged in the lungs, continued iron accumulation may occur [19]. This phenomenon could occur in Bakhour as well with iron or any other existing metals that are bioavailable, like cadmium and lead.

The objective of this study was therefore to obtain fundamental information on the chemical composition of various brands of raw Arabian Bakhour and then evaluate the potential impact of the identified species on human health and the environment. Advanced techniques for sampling and analysis were applied in order to obtain a detailed chemical profile of all organic and inorganic compounds present in raw Bakhour samples. The techniques used for analysis are gas chromatography–mass spectrometry (GC–MS), inductively-coupled plasma–optical emission spectroscopy (ICP–OES) and scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDS).

2. Materials and methods

2.1. Bakhour samples

Three Bakhour samples were obtained and used in this study from a domestic producer in Sharjah, UAE. The samples were labeled as B1, B2 and B3. The choice of the Bakhour samples was based on their price and popularity in the market.

2.2. Ash content

The Bakhour samples were ashed in a furnace (Barnstead/Thermolyne Type 6000, USA). Four 1.0 g replicates of each Bakhour sample were weighed and placed in separate ceramic crucibles and ashed at 575 °C for 6 h before being cooled in a desiccator [20]. The mass of the remaining residue was used to determine the ash content for each Bakhour sample.

2.3. CHN elemental analysis

An elemental analyzer (EuroVector EA3000, Italy) equipped with a thermal conductivity detector (TCD) was used to determine the carbon, hydrogen and nitrogen (CHN) content of the three Bakhour samples. 2 mg of each Bakhour sample was placed in a separate tin foil vial and then combusted completely in a high purity oxygen environment at 980 °C. Helium was used as a carrier gas. The reduced gases were then separated in a gas chromatography column and quantified. The instrument was calibrated using high purity acetamide standard.

2.4. SEM/EDS analysis

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDS) was used to provide a qualitative and semi-quantitative elemental analysis of the surface of the ashed Bakhour samples. Prior to analysis, each of the ashed Bakhour samples was mixed with water and alcohol solution of equivalent volume ratio. A portion of the suspension was then placed on carbon films taped to a metal disk and allowed to dry overnight in a desiccator. The disks were then placed inside the SEM instrument (Tescan VEGA3 SEM, Czech Republic) followed by analysis using an X-ray detector (Oxford instruments INCA X-act, UK). A highly-focused scanning electron beam (10–30 keV) was used [21]. The electron beam was focused on multiple areas on the surface of the same sample to ensure representative analysis.

2.5. ICP analysis

Further quantitative analysis for the three Bakhour samples was done using Varian Liberty AX sequential inductively-coupled plasma–optical emission spectroscopy (ICP–OES) analysis [22]. Trace metals of potential health concerns were selected and they are aluminum, cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc. Three 1 g replicates of each Bakhour sample were weighed in separate ceramic crucibles and ashed at 500 °C in the furnace for four hours then cooled in a desiccator [20]. The ashed samples were re-weighed after cooling and then transferred into Teflon tubes for microwave digestion. The ashed Bakhour samples were digested in mixtures of 9 mL nitric acid (HNO_3) and 3 mL hydrochloric acid (HCl) using a microwave reaction system (Anton Paar Multiwave 3000 SOLV Microwave Reaction System). The digestion occurred at 175 °C for 55 min (15 min hold) at a power of 800 W [18]. After microwave-assisted sample digestion, all replicates were filtered and then transferred to a 50 mL volumetric flask followed by dilution with deionized water. The solutions were then transferred into separate tubes for ICP–OES analysis.

Sigma-Aldrich TraceCERT® mixed metal solution was used to prepare standard solutions of concentrations in the range of 0.001–20.0 mg/l. The metals included in this experiment were aluminum cadmium, cobalt, copper, iron, manganese, nickel, lead, and zinc.

To ensure the accuracy and validity of the results, the limit of quantitation (LOQ) and limit of detection (LOD) of the ICP–OES instrument were determined. Three standard solutions containing the ten trace metals and seven deionized water blanks were used.

2.6. GC–MS analysis

The volatile and semi-volatile organic compounds in raw Bakhour were analyzed using gas chromatography–mass spectrometry (GC–MS). Each of the three Bakhour brands was extracted using three solvents of different polarity: acetone, dichloromethane (DCM), and toluene. Extraction was done in two different methods: (1) using a Multiwave 3000 SOLV Microwave Reaction System (Anton Paar, Germany) and (2) using an orbital shaker (Edmund Buhler GmbH, Germany).

0.5 g of each Bakhour sample was mixed with 9 mL of acetone in the extraction tubes. The temperature of the microwave was ramped at 10 °C/min to 110 °C, and then held constant for 10 min. A power of 600 W was applied. After extraction, the microwave was left to cool for 10 min. On the other hand, 0.5 g of each of the Bakhour samples was also extracted in separate 10 ml aliquots of toluene and DCM, and shook in the orbital shaker set at 50 °C for 24 h. After extraction, the samples were filtered using Spartan 3010 45 RC filters (Schleicher & Schuell, Germany) and transferred into vials in preparation for GC–MS analysis.

The extracts from the raw Bakhour samples were loaded to the auto sampler of the QP-2010 Ultra GC–MS (Shimadzu, Japan). The analysis was done using the following conditions: an injection temperature of 280 °C with a split ratio of 20:1. Initially, the column temperature was maintained at 40 °C for 3 min and then ramped at a rate of 5 °C/min to 300 °C, which was kept constant for 5 min. The column used for analysis is Rtx-5MS (crossbond® diphenyl dimethyl polysiloxane) from Restek (30 m in length, 0.25 μm in thickness, and 0.25 mm in diameter). The carrier gas used in the analysis was helium with a column flow of 1.24 mL/min.

3. Results and discussion

3.1. CHN content

The percentage by weight (wt.%) for carbon, hydrogen, and nitrogen in all raw Bakhour samples was determined and the results are presented in Table 1. As expected, carbon has the highest weight percentage (wt.%) in all three Bakhour samples followed by hydrogen and then nitrogen. The weight percentages for each element are similar among the three Bakhour samples. It is worth noting the presence of nitrogen in the three samples, which could possibly lead to the emission of oxides of nitrogen (NO and NO_2), amines, nitrates and nitrites upon combustion. These compounds are known to be hazardous to both human health and the environment [8,23]. Exposure to nitrogen oxides can result in adverse respiratory effects, lung irritation, alterations in the lung's defense system, and aggravation of existing cardiovascular diseases [8]; as well as being a precursor to the formation of ground-level ozone and smog [14]. Nitrites are known to impact blood hemoglobin and induce methemoglobinemia which causes cyanosis (blue skin), weakness, and rapid heart rate [23].

3.2. Ash content

The weight percentage, of the remaining ash for all Bakhour samples is summarized in Table 1. The mean weight percentage (wt.%) of the ash is consistent for the three Bakhour samples with average mean values below 5%. The ash content is assumed to be inversely proportional to the amount of PM of small sizes in smoke that are easily inhaled, ingested, and

Table 1 The weight percentages (wt.%) of carbon, hydrogen, and nitrogen (CHN) and the ash content in the Bakhour samples B1, B2, and B3.

Bakhour sample	Carbon (wt.%)	Hydrogen (wt.%)	Nitrogen (wt.%)	Ash content (wt.%)
B1	55.9	6.95	3.61	2.82
B2	55.6	6.81	3.73	3.56
B3	54.3	6.51	2.18	3.12

dispersed [17]. As a result, it is expected that the three Bakhour samples will produce similar amounts of PM into the environment. The type and amount of metals in the ashed samples were determined by further analysis using SEM/EDS and ICP–OES methods.

3.3. SEM/EDS elemental analysis

Table 2 shows the SEM/EDS elemental analysis results of the ashed Bakhour samples. The weight percentage (wt.%) of the elements was then converted to mg/kg of raw Bakhour using the ash content results reported in Table 1. The observed differences in the trends between the wt.% and the calculated mg/kg results are due to the differences in the mass of ash produced from the three types of Bakhour. The elements that were detected using EDS as shown in Table 2 include: carbon (C), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), oxygen (O), phosphorus (P), sulfur (S), silica (Si), strontium (Sr) and titanium (Ti). The results show that elements O, C and Ca have the highest amounts and their weight percentages contribute to about 94% of the total weight of the ashed samples. When compared to the results in Table 1, the weight percentage of carbon is expected to decrease and that of oxygen to increase upon ashing due to combustion reaction. Since Bakhour consists of oud woodchips, the constituents of its ash should be similar to those of wood ash. Therefore, the presence of carbon, oxygen, calcium and silicon in the analyzed samples would then be expected. Calcium is more likely to exist in the form of calcium oxide (CaO) originated from calcium carbonate ($CaCO_3$) [24]. $CaCO_3$ salt is known for its use as mineral filler in Asian incense [17,18]. It has been reported that the addition of $CaCO_3$ to incense can effectively suppress the $PM_{2.5}$ emission by as much as 40%. Therefore, it is most likely that $CaCO_3$ was added to the Bakhour ingredients during the production process. Since sample B1 showed the lowest Ca content, it may be inferred that its $PM_{2.5}$ emissions might be the highest and hence it would potentially be the most hazardous to the respiratory system in that respect. Other common and essential elements such as Mg, Na, K, P, Zn were detected by the EDS analysis and are also expected to be present in plants due to their role in plant sustenance and growth [24]. Earth minerals and metals such as Al, Fe, Sr, and Ti are found abundantly in the earth's crust [24]; their presence in plants is therefore not unusual and can be reasonably anticipated in raw Bakhour.

3.4. Trace metals' analysis

Since EDS is viewed as a surface analysis technique, ICP–OES were then applied to analyze the Bakhour samples for metals

Table 2 The weight percentages of elements (wt.%) in the ashed Bakhour samples (B1, B2, and B3) and their relative concentrations in the raw Bakhour (mg/kg) measured using SEM/EDS.

Element	B1		B2		B3	
	wt.%	mg/kg	wt.%	mg/kg	wt.%	mg/kg
Oxygen	44.1	**	45.2	**	43.0	**
Carbon	28.1	7910	27.3	9730	27.5	8570
Calcium	21.5	6050	22.3	7940	23.9	7450
Silicon	1.35	381	0.90	320	1.48	462
Iron*	0.91	257	0.65	231	1.03	321
Potassium	0.79	223	0.98	349	0.71	222
Aluminum*	0.91	257	0.55	196	0.55	172
Sodium	0.58	164	0.64	228	0.48	150
Sulfur	0.65	183	0.47	167	0.42	131
Magnesium	0.49	138	0.48	171	0.50	156
Cupper*	0.17	47.9	0.19	67.6	0.12	37.4
Phosphorus	0.18	50.8	0.14	49.8	0.12	37.4
Manganese*	0.10	28.2	0.09	32.0	0.05	15.6
Lead*	0.11	31.0	0.05	17.8	0.07	21.8
Strontium	0.02	5.64	0.01	3.56	0.08	25
Titanium	0.05	14.1	0.03	10.7	0.03	9.36
Zinc*	0.04	11.3	0.01	3.56	0.04	12.5
Chromium*	0.02	5.64	0.00	0.00	0.00	0.00

* Metals that were chosen for further analysis using ICP–OES.
** Not applicable due to introduction of oxygen from air during the ashing process.

that are possibly found in trace amounts, such as Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. Following the acid digestion of the ashed Bakhour, the concentrations of the selected metals were measured in the solutions. To ensure the accuracy of the results and validate the sensitivity of the method, the limit of quantitation (LOQ) and limit of detection (LOD) of the ICP–OES instrument were determined and presented in Table 3. The measured concentrations for all metals were above the LOD and LOQ of the instrument except for Co which is present in concentrations (1.0 μg/L) below the LOD (10.6 μg/L) and LOQ (106 μg/L). As a result, the amounts of Co present are too low to be considered in this study and that explains the high standard deviation for this metal. The concentrations of the other metals were then converted to mg of metal/kg of Bakhour and are reported along with their standard deviations in Table 3. The concentrations of the trace metals in the three Bakhour samples varied significantly ranging from around 0.0860 mg/kg to 438 mg/kg. The amount of Fe in all three samples is the highest compared to the other metals, especially in B3 where its concentration is reported at 438 mg/kg. On the other hand, Cd is found to have the lowest concentration – aside from Cobalt (Co) – relative to the other metals. It is also worth noting the presence of lead, highly toxic metal, in all three Bakhour samples.

A comparison between the ICP–OES results (Table 3) and SEM/EDS results (Table 2) was made. The metal concentrations calculated in mg/kg from both methods were plotted as shown in Fig. 1. The error analysis was taken into consideration where the metals present below LOQ or those quantified but with high standard deviation were excluded. A positive and strong linear correlation between the two methods is observed with regression coefficient of $R^2 = 0.98$.

The concentrations of the metals obtained in this study were compared to those reported in the literature and evaluated for their possible effects on human health and the environment (Supporting information Table A.1) [19,25,26]. Fang et al. proved the presence of Fe on fine ($PM_{2.5}$) and coarse ($PM_{2.5–10}$) particles produced from the mass-burning of Asian incenses similar to Bakhour [25]. Therefore the high Fe content in Bakhour (346 mg/kg on average) is of utmost concern since the average $PM_{2.5}$ concentration from burning Bakhour is expected to be about 1.35 mg/m^3 [11]. Inhaled Bakhour particulates that transport iron into the lungs could cause oxidative-stress, irritate lung cells and cause inflammation, similar to that observed in cigarette smoke [19,23].

Due to the absence of regulations on the allowed metal content in raw Bakhour, the average concentration of the metals obtained using ICP–OES was compared to those in cigarette tobacco listed in a World Health Organization (WHO) bulletin report [26] (Table 4). Several metals in Bakhour, Al, Cd and Mn, were found to be considerably below the provided ranges shown in Table 4. These metals were found to be approximately five times below the ranges reported in cigarettes. Other trace metals in Bakhour such as Fe, Ni, Pb, and Zn are found within similar ranges to those reported for cigarettes. It is important to indicate that the Cu concentration in Bakhour (43–75 mg/kg) exceeded that in cigarettes (16–21 mg/kg) considerably. The presence of Cu is known to cause respiratory irritation when inhaled [23]. The presence of toxic trace and heavy metals, some of which have a long biological half-life, in Bakhour in similar quantities to that found in cigarettes indicates that prolonged exposure to such metals will impart adverse effects on human health [26]. Although the inhalation intake rates from cigarette smoke and incense smoke differ, Bakhour's emission rate of 5.9 mg/min is significantly higher [11].

3.5. Organic compounds

GC–MS analysis was done on the raw Bakhour samples extracted using each of the acetone, toluene and dichloromethane solvents. The GC–MS chromatograms for acetone, DCM and toluene extracts indicate the presence of a wide range of peaks that varied in their relative areas. The presence of peaks (Supporting information Fig. A.1–A.4) provides indirect information on the chemical composition of the sample under investigation. The mass spectra for each peak were refined and subjected to baseline corrections. The mass spectra for all peaks were then analyzed and the chemical compounds were identified using the standard NIST (National Institute of Standards and Technology) library database. In cases of overlapping peaks in the spectra, correction was made by ion fragment refining and separation. GC–MS analysis resulted in the identification of a total of 450 organic compounds in the three Bakhour samples. These compounds were then categorized according to their possible health risks into carcinogens, toxic substances, irritants and other miscellaneous health effects. These extremely harmful compounds are likely to be emitted into the atmosphere upon combustion of the Bakhour bricks and consequently inhaled by individuals. To

Table 3 The mean ± standard deviation of the concentrations (mg/kg) of trace metals in the Bakhour samples (B1, B2, and B3) using ICP–OES. Limits of quantitation (LOQ) and detection (LOD) for the ICP–OES instruments are presented.

Element	Mean concentrations of Trace metals in Bakhour samples (mg/kg)			LOQ (μg/L)	LOD (μg/L)
	B1	B2	B3		
Iron	349 ± 5.3	252 ± 5.9	438 ± 15.3	18.7	10.9
Aluminum	304 ± 4.8	217 ± 0.9	237 ± 13.5	20.1	4.30
Cupper	75.1 ± 0.4	62.8 ± 3.7	42.5 ± 1.1	54.8	39.1
Manganese	37.6 ± 0.02	33.9 ± 1.8	23.3 ± 0.3	2.20	0.80
Zinc	33.5 ± 13.5	15.7 ± 3.6	17.5 ± 2.9	4.30	3.40
Chromium	3.13 ± 0.26	2.19 ± 0.23	2.97 ± 0.16	11.1	4.30
Lead	3.24 ± 1.09	2.99 ± 1.10	2.03 ± 0.17	42.1	16.8
Nickel	1.67 ± 0.62	1.04 ± 0.08	1.82 ± 0.34	51.5	18.0
Cadmium	0.102 ± 0.011	0.103 ± 0.008	0.086 ± 0.022	2.80	1.40
Cobalt	0.045 ± 0.063	0.034 ± 0.023	0.036 ± 0.051	106	10.6

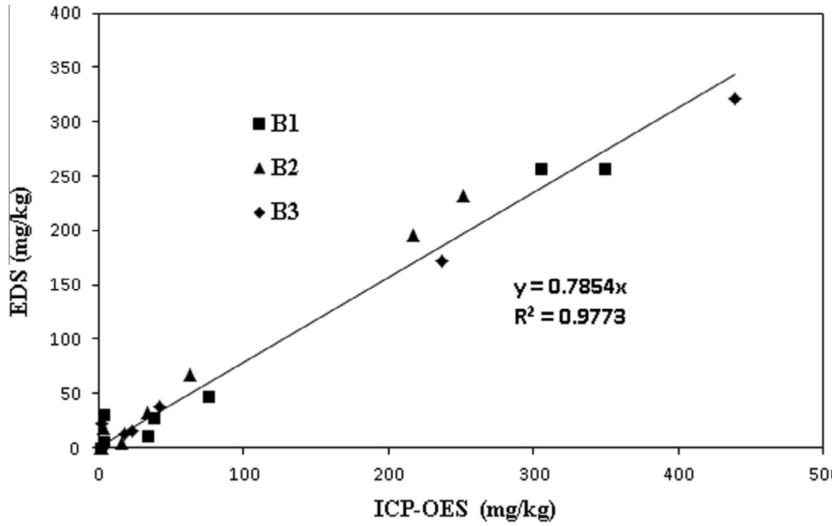

Figure 1 The relationship between the SEM/EDS and ICP–OES analysis results for the three Bakhour samples.

Table 4 Comparison of the concentration ranges of trace and heavy metals in Bakhour samples to those reported for raw cigarette tobacco [26].

Element	Concentration range of trace metals (mg/kg)	
	Cigarette tobacco [26]	Bakhour samples
Aluminum	699–1.20 × 10^3	217–304
Cadmium	0.500–3.50	0.0864–0.103
Cadmium	< 0.100–3.45	2.19–3.13
Cupper	15.6–21.1	42.5–75.1
Iron	325–520	252–438
Manganese	155–400	23.3–37.6
Nickel	< 2.00–400	1.04–1.82
Lead	0.600–2.40	2.03–3.24
Zinc	16.8–30.5	15.7–33.5

a lesser extent, individuals may be exposed to these in the raw material as well. The compounds found in the raw Bakhour samples and their respective health effects are summarized in the following sections. It is important to mention that many other compounds were identified in the raw Bakhour samples but lacked sufficient clinical studies or evidence to make a complete accurate health risk assessment.

3.6. Carcinogens

Table 5 presents a total of eight known and suspected carcinogenic compounds identified in the raw Bakhour products. Some of the compounds are found in multiple Bakhour samples while others are found in only one sample. For example, acetic acid phenylmethyl ester was found in both B2 and B3, but was not detected in B1. Although esters are perceived to be relatively safe to human health, acetic acid phenylmethyl ester has been identified as a mutagen, and also known to produce tumorigenic effects that can lead to the formation of cancers, specifically gastrointestinal and reproductive system cancers [27]. In addition, 9-dodecyl-tetradecahydro-anthracene and 1,2-dimethoxy-4-(1-propenyl)-benzene, classified as suspected carcinogens, were identified in all three Bakhour samples. Clinical studies showed that laboratory animals exposed

Table 5 List of known/suspected carcinogenic compounds identified in three raw Bakhour samples (B1, B2, and B3). The symbol (X) indicates the presence of the compound.

Name	B1	B2	B3
Acetic acid phenylmethyl ester	–	X	X
Benzoic acid hydrazide	X	X	X
1,2-Dimethoxy-4-(1-propenyl)-benzene	X	X	X
9-Dodecyl-tetradecahydro-anthracene	X	X	X
Ethylbenzene	X	X	X
α-Methyl-benzenemethanol	–	X	X
α-Methyl-acetate-benzenemethanol	–	X	–
Styrene	X	–	–

to these two compounds developed cancerous tumors and a buildup of fluid in their tissues [28,29]. Another suspected carcinogen present in both B2 and B3, but not in B1 is α-methyl-benzenemethanol, correlated with the development of lung and skin cancer, as well as an increased formation of benign kidney tumors in rats [30]. Furthermore, α-methyl-acetate-benzenemethanol, which was only found in B2 is a suspected carcinogen based on animal studies [31,32]. Benzoic acid hydrazide, classified as a known carcinogen, was found in all of the three Bakhour brands. Other suspected carcinogenic compounds identified include ethylbenzene and styrene. Ethylbenzene is classified as a possible carcinogen by the International Agency for Research on Cancer (IARC) [33] and was also found to be mutagenic in mammalian somatic cells, bacteria, and yeast [34]. On the other hand, the US Environment Protection Agency (EPA) does not have a carcinogen classification for styrene but the chemical is currently being subjected to an EPA Integrated Risk Information System (IRIS) review to establish such a classification [35]. Styrene, which was found only in B1, is reasonably anticipated to be a human carcinogen based on several epidemiologic studies that suggested an association between styrene exposure and an increased risk of leukemia and lymphoma; although this evidence is deemed inconclusive [35,36].

Out of the three Bakhour brands, B2 seems to contain the highest number of suspected/known carcinogens whereas B1 contained the least number of those harmful compounds. The presence of suspected or known carcinogenic compounds in the raw Bakhour products should be of serious concern. Historically carcinogenic compounds were believed to be produced as a result of the combustion process; however this study demonstrates the presence of these compounds in the raw material.

3.7. Toxins

Table 6 lists a total of 18 toxic compounds found in the raw Bakhour brands. These compounds have been classified as toxins based on their adverse effects to a multitude of organs leading to their function failure or damage, in addition to adverse effects on the nervous system. The inhalation of α-farnesene at high concentrations causes central nervous system (CNS) depression in addition to lung irritation with coughing and nausea, headaches and dizziness, slowing of reflexes, fatigue and incoordination [37]. Other compounds, such as 3-7-dimethyl-1-7-octanediol and tonalid have been reported to cause liver damage and decreased liver function and weight, at high-doses in animal experiments [38,39]. 4-Hydroxy-4-methyl-2-pentanone, present in all three Bakhour samples, was found to cause liver and kidney damage [40,41]. Moreover, compounds such as benzaldehyde, diisooctyl phthalate, 4-hydroxy-4-methyl-2-pentanone, acetic acid hexyl ester, 1-2-dimethyl-cyclohexane, 1-methyl-4-(1-methylethenyl)-acetate-cyclohexanol, ortho- and para-xylene, musk ambrette, and phenylmethyl alcohol were all found to produce CNS depression [42–46]. CNS depression can result in decreased rate of breathing, decreased heart rate and, in severe cases, loss of consciousness possibly leading to coma or death [47]. In addition, exposure to N-methyl-formamide was correlated with teratogenic and embryotoxic effects, hence posing a serious threat to pregnant women and the developing fetus [48].

Moreover, musk ambrette, which is found in all three raw Bakhour samples, is a white to yellow powder with a heavy musky aroma that is commonly used as a perfume fixative and a flavoring agent [42]. Health effects associated with musk ambrette include evidence of photosensitivity, neurotoxicity, CNS depression, penetration through human skin with slow excretion, muscle weakness, weight loss, and brain and spinal cord demyelination in repeated-dose oral studies of rats [42]. The use of musk ambrette in fragrances is prohibited within the European Union [42].

Comparing the three Bakhour brands, B2 seems to contain the highest number of compounds with known toxicities whereas B1 contained the least number of these toxins. This finding is consistent with the results portrayed in Table 5 where B2 contained the highest number of carcinogens and B1 the least. Such compositional differences between the Bakhour brands illustrate the variability found among incenses, even if they are of the same type.

Table 6 List of toxic compounds identified in three raw Bakhour samples (B1, B2, and B3). The symbol (X) indicates the presence of the compound.

Name	B1	B2	B3
Acetic acid hexyl ester	–	X	X
Benzaldehyde	–	X	X
Benzyl alcohol	X	X	X
Diisooctyl phthalate	X	–	–
1-2-Dimethyl-cyclohexane	X	X	–
3-7-Dimethyl-1-7-octanediol	–	X	X
Ethylbenzene	X	X	X
α-Farnesene	X	X	X
3-(2-Hydroxyphenyl)-(E)-2-propenoic acid	X	X	X
4-Hydroxy-4-methyl-2-pentanone	X	X	X
1-Methyl-4-(1-methylethenyl)-acetate-cyclohexanol	X	X	X
α-Methyl-acetate-benzenemethanol	–	X	–
α-Methyl-benzenemethanol	–	X	X
N-methyl-formamide	X	–	–
Musk ambrette (artificial)	X	X	X
Phenylethyl alcohol	X	X	X
Tonalid	X	X	X
p-Xylene	X	X	X

3.8. Irritants

In addition to carcinogens and toxins, a total of 52 irritants were also found in the smoke of Bakhour (Supporting information Table A.2). These irritants affect different parts of the body, mainly the eyes, skin, respiratory and digestive tracts.

3.9. Compounds with miscellaneous health effects

Some of the compounds identified in the three raw Bakhour brands are not classified under carcinogens, toxins and irritants but can still produce various adverse health effects that vary in their severity from slight dizziness to potential organ damage. Table 7 lists 11 of these compounds. The presence of 1,2-dimethyl cis-cyclohexane as well as 3,7-dimethyl-formate,2,6-octadien-1-ol and tert-butyl-benzene may lead to drowsiness, dizziness, sleepiness, reduced alertness, loss of reflexes, lack of co-ordination, and vertigo [49,50]. In addition, the chronic exposure to diethyl phthalate can result in target organ damage [45,46]. In addition, diethyl phthalate can produce other chronic health effects such as decreased growth rate, food consumption and altered organ weights [47,49,50]. Studies on animals showed that the exposure to α-terpineol-L causes flaccid paralysis, somnolence, ataxia and skin irritation. Eye discomfort, transient conjunctivitis, nausea, vomiting, headache and dizziness may be the result of exposure

Table 7 List of compounds identified with miscellaneous health effects in the three raw Bakhour samples (B1, B2, and B3). The symbol (X) indicates the presence of the compound.

Name	B1	B2	B3
2-Bornanone	X	X	X
1,2-Dimethyl cis-Cyclohexane	X	X	–
3,7-Dimethyl-formate, (E)-2,6-Octadien-1-ol	–	X	–
Tert-butyl-benzene	X	–	–
Diethyl phthalate	X	X	X
3,7-Dimethyl-1,6-Octadien-3-ol	X	X	X
1,4-Dione,2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene	–	X	–
Diphenyl ether	X	X	X
1-Methoxy-4-methyl-benzene	X	–	–
1,1′-Oxybis-2-propanol	–	X	X
α-Terpineol-L	X	X	X

to 1,1′-oxybis-2-propanol. Burning sensations in the throat, coughing, wheezing, sore throat, and skin irritation are the result of exposure to 1-methoxy-4-methyl-benzene. Acute exposure to diphenyl ether results in symptoms that include loss of appetite, increasing weakness, congestion of the lung and liver, lesions of the liver, spleen, kidneys, thyroid and gastrointestinal tract irritation. In addition, the presence of 1,4-dione,2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene may lead to ulceration of the nasal septum and hemorrhage. 3,7-dimethyl-1,6-Octadien-3-ol may cause weaknesses and weight loss and erythema while 2-bornanone may result in slight eye irritation and drowsiness [50].

4. Conclusions

Raw Arabian Bakhour was found to contain a wide range of metals and chemical compounds that are classified as hazardous to human health as well as to the environment by significantly altering indoor air quality upon burning. Nitrogen was found in three different locally produced Bakhour samples and its presence should be of concern. Multiple methods for metal analysis (ICP–OES and SEM–EDS) were developed. Results showed that sample B1 has a relatively higher metallic content than the other two Bakhour samples in spite of the fact that B1 is classified as the premium brand. Iron, aluminum, and copper were present in the highest concentrations in all Bakhour samples. A GC–MS analysis of the raw Bakhour materials revealed the presence of organic compounds, many with potentially severe health effects. Overall, 8 known/suspected carcinogens, 18 toxins, and 11 other compounds were identified in the raw Bakhour bricks. This study is the first of its kind to highlight the source of the harm associated with Bakhour as a raw material for incense. When it comes to rectifying the problem created by burning Bakhour, it is important to address the source of these harmful chemicals present in the raw Bakhour material. By profiling the chemical components present in raw Bakhour, further steps can be taken to use alternative, less hazardous ingredients, which could make this traditional practice safer to human health and the environment.

Acknowledgement

The authors would like to express their appreciation to the American University of Sharjah for funding the work. Also, they would like to convey appreciation to Mr. Ziad Sara, Mr. Nidhal Abu Farha, and Mr. Thomas Job for their technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2014.10.005.

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