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The Hookah Series Part 2: Elemental Analysis and Arsenic Speciation in Hookah Charcoals

Ryan Saadawi, Oliver Hachmöeller^, Matthew Winfough, Traci Hanley, Joseph A. Caruso*, Julio Alberto Landero Figueroa

Department of Chemistry, University of Cincinnati/ Agilent Technologies, Metallomics Center of the Americas, McMicken College of Arts and Sciences, University of Cincinnati, Cincinnati, OH, 45221-0172, USA

*Corresponding author: Caruso, J.A. Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, United States

Email: joseph.caruso@uc.edu

^Current address: University of Muenster, Muenster, Germany

Abstract

The use of water pipes or hookahs to smoke tobacco formulations has gained great popularity among young people around the world, but the potential health hazards have not yet been adequately evaluated. The complexity of a multi component hookah apparatus, compared with cigarettes and cigars, makes it difficult to study under laboratory conditions. For this reason the detailed study of its components simplify the task. In this study the charcoal, which is traditionally used as the heat source, was analyzed for metal content before and after combustion. Sixteen different hookah charcoals were analyzed representing different compositions and manufacturing processes as well as different geographic origins. ICP-MS was used to measure 24 elements: Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Sb, Ba, Tl, Pb, Th, U. The total concentration ranges of toxic elements in native (un-burned) charcoals was: arsenic 14.8 – 10,300 ng g^{-1}, cadmium 3.3 – 2,100 ng g^{-1}, and lead 95.2 – 55,600 ng g^{-1}. The mass-loss-corrected content of elements in combusted charcoals shows that most of the metals remain in the ash, with iron, cadmium and lead as exceptions. Because of the high content of arsenic in some samples an extraction and speciation method was developed to quantify four chemical forms of arsenic. Nitric acid, and phosphoric acid were evaluated as extractants used in a heating block, and ascorbic acid was used to minimize oxidation of inorganic As^{+3} to As^{+5}. Anion exchange chromatography coupled to ICP-MS was used to carry out the separation and quantification of arsenic species. The best conditions in terms of extraction efficiencies and species conservation was 1.2 mol L^{-1} H_3PO_4, with 0.2 mol L^{-1} ascorbic acid. As^{5+} was the dominant arsenic species in charcoal. Concentrations ranged from 0.08 – 2.42 mg kg^{-1}, for As^{+3} and 0.46 – 8.36 mg kg^{-1} for As^{+5}. The results show high variation depending on the sample origin and composition. The possibility of volatile cadmium and lead contributions to the primary and second hand smoke by the charcoal are suggested and the high levels of arsenic suggest that for certain charcoals there may be more hazard from them than from the tobacco formulation.

Introduction

The hookah has been used for centuries, primarily in eastern cultures. Recently hookah use has become increasingly popular in western culture[1] and trendy with younger populations[2] using hookah tobacco flavors such as grape, bubble gum and double apple. The hookah is smoked by lighting a hookah tobacco formulation, e.g. mo'assel, with smoldering charcoal, passing the smoke through a "water filter" and inhaling through a hose attached to the water chamber that draws the smoke to the consumer. Numerous studies have shown tobacco consumption exposes the consumer to potentially toxic chemicals[3-6], however, the metals and organic toxic species produced by keeping the tobacco formulation lit with the smoldering charcoal require rigorous studies to begin to assess toxic potential, since the smoke is a mix of charcoal smoke, tobacco smoke and smoke from other parts of the tobacco formulation, e.g. glycerin and molasses.

The chemicals the hookah consumer is exposed to will ultimately reflect not only the tobacco formulation, and effects of the various hookah compartments but also the materials from which the charcoal is made, as well as the pyrolysis methods used in its production[7]. Traditionally, hookah has been lit with natural charcoal, meaning the charcoal came from pyrolized embers of a wood fire. With hookah's increase in popularity, many different types of charcoals have emerged. To name a few, there are now quick light disks, coconut cubes, briquettes, and sticks; all claiming to be natural. These charcoals are made by using a wood source (trees, coconuts, dried cane, scrap lumber, likely toxic metal treated scrap lumber, etc.) and in many cases mixed with a casing agent such as unrefined molasses from sugar cane or starch from flour or corn. The effect to the smoker is then a combination of all the contributions to the smoke from various hookah apparatus chambers, the individual manner of smoking including length of time and puff intensity, the tobacco formulation and the charcoal, which is the subject of this study.

It should be no surprise that charcoal contains a variety of metals and metalloids, including toxic metals and metal species from elements such as arsenic, cadmium, lead, and chromium to name a few[8, 9]. In fact, the science of phytoremediation is driven by hyper-accumulation of metals by plants, some of which become charcoal. The presence and concentrations of toxic metals and organic substances in the charcoal is highly dependent on the origin and type of wood used the growth media (typically soil), post-harvest treatment of the wood and different production processes. The term “wood” is used generically in this report as charcoal comes from tree parts, coconut shells, dried sugar cane stalks, lumber, scrap lumber, etc. Toxic substances are introduced into the environment through natural uptake (trees) and anthropogenic means (toxic metal treated lumber). For trees and plants, these are taken up by the roots and may be translocated to different aerial parts of the plants and, to some degree, the fruit[10]. The wood and fruit (such as coconuts) are then processed and formulated into charcoal. Additionally during the manufacturing process, other chemicals may be added to aid in lighting or encasing the charcoal power into some 3-D block. The variations in the charcoals’ origins and manufacturing processes ultimately affect the types of toxic elements and organic compounds plus their concentrations to which a hookah smoker is exposed.

When the smoldering charcoal ignites the hookah tobacco formulation (more of a charring), the smoker is exposed to putative hazardous metals and organics from both sources. The degree of exposure greatly depends on the charcoal type, the metal volatility, and the length of exposure. The combined toxic exposure from charcoal and the tobacco formulations associated with hookah consumption has yet to be studied in detail and is important as a step towards understanding the hazardous risks the consumer is subjected to while smoking hookah tobacco, not to mention the side-stream smoke affecting non-consumers.

Tobacco and charcoal are both known to contain arsenic, cadmium, lead, and chromium, among other toxic metals[4, 8, 10, 11] and the focus here is on metals and elemental speciation. Toxicity from exposure to these elements can occur at low concentrations[12-15]. When the consumer smokes hookah they are exposed to metals from both the charcoal and the tobacco. The effects of tobacco are better understood than charcoal, but to our knowledge no studies have been done on metal exposure from hookah charcoals. Metal toxicity often varies with the specific metal form. Arsenic speciation has been performed on a wide variety of matrices including tobacco[16-19] but never hookah charcoal. It is important to speciate the arsenic and to determine if the inorganic forms (most toxic forms[20]) or other species such as organoarsenicals are present[21, 22].

Arsenic speciation has been important to a wide variety of areas, ranging from foods[23] to environmental[24]. In fact various agencies have established methods for arsenic speciation such as EPA method 1632 and FDA Elemental Analysis Manual: Section 4.11. Fast and robust speciation methods include acid extraction of arsenic followed by anion exchange chromatography (AEX), with inductively coupled plasma mass spectrometric detection (ICP-MS). These methods provide detection limits at sub-ppb and even ppt levels; with some modification they are applicable to a variety of different matrixes including charcoal and tobacco[25].

This study focuses on determining the trace elements present in a number of different charcoal matrices marketed for hookah consumption and any arsenic discovered will be further speciated, so toxicity inferences can be made. Sixteen different charcoals were analyzed representing different charcoal material with different origins and manufacturing processes. Also fourteen of the charcoal samples were burned to ash and the ash analyzed. The study includes the following elements Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Sb, Ba, Tl, Pb, Th, U.

Materials and Methods

Instrumentation

The XL 30 ESEM scanning electron microscope - energy-dispersive X-ray spectroscopy (SEM-EDX) SEM (FEI Company, Hillsboro, Oregon, USA) EDX (EDAX, Mahwah, NJ, USA) was used for charcoal images as well as detection of silica in the charcoal matrix.

An Agilent 8800x inductively coupled plasma triple quad mass spectrometer (ICP-QQQ, Agilent Technologies, Santa Clara, CA, USA), equipped with a CETAC Micromist nebulizer (CETAC, Omaha, NE, USA), was utilized for the determination of total metals in charcoal formulations intended to be used for hookah smoking. The instrument was set to monitor the following metal isotopes: ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{43}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{68}Zn, ^{75}As, ^{88}Sr,

^{95}Mo, ^{109}Ag, ^{111}Cd, ^{121}Sb, ^{137}Ba, ^{205}Tl, ^{208}Pb, ^{232}Th, ^{238}U. As internal standards (ISTD) ^{6}Li, ^{45}Sc, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb ^{209}Bi were used to correct over the broad elemental mass range.

Instrumentation used for arsenic speciation

The same ICP-MS system was used for arsenic speciation. Chromatographic separations were performed with an Agilent 1100 high performance liquid chromatography (HPLC) and a Hamilton PRP-X100 anion exchange column (Hamilton, Reno, NV, USA). The HPLC was equipped with an autosampler, a degasser, a binary pump, a column compartment and a six-port switching valve with a 50 µl PEEK loop to inject a post column internal standard (PCIS; c(As) = 10 ppb). To adjust pH values a pH meter AB15 (Fisher Scientific, Fair Lawn, NJ, USA) was used. For mixing the samples a VortexGenie2 (Fisher Scientific, Fair Lawn, NJ, USA) was applied.

Reagents and Standards

Trace metal grade nitric acid (HNO_3), hydrogen peroxide (H_2O_2), phosphoric acid (H_3PO_4), ascorbic acid, ammonium phosphate dibasic ($(NH_4)_2HPO_4$,) and ammonium hydroxide (NH_4OH) were obtained from Fisher Scientific (Pittsburg, PA, USA). Doubly deionized water (DDIW) 18 MΩ generated from a Milli-Q system (Bedford, MA, USA) was utilized. Ultrex II ultra-high purity nitric acid (HNO_3) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Multi-elemental standards, 1000 μg ml^{-1} and 10 μg ml^{-1} stock solutions used for both spiking and calibration curves were obtained from Spex Certiprep (Metuchen, NJ, USA). Internal standard mix ICP-MS-IS-3 and trace metals in drinking water (TMDW) certified reference materials (CRM) were obtained from High-Purity Standards (Charleston, SC, USA). Trace elements in coal material

(CRM-COAL-Al) and marine sediment (CRM-MS-S) were obtained from High-Purity Standards (Charleston, SC, USA). We did not find and CRM charcoals.

Sample collection and preparation

Sixteen charcoal samples were purchased online or from various hookah shops for the experiment and are of USA, Jordan, Indonesia, China, Japan and the Netherlands origins. Charcoal samples vary in size shape and properties. Some are cubes, briquettes, quick light disks, squares and natural (tree branch appearance) just to name a few. All charcoal samples were homogenized using acid washed pestle and mortar into a fine powder and sieved through a 0.175 mm fine-mesh sieve. Approximately 100 g of charcoal was ground and stored in 50 mL metal free polypropylene vials and capped and stored until analysis. Fourteen of the charcoal samples were combusted using a muffle furnace at 600 ˚C for 30 minutes, removed and allowed to burn until only ash remained and then analyzed for total metals.

Sample digestion for total metal analysis of finely ground hookah charcoal

All samples were prepared and analyzed in quadruplicate, fortifying the fourth sample with 50µL of a 20 mg g^{-1} of multi elemental standard for a final concentration of 16.7 ng g^{-1}. For total metal analysis on charcoal and ash samples the Lepri et al[26]. method was adapted. Prepared samples (0.25 -0.30 g) were weighed directly into acid washed 35 mL pyrex digestion vessels and 2.5 g of 30% H_2O_2 was added to each sample vessel and allowed to predigest for 24 hours in a laminar flow hood. Covered sample vessels with 5 g concentrated HNO_3 added to each vessel, were allowed to pre-digest overnight prior to microwave digestion. Samples were subjected to microwave digestion using a CEM Discover SP-D microwave system (CEM, Matthews, NC, USA). Digestion occurred in two steps. The sample vessels were first ramped to 120 °C over 10 minutes and held for 5 minutes. Then samples were ramped to 200 °C over 15 minutes then held for 15 minutes before they were allowed to cool and subsequently vented. The digested solution was then diluted to 30 g with doubly deionized water, DDIW. Prior to analysis samples were diluted a second time taking 5 g of the first dilution and diluting to a final weight of 10 g. High-Purity Standards CRM-COAL-Al and Marine Sediment CRM-MS-S were digested with each sample set to assure as much as possible that the method was giving a correct response for the charcoal, since no CRM hookah charcoals are available.

Sample digestion for total metal analysis of hookah charcoal ash

All samples were prepared and analyzed in quintuplicate, fortifying the fourth sample with 40 µL of a 0.5 mg g^{-1} of multi elemental standard and fortifying the fifth sample with 40 µL of a 5 mg g^{-1} of multi elemental standard. Prepared ash samples (0.05 g) were weighed directly into acid washed 10 mL pyrex™ digestion vessels and 0.5 g of 30% H_2O_2 was added to each sample vessel and allowed to pre-digest for 24 hours in a laminar flow hood. Prior to microwave digestion 3g 20% HNO_3 was added to each vessel. Samples were subjected to microwave digestion using the CEM Discover SP-D microwave system. Digestion occurred in two steps. The sample vessels were first ramped to 120 °C over 10 minutes and held for 5 minutes. Then samples were ramped up to 200 °C over 15 minutes then held for 15 minutes before they were allowed to cool and subsequently vented. The digested solution was then diluted to 10 g with DDIW. Prior to analysis samples were diluted a second time taking 5 g of the first dilution and diluting to a final weight of 10 g. High-Purity Standards CRM-COAL-Al was digested with each sample set to assure a valid response to charcoal material.

Sample preparation for Arsenic speciation

Five different charcoal brands, produced in China and USA for hookah smoking, were investigated. A random selection of charcoal pieces was manually ground by an acid washed mortar and pestle and sieved trough a 0.175 mm fine-mesh sieve. Samples were stored in metal-free polypropylene vials. In this report, the different charcoal brands are named as samples #1, #2, #3a, #3b, #4 and #5. Sample #3a and #3b originate from the same charcoal brand: #3a was used for the method development, #3b for the final measurement of the samples. Additionally, CRM-Coal-A1 was measured as a reference material.

Reagents and standards used for Arsenic speciation

HNO_3, H_2O_2, H_3PO_4, multi-elemental standard, ICP-MS-IS-3, TMDW and CRM-Coal-A1 are the same as above. HNO_3, H_2O_2, H_3PO_4 were used for extraction and total metal analysis. Ascorbic acid used as an antioxidant, ammonium phosphate dibasic $(NH_4)_2HPO_4$ and NH_4OH used for the preparation of the mobile phase. Multi-elemental standard was used for calibration curves and spiking for total arsenic analysis and total extraction optimization.

The following arsenic compounds were used for spiking and calibration curves for the speciation analysis: sodium m-arsenite ($NaAsO_2$, 97.0%), potassium arsenate (KH_2AsO_4) from Sigma-Aldrich (St. Louis, MA, USA), monomethylarsonic acid disodium salt ($CH_3AsO_3Na_2{\cdot}6H_2O$) and dimethylarsinic acid $(CH_3)_2As(O)OH$, >99%, Fluka (Buchs, Switzerland).

Nitric and phosphoric acid as arsenic extractants

As extractants for the charcoal matrices, different concentrations of HNO_3 and H_3PO_4 were tested as follow: 0, 0.2, 0.6, 1.0, 1.2, 1.4 and 1.6 mol L^{-1}. Each sample was prepared in duplicate: 100 mg of charcoal sample #3a was weighed and 2.5 g of the respective acid was added. The extraction was performed on a heat block at 95 °C for 90 minutes. Following this, the extraction solutions were diluted to 10 g with DDIW and centrifuged. Prior to analysis, the samples with HNO_3 were diluted by a factor of 20. In order to minimize interface damages at the ICP-MS, samples with H_3PO_4 were diluted to a final concentration of 20 mmol L^{-1} phosphate. Moreover, ICP-MS ISDT (internal standard) was added in this step with a final concentration of 5 ng mL^{-1} of the internal standard mixture (Li, Sc, Ge, Y, In, Tb, Bi). Calibration was carried out by the standard addition method: Each set of samples extracted with HNO_3 was split into three aliquots. One aliquot was not spiked, while two were fortified to a final concentration of 25 and 50 ng mL^{-1}, respectively, with a multi-elemental standard. When H_3PO_4 was used as extractant, the final concentrations of the multi-elemental standard were correspondingly lower due to the higher dilution factor. The settings for the ICP-MS parameters for the total extraction optimization are listed in *table 1*.

Preventing conversion of As^{3+} to As^{5+} for sample preparation and speciation

To prevent conversion between As^{3+} and As^{5+}, ascorbic acid was added as an antioxidant[17, 27]. HNO_3 at 1.4 mol L^{-1} and H_3PO_4 at 1.2 mol L^{-1} were chosen and ascorbic acid was added at concentrations of 0, 0.05, 0.1, 0.2 and 0.3 mol L^{-1} to each acid. Additionally, a solution of 0.5 mol L^{-1} HNO_3 with 0.2 mol L^{-1} ascorbic acid was tested as an extractant as shown in *figure 6*.

Each sample was prepared in quadruplicate and 100 mg of hookah charcoal sample #3a was weighed and 2.5 g of the acid and ascorbic acid concentration was added, whereas two samples were fortified with 100 mg of a solution containing 10.0 mg L^{-1} As^{3+} and As^{5+}. The extraction was carried out on a heat block at 95 °C for 90 minutes. Following this, the samples were centrifuged and the supernatant was used for further dilutions. Prior to analysis, samples prepared with HNO_3 were diluted by a factor of 20 with a buffer solution of pH 10.25. Samples prepared with H_3PO_4 were diluted by a factor of 30 with a buffer solution at pH 10.00. The buffer solutions were prepared by adding ammonium hydroxide to the mobile phase (10 mmol L^{-1} $(NH_4)_2HPO_4$, pH 8.25). The parameters for the speciation analysis are listed in *table 1*.

Arsenic speciation on charcoal samples

Each sample was prepared in quintuplicate and 100 mg H_2O_2 were added to the fourth sample after the extraction to oxidize As^{3+} to As^{5+} and show that there are no interferences with As^{3+}. The fifth sample was fortified with 100 mg of a 10.0 mg L^{-1} As^{3+} and As^{5+} solution (sample #3b and CRM-coal-A1) or with 100 mg of a 2.0 mg L^{-1} As^{3+} and As^{5+} solution (samples #1, #2, #4, #5). 100 mg charcoal was weighed and 2.5 g of 1.2 mol L^{-1} H_3PO_4 / 0.2 mol L^{-1} ascorbic acid mixture was added. The extraction was run for 90 minutes at 95 °C on a heat block. After the extraction, the samples were centrifuged and the supernatant was used for further dilutions. For analysis, the samples were diluted by a factor of 30 with a buffer solution with pH 10.00. The buffer solution was prepared by adding ammonium hydroxide to the mobile phase of 10 mmol L^{-1} $(NH_4)_2HPO_4$, pH 8.25.

Results and Discussion

The aim of this initial study is to better understand the possible toxic hazards of charcoal, the traditional heat source used in hookah smoking. As far as we know, this is the first study performed on hookah charcoal formulations with an interest in a variety of elements, particularly metals. An arsenic speciation method has been developed and performed on five samples reporting the highest total As values and on a coal CRM, since no hookah charcoal CRMs are available.

The hookah is a multi-component apparatus used for consumption of the smoke from a tobacco matrix (consisting of wet leaf tobacco and up to 50% other ingredients). The tobacco matrix is lit (primarily pyrolized) using smoldering charcoal and the smoke is drawn down the hookah apparatus, through water in the bowl, and out the hose to the consumer as depicted in *figure 1*. Previous studies indicate the hookah tobacco formulation contains potentially toxic elements such as As, Cd, and Pb[11]. With charcoal in the smoking routine the consumer is exposed to a second source that may contain potentially toxic elements in addition to those in the tobacco formulation. The charcoal component of this double jeopardy paradigm was investigated to ascertain the extent of toxic metals the consumer might be exposed to resulting solely from the charcoal and to see if there might be analytical evidence to support or discredit the notion that hookah smoking is a “safer” or “healthier” than cigarette smoking. In our previous study “The hookah series part 1…” we investigated hookah tobacco formulations [11].

Total Elemental Analysis of Finely Ground Hookah Charcoals

Hookah charcoal typically comes in two forms, lump which is pyrolized natural wood pieces or manmade (disks, cubes, briquettes etc.). Some are shown in *figure 2*.

Charcoal used to light the hookah comes from a variety of sources, mostly from China and Indonesia as shown in *tables 2-4.* The elemental composition and concentrations reflect the geographic origin and manufacturing process of the charcoal. Charcoal from renewable sources such as coconut husks and wood embers are expected to contain a different elemental profile than lump charcoal. The manmade charcoals are made up from a variety of sources then modified with an agent that aids with caking and in many cases an infused ignition source (quick light types). SEM Images show great differences in lump charcoal vs. the manmade forms as depicted in *figure 3.* Lump charcoal resembles wood, with cell walls still visible. The manmade forms appear to be ground wood material mixed with a casing agent acting like glue holding it together. The total elemental profile for sixteen charcoal samples and two certified reference materials was obtained as an initial step in understanding how the charcoal matrix contributes to the first and second hand smoke (*tables 2-4*) for spike recoveries on selected elements see Supplement *table T1*. After the digestion of the charcoal samples, a white sand like substance remained. This has been shown to be silica by using SEM-EDX, and is shown in Supplement *figure F1*

Finely ground charcoal from a variety of geographic origins was extracted and total elemental analysis was performed. The results are summarized in *tables 2-4*. The concentrations vary from one charcoal brand to another and are reported as the average of triplicate analysis ± 1 SD. The charcoal samples contain widely varying trace element ranges, e.g. as for the toxic elements: arsenic 14.8 – 10,300 ng g^{-1}, cadmium 3.3 – 2,100 ng g^{-1}, lead 95.2 – 55,600 ng g^{-1}. Finding a nail in one sample (Supplement image I1) suggests that scrap or painted lumber may be responsible for a number of elevated results. For example, in the USA arsenic/copper treated lumber was phased out in 2003, with certain exceptions. That notwithstanding, there remain large amounts of this lumber from various construction types, which as scrap (likely free starting material) may be utilized to make charcoal, carrying the heavy metal burden with it as depicted in table 4, item 6. This situation carries over to most countries where hookah charcoal is produced, and to our knowledge there are few regulations on starting materials for hookah charcoal production, even though it may carry as much or more of the heavy metal burden than the tobacco formulation. Elements such as lead[28] and cadmium[29] are known to be toxic above a certain threshold at any inorganic form while arsenic toxicity is species dependent[20]. And it should be noted that lead and cadmium species are sufficiently volatile to be carried in the smoke.

Combusted charcoal analysis

The charcoal samples were combusted and comparisons were made between the elemental compositions of the neat (or as depicted here, original charcoal samples) versus the combusted or ash samples. The mere presence of an element is not enough to indicate toxicity to anyone using the charcoal. The concentrations of these elements that volatilize and reach the consumer provide basic information that may relate to ultimate toxicity to be determined by toxicological studies. Elements such as cadmium and lead are known to volatilize[30]. The comparisons between total elements in the finely ground charcoal *vs.* the corrected ash are shown in *figures 4 and 5*. Ash concentrations were corrected to account for sample loss during consumption so that comparisons can be made between unconsumed and consumed charcoal matrices. Numbered samples in *figures 4 and 5* correspond to concentrations found in tables 2-4 for finely ground charcoal and in Supplement *Tables T2-T4* for the ash. The majority of the total contents remain constant between the two states indicating these elements do not volatilize. However, Fe, Cd and Pb total masses are higher in the finely ground charcoal than the ash indicating portions of these elements can enter into the smoke, once they are used in a hookah apparatus. It is important to note that the method used for digestion of the charcoal samples is more of an extraction than a total digestion. The extraction efficiencies of each element were not performed when the muffle furnace was used. The corrected concentrations of each element are provided in the Supplement, *Figures F2-F6*. The metal profiles for the different charcoal samples vary greatly from brand to brand within similar types (i.e. cubes, disk etc.). We speculate that the combination of different starting materials; wood, coconut, recycled woods, etc., and the casing agents molasses, starch etc., are likely the causes. Interestingly, in many cases the metals do not always follow the same patterns. For instance in *figure 5*, for Cu the majority of the samples show that the metal remains in the ash, but samples 6 and 11 do not follow this pattern. This again may be attributed to the complex matrices of the samples, which contribute positively or negatively to the elements' volatility.

Arsenic Speciation

Arsenic speciation has been performed on a wide variety of sample types including seafood, tobacco, rice, plants, tissues, body fluids and apple juice, to name a few[19, 21, 31-37]. To the authors' knowledge, to date this is the first study where arsenic was speciated in hookah charcoal. The USFDA Elemental Analysis method 4.11 was adopted and modified to extract then analyze for several arsenic species. A major challenge for the arsenic species determination in charcoal is the extraction, which is necessary to avoid changes in arsenic oxidation states as would occur with total digestion. As a similar matrix, Sun et al[17]. developed a method for arsenic species determination in coal by HPLC hydride generation atomic fluorescence spectrometry, HPLC-HG-AFS[17]. Extraction was carried out by 1.0 mol L^{-1} H_3PO_4 with 0.1 mol L^{-1} ascorbic acid. Ascorbic acid was added as an antioxidant to prevent an oxidation of As^{3+} to As^{5+}. The approach of using an antioxidant can also be found for the arsenic species determination in soil, for which the same ascorbic acid extractant was used by Garcia-Manyes et al[27]. In addition to ascorbic acid, sodium bromide, oxalic acid and hydroxyl ammonium chloride were also screened as antioxidants[27].

In this study the different arsenic species in charcoal were separated chromatographically by an anion exchange HPLC column and detected by ICP-MS as a highly sensitive and low detection level detector. In developing the method, the extraction was optimized, nitric acid (HNO_3) and phosphoric acid (H_3PO_4) were evaluated as extractants and ascorbic acid was added as antioxidant. Finally, 1.2 mol L^{-1} H_3PO_4, with 0.2 mol L^{-1} ascorbic acid was chosen as an extractant for the charcoal and the method was applied to determine arsenic species in five different charcoal brands used for hookah smoking.

In addition to the extraction efficiency, the correct determination of the different arsenic species is an important requirement for the analytical method. During sample preparation, especially by using an oxidizing acid like HNO_3 as extractant, a species conversion can take place if the redox potential is favorable. Because of this, ascorbic acid was added as an antioxidant to minimize conversion from As^{3+} to As^{5+}[17, 27]. Due to the fact that there is no reference material for arsenic species in charcoal available, charcoal samples were spiked with a 10.0 mg L^{-1} solution of As^{3+} and As^{5+}. The samples were measured directly following the sample preparation. Parameters and settings for totals and speciation analysis are listed in *table 1.*

A digestion for arsenic speciation totals was rerun because more samples were needed for method development, to ensure the best reproducibility possible. The results for the total arsenic concentrations by total digestion of the different charcoal samples and CRM-coal-A1 are listed in *table 5.* Supplemental *table T5* shows details on assigned numbers for samples used in the charcoal experiments for both totals and speciation; also, which samples are quick-lights and not quick-lights.

The results ranged between 0.71 and 15.1 mg kg^{-1} in the different charcoal samples. An arsenic concentration of 16.5 mg kg^{-1} was obtained for the CRM-Coal-A1 instead of 12 mg kg^{-1}, given by the vendor. However, the vendor value provided is not a certified value, but only given for information proposes. Furthermore, it should be noted, that the determination of the arsenic concentrations were carried out from an extraction and not from a complete digestion.

Table 6 shows the results for the total arsenic extraction by H_3PO_4 and HNO_3. An increasing acid concentration led to a higher concentration of extracted arsenic and subsequently, to a higher extraction efficiency. With H_3PO_4, higher extraction efficiency at low acid concentrations was obtained when compared to HNO_3.

However, H_3PO_4 as well as HNO_3 can be used for a complete arsenic extraction from the charcoal with HNO_3 only when the total arsenic is required. In this work, H_3PO_4 was applied at a concentration of 1.2 mol L^{-1} and HNO_3 at a concentration of 1.4 mol L^{-1}.

Different concentrations of ascorbic acid in 1.4 mol L^{-1} HNO_3 were used to evaluate species interconversion. The results of the spike recovery are shown in *table 7.* Regardless of the concentration of ascorbic acid, an almost complete conversion from As^{3+} to As^{5+} took place. The concentrations of As^{3+} were below the limit of quantification (LOQ). LODs and LOQs are determined by the signal to noise ratio (S/N). The ICP-MS response was compound independent as the calibration curves constructed by peak areas show the same slope for both signals. The difference in LOD estimations is due the different chromatographic behavior between the two species signals, the As^{3+} chromatographic signal elutes earlier than the As^{5+} one, and therefore the efficiency of the first one is considerably larger (less band broadening). The difference in chromatographic efficiencies is reflected in the signal to noise ratios (S/N). The LOD estimation was carried out by following the IUPAC recommendations, 3 x SD of the blank (base line of the chromatograms) divided by the slope of the calibration curve constructed by signal height; and therefore the calculated values are different, as the As^{5+} signal will fall under the chromatographic noise before the higher As^{3+} signal does, as the concentration decreases for both species. In short the ICP-MS response is compound independent, but the HPLC signal behavior is not. We felt compelled to use the IUPAC definition requiring signal height, although the area measurement is better. The LOD was calculated as three times the S/N ratio, the LOQ is 10x the S/N ratio.

Based on the results, 1.4 mol L^{-1} HNO_3 cannot be used as an efficient extractant to speciate arsenic in charcoal if preservation of species is intended. It is too strong of an oxidant at this concentration and conversion takes place from As^{3+} to As^{5+}.

Because of this, HNO_3 with a concentration of 0.5 mol L^{-1} with 0.2 mol L^{-1} ascorbic acid was tested as an extractant see *Table 6*. Also seen in *table 6*, an extraction efficiency of about 90% can be realized by using a HNO_3 concentration of 0.5 mol L^{-1}. This could be used as a compromise between sufficient extraction efficiency and the avoidance of H_3PO_4 as extractant to prevent interface issues at the ICP-MS. However, the oxidation of As^{3+} to As^{5+} was considerable (91.3%). Thus, the use of HNO_3 even at a concentration of 0.5 mol L^{-1} is not an alternative to the use of H_3PO_4 because of its strong oxidative effect.

The results for the use of ascorbic acid in 1.2 mol L^{-1} H_3PO_4 are listed in *table 8*. The spike recovery of As^{3+} increased from 8.10% extraction efficiencies with no ascorbic acid added, to a maximum of 81.2% extraction efficiency with addition of 0.2 mol L^{-1} of ascorbic acid. The spike recovery of As^{5+} showed an opposite development: It decreased from 161% extraction efficiencies with no ascorbic acid added to its minimum of 114% with addition of 0.2 mol L^{-1} of ascorbic acid. At a higher concentration of ascorbic acid the spike recovery of As^{3+} decreased and the spike recovery of As^{5+} increased again.

Based on these results represented in *figure 6,* 1.2 mol L^{-1} H_3PO_4 with 0.2 mol L^{-1} ascorbic acid, was chosen as an extractant for this method. It should be noted that a spike recovery close to

100% for both species could not be achieved. Furthermore, the use of H_3PO_4 in comparison to HNO_3 also requires a higher dilution of the samples so less phosphate is introduced into the ICP-MS leading to a higher LOD and LOQ. Additionally, a platinum skimmer cone should be used for the ICP-MS, when H_3PO_4 is used at the proposed concentrations to prevent premature cone degradation.

The method is capable of separating the four arsenic species As^{3+}, MMA, DMA and As^{5+}. In *figure 7* a chromatogram for the separation of a standard solution of 5 µg kg^{-1} is shown for the four arsenic species.

Table 9 lists the results for the charcoal LODs and LOQs of As^{3+}, MMA, DMA and As^{5+} as well as the retention times and not the instrument's LODs and LOQs. The results for the determination of arsenic species in charcoal are presented in *Table 10.* The concentrations of As^{3+} found in the charcoal samples were 0.08 – 2.42 mg kg^{-1}, the concentrations of As^{5+} 0.46 – 8.36 mg kg^{-1}. The wide range in concentrations is due to the large variation between the different charcoal sample types, their geographic origins, the manufacturing process, etc. A portion of arsenic may come from natural means (i.e. uptake from the soil) and other portions may be from anthropogenic contaminants such as those produced by pyrolysis of treated lumber to form charcoal. DMA and MMA were not detected in the charcoal extracts. As^{5+} was the dominant arsenic species in charcoal. The extraction efficiencies for the charcoal samples varied from 69.7 to 87.6%.

The column recoveries are shown in *table 10.* They ranged between 78.9% and 84.9%. For the different charcoal samples a small arsenic peak could be found within the void volume (Retention time of 2.0 minutes, see *figure 6*) indicating further neutral or positively charged arsenic compounds requiring additional chromatographies to separate. However, these compounds appear at apparent levels below LOQ.

This study indicates that the interconversion of the two inorganic arsenic species is strongly influenced by the respective sample matrix and the sample workup. The correct determination of the arsenic species in all measured samples would require a method modification for each sample. On the other hand, the determination of As^{3+} and As^{5+} as inorganic arsenic seems to be sufficient because of similar high median lethal doses (LD_{50}) of As^{3+} and As^{5+} and the greater concern for these. The speciation of the inorganic arsenic species in charcoal with its complex matrix, was more complex than anticipated.

The method developed for arsenic speciation in charcoal is compared to several other methods in different samples found in the literature *(Supplement table T6[16-18, 27, 38-40]).* The determined LODs and LOQs are higher than those in the reported methods. This is likely because of the high nominal dilution factor of 750 for the method presented here leading to a sub-optimal S/N, since high dilution was necessary to reach a low phosphate concentration in the samples because of ICP-MS sample introduction requirements. However, the LOQs are low enough to quantify all As^{3+} and As^{5+} concentrations in the charcoal samples. The extraction efficiencies presented here are comparable to other reported methods.

Another important issue with arsenic speciation in charcoal is the recovery of the different species, especially As^{3+} and As^{5+}, to judge the method. It was shown that the determination of the inorganic arsenic species, particularly in the charcoals complex matrix, is not a routine task and spike recoveries are dependent on each sample. However, Sun et al.[17] determined the spike recovery of the arsenic species for one coal sample, but not for all samples[17]. Therefore, one reported spike recovery cannot be extended to all samples without some method modification.

Conclusion

Since the ignition charcoal used in most hookah smoking leads to an important portion of the smoke, we have initiated a study to characterize metal content in sixteen samples of charcoal by ICP-MS, from different compositions and geographic origins,. Arsenic speciation was performed on five samples with the highest levels of toxic metals. The results show a large degree of variation between samples. The quick lighting charcoals appear to be the most contaminated with toxic metals. This is not surprising as the materials to produce the charcoal sample will drive the exposure to high concentrations of heavy metals from natural or anthropogenic activities. The analysis of combusted charcoal samples revealed that Fe, Cd and Pb are the only elements with considerable loss during the ashing process, as their content is lower in the ash than in the un-burned material.

An extraction of arsenic for speciation analysis was developed and applied to all samples with recoveries of 79-85%. This analysis shows that all the arsenic was present in its inorganic forms, and mainly as As^{+5}. Since arsenic may remain in the ash, how one disposes of the ash needs to be considered.

The possibility of exposure to Cd and Pb from the first and second hand smoke are suggested. Further studies on levels released in the side-stream smoke need to be done to suggest what risks need further investigation.

As part of a comprehensive study of the possible harmful effects of hookah smoking, the charcoal represents a major contributor to toxic metals exposure risks. Yet more compartments of the smoking apparatus have to be closely studied followed by toxicological studies before a general conclusion can be made in the relevant topic of hookah smoking.

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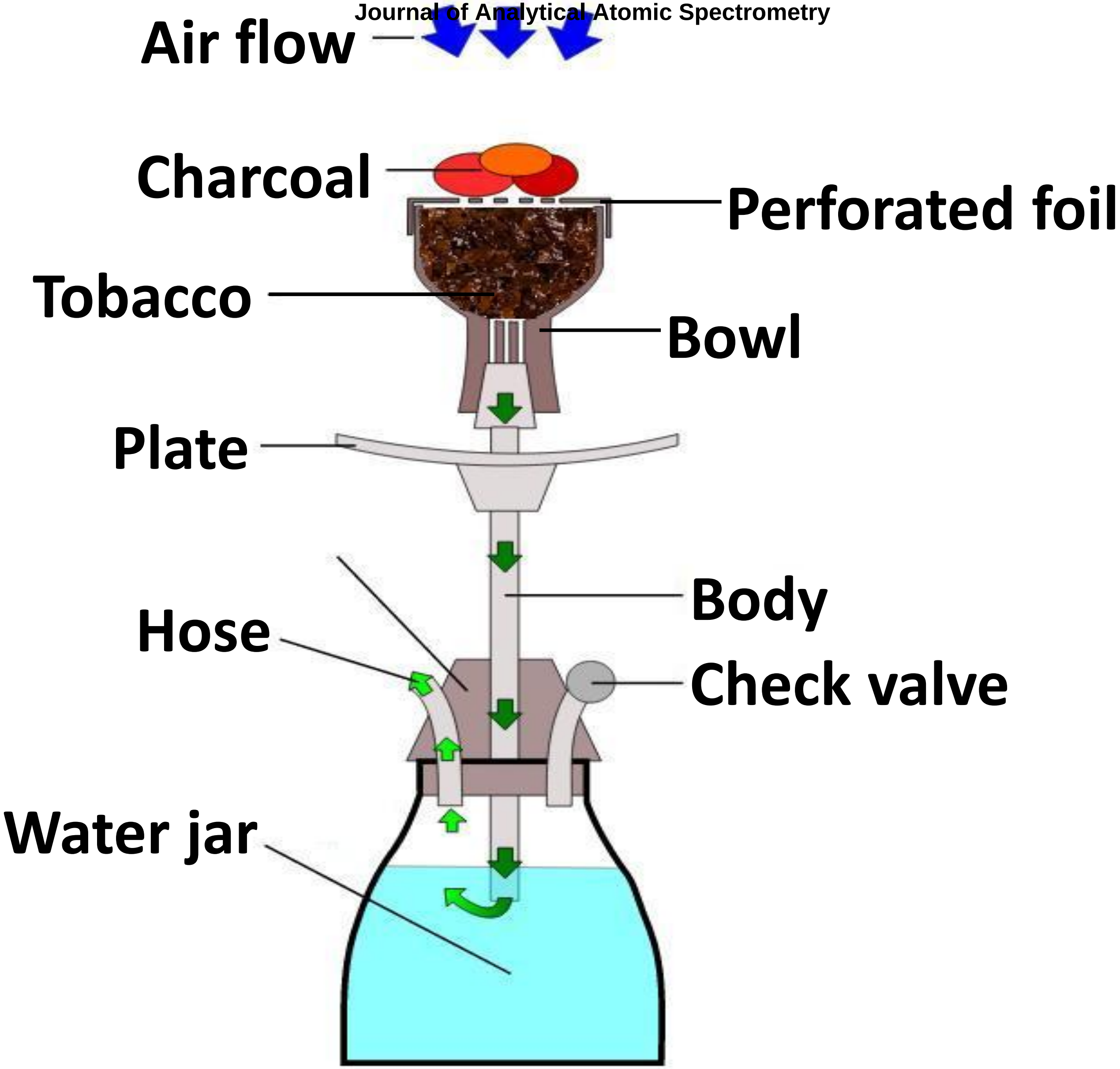

Figure 1. Hookah depiction. Green arrows portray smoke flow through the body, water and out the hose.

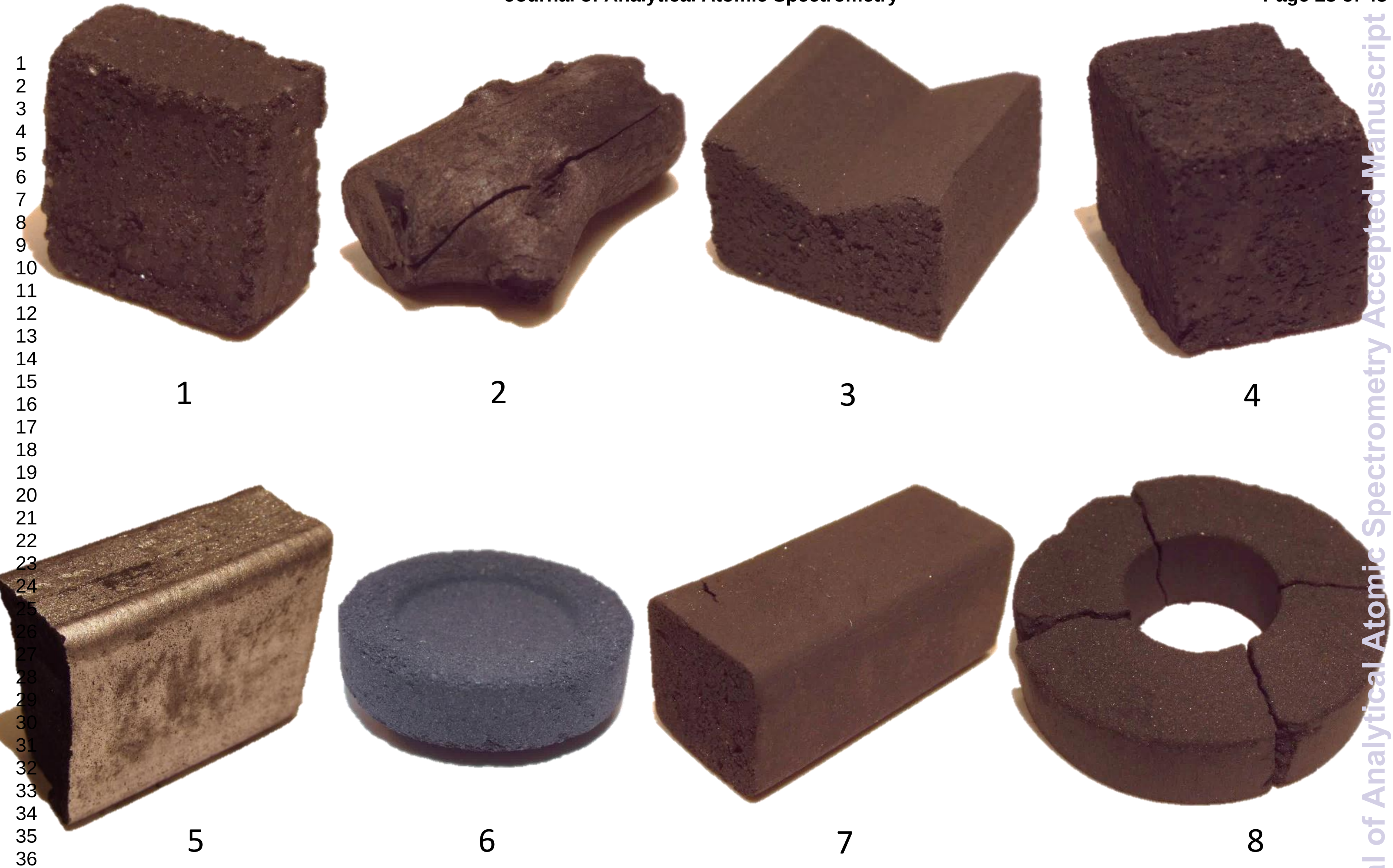

Figure 2. Hookah charcoal images. 1,3,4 depict cube style charcoals. 2 natural lump charcoals. 5 Japanese quick lights. 6 quick light disks. 7 briquettes. 8 bamboo style.

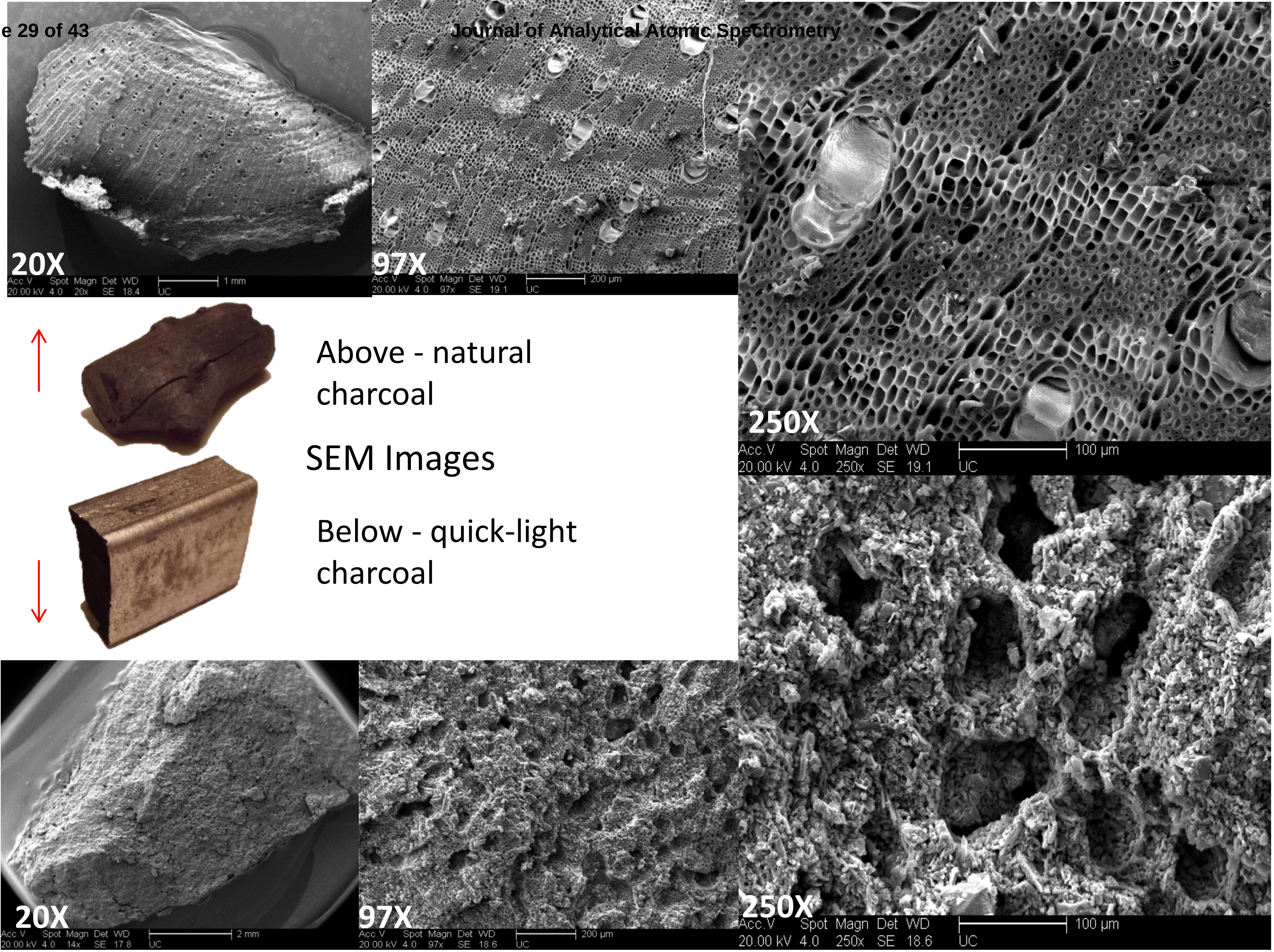

Figure 3. SEM image of hookah charcoal with different magnifications. Top image is lump charcoal and bottom image is manmade quick-light.

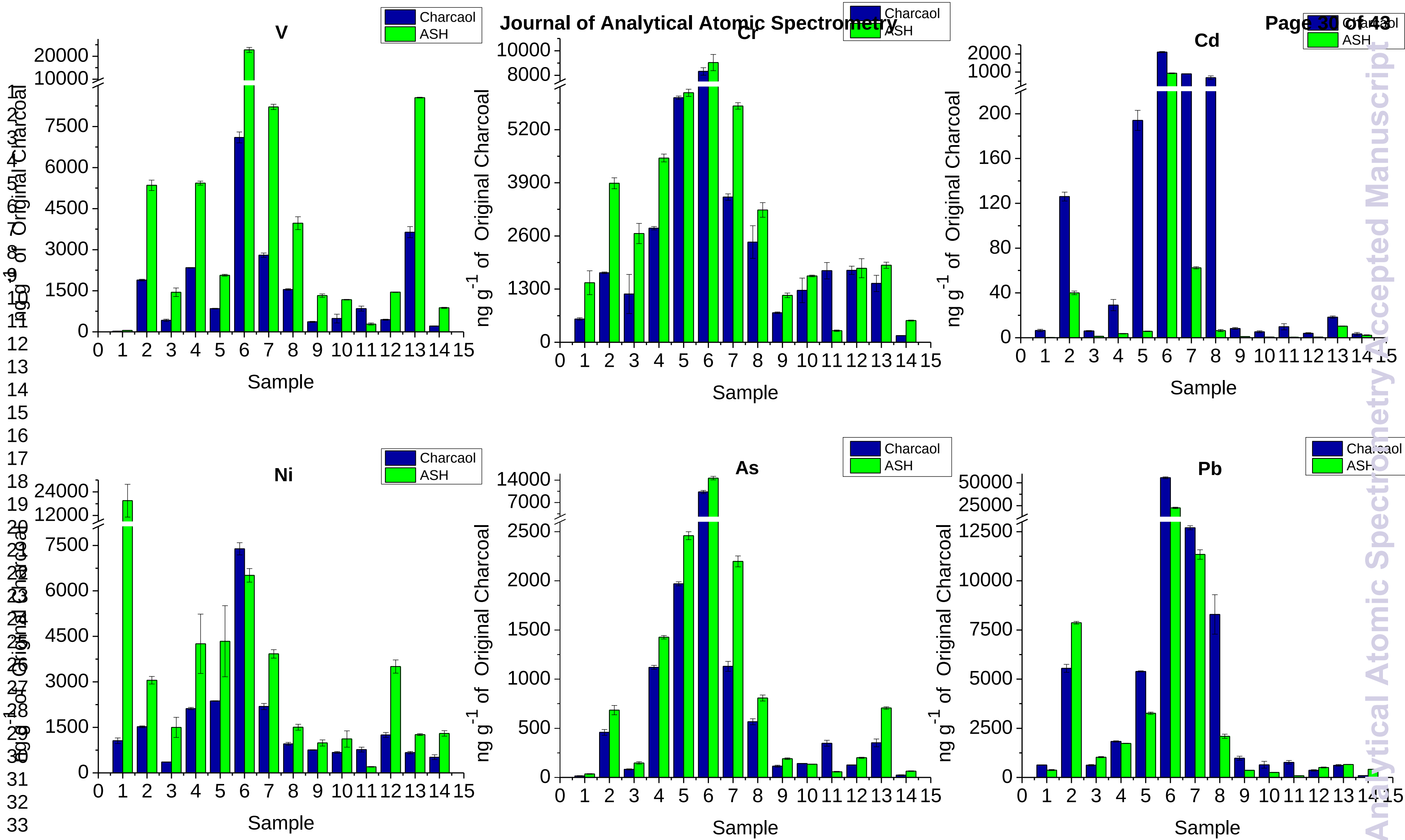

Figure 4. Total elemental analysis was performed on homogenized charcoal (blue) and the ash formulation remaining after combusting the charcoal (green). The reported ash concentrations were corrected to account for sample loss during consumption so that comparisons can be made between unconsumed and consumed charcoal matrices. Results are reported as an average of 3 replicates in ng g^{-1} (ppb) ± 1 SD.

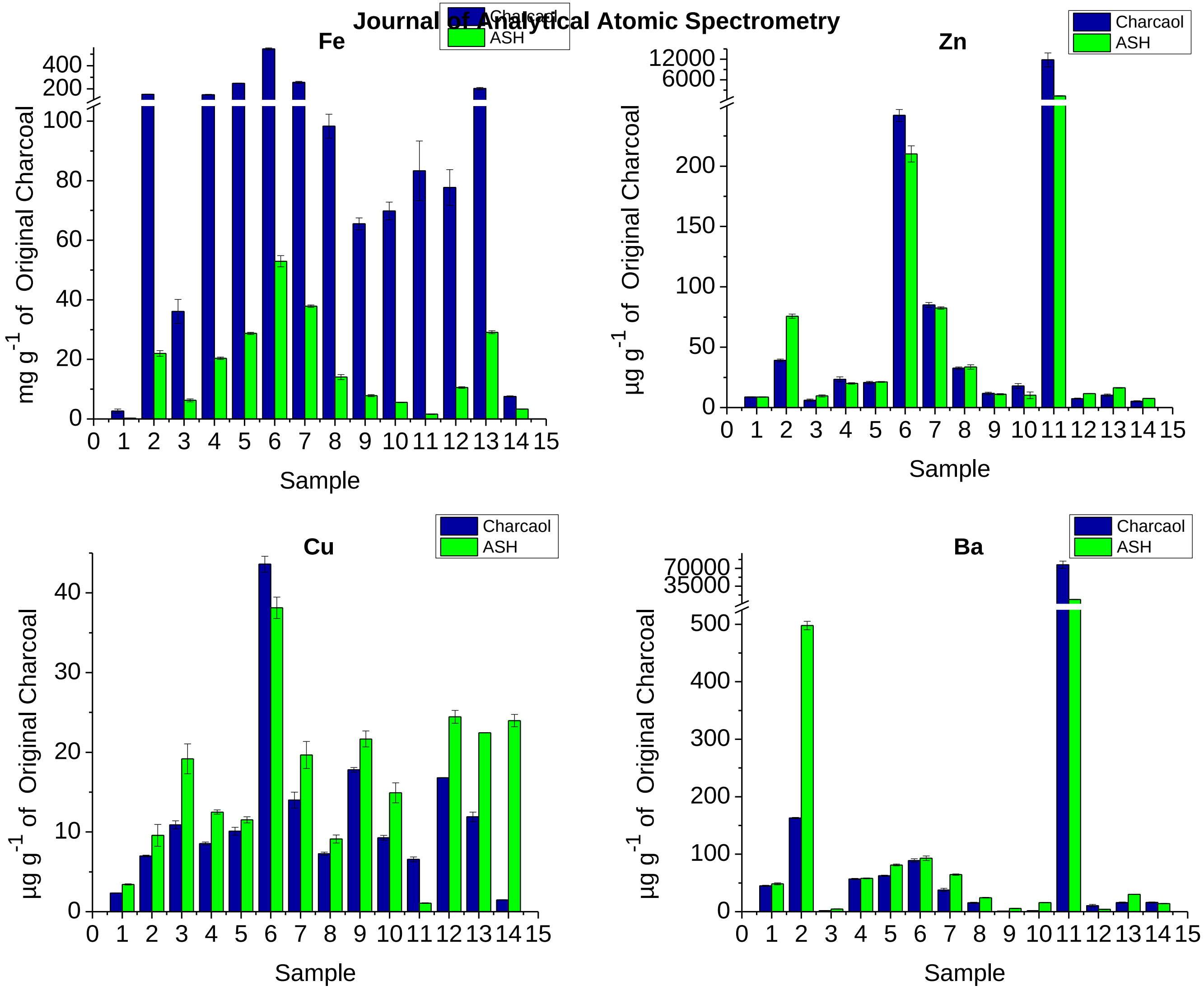

Figure 5. Total elemental analysis was performed on homogenized charcoal (blue) and the ash formulation left over after combusting the charcoal (green). The reported ash concentrations were corrected to account for sample loss during consumption so that comparisons can be made between unconsumed and consumed charcoal matrices. Results are reported as an average of 3 replicates in μg g^{-1} (ppb) ± 1 SD and in mg g^{-1} for Fe.

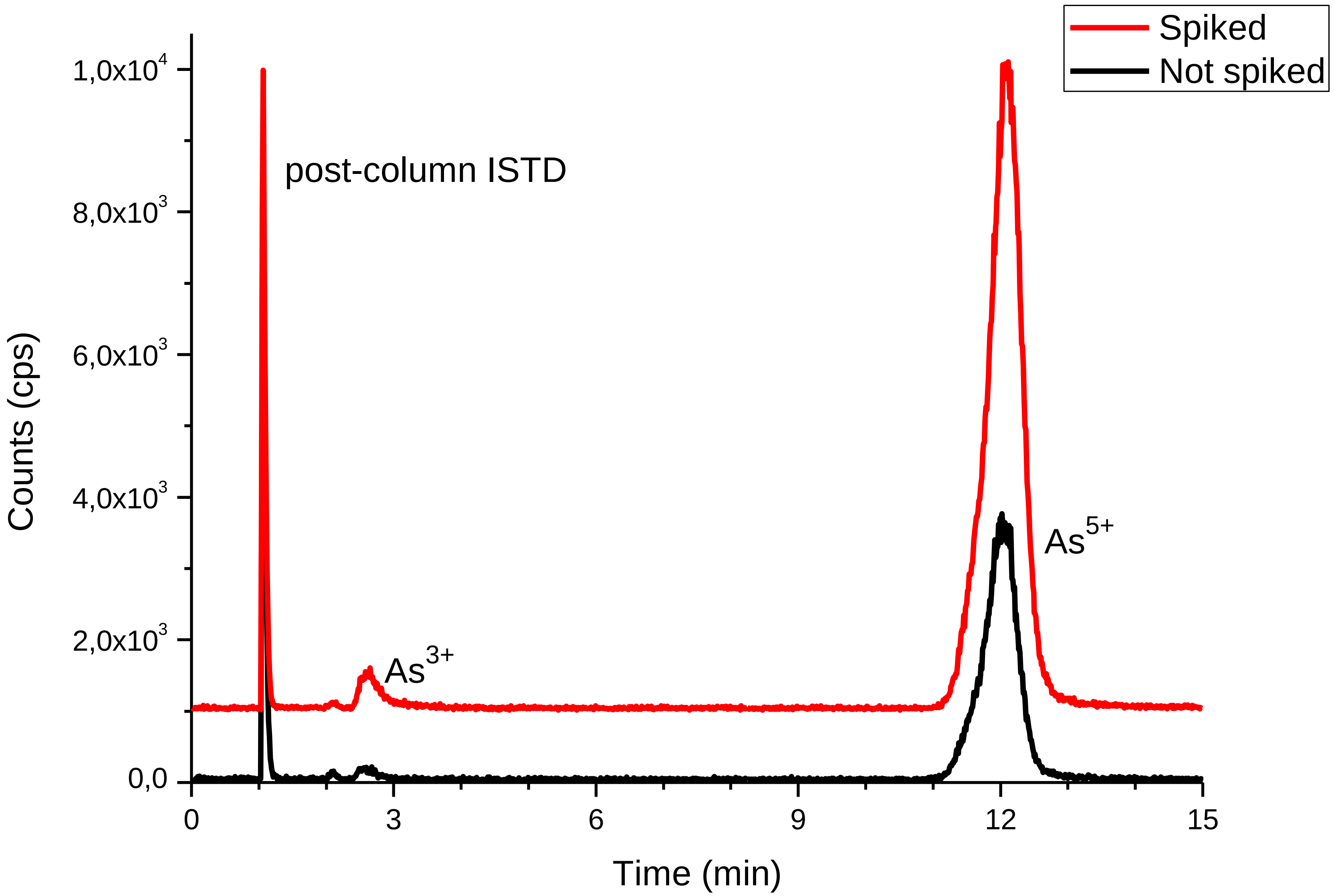

Figure 6. Chromatograms for extraction by 0. 5 mol L^{-1} nitric acid with 0.2 mol L^{-1} ascorbic acid, sample # 3a; not spiked **in black** and spiked with 10.0 mg L^{-1} of As^{3+} and As^{5+} **in red**; graphs stacked with a y-offset of 1000 cps.

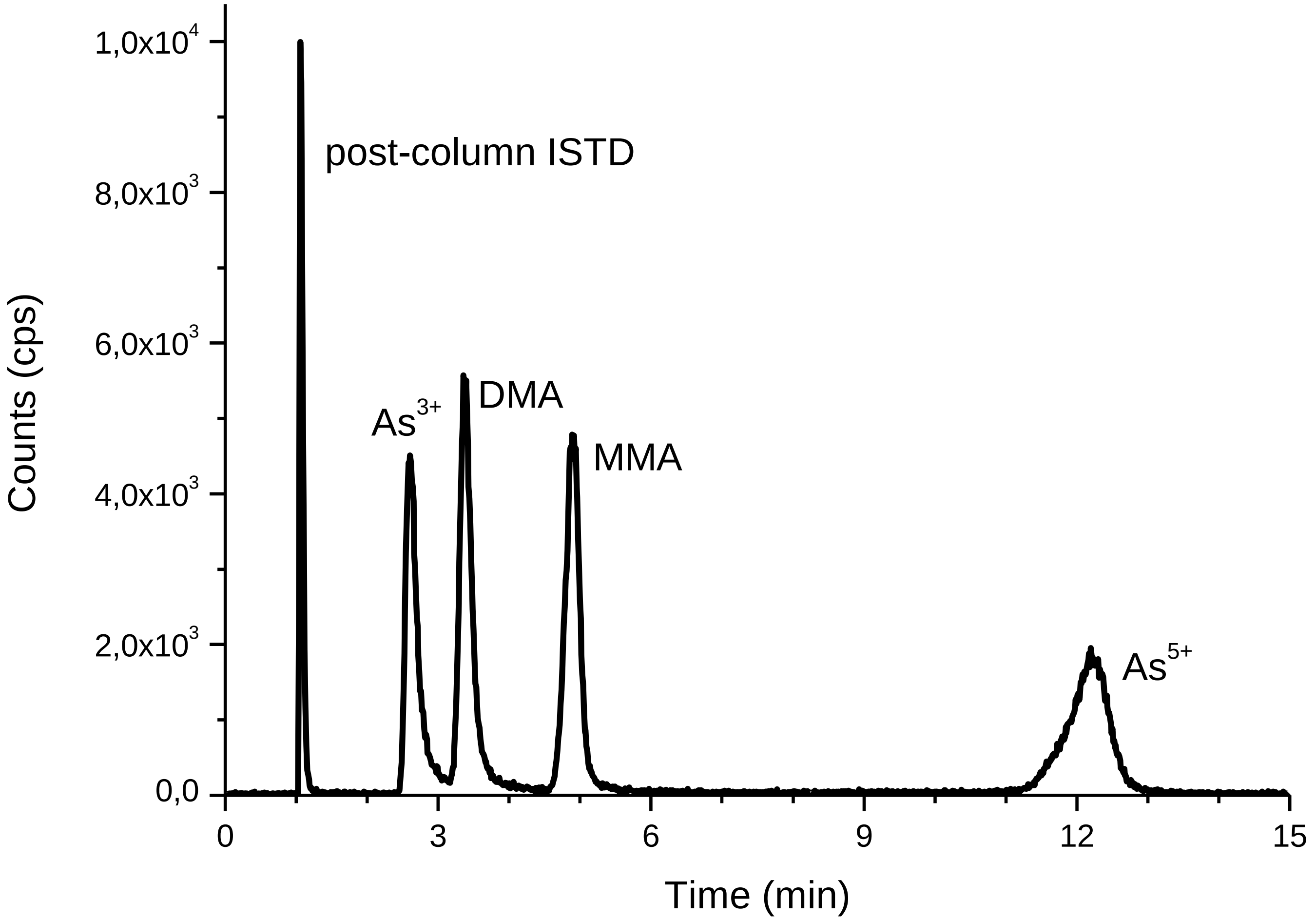

Figure 7. Chromatogram for separation of standard solution with 5 µg kg^{-1} As^{3+}, DMA, MMA and As^{5+}.

Table 1. Settings of HPLC and ICP-MS parameters used for the determination of the total arsenic concentration, total extraction optimization and speciation.

Parameter	Setting for		
	Total arsenic concentration	**Total extraction optimization**	**Speciation analysis**
HPLC (Agilent 1100)			
Column			Hamilton PRP-X100 anion exchange column (250 mm x 4.1 mm i.d., 10 µm)
Mobile phase			10 mmol·L^{-1} $(NH_4)_2HPO_4$, pH 8.25
Flow rate			1.0 mL·min^{-1}
Injection volume			100 µL
Acquisition time			15 min
Six-port valve time table for introduction of post-column ISTD			0.1 min, main pass 1.0 min, bypass 1.8 min, main pass
ICP-MS (Agilent 8800)			
RF power	1550 W	1600 W	1550 W
Plasma gas flow	15.00 L·min^{-1}	15.00 L·min^{-1}	15.00 L·min^{-1}
Auxiliary gas flow rate	0.15 L·min^{-1}	0.13 L·min^{-1}	0.15 L·min^{-1}
Carrier gas flow rate	1.00 L·min^{-1}	1.02 L·min^{-1}	1.00 L·min^{-1}
Nebulizer type	Micromist nebulizer, glass concentric	Micromist nebulizer, glass concentric	Micromist nebulizer, glass concentric
Sampling depth	8.0 mm	8.5 mm	8.0 mm
Sampling cone	Nickel	Nickel	Nickel
Skimmer cone	Nickel	Platinum	Platinum
He flow rate (collision cell)	3.0 mL·min^{-1}	3.5 mL·min^{-1}	3.0 mL·min^{-1}
Isotopes monitored (dwell time)	$^{45}Sc^+$, $^{72}Ge^+$, $^{75}As^+$, $^{77}ArCl^+$, $^{89}Y^+$, $^{115}In^+$, $^{159}Tb^+$	$^{45}Sc^+$, $^{72}Ge^+$, $^{75}As^+$, $^{77}ArCl^+$, $^{89}Y^+$, $^{115}In^+$, $^{159}Tb^+$	$^{75}As^+$ (0.5 s), $^{77}ArCl^+$ (0.2 s)

Table 2. Total elemental analysis of homogenized hookah charcoal formulations. The results are the average of 3 replicates in mg g^{-1} with ± 1 SD

Assigned #	**Origin**	**Na**	**K**	**Ca**	**Fe**
1	Netherlands	23.3 ± 0.9	2.45 ± 0.04	0.704 ± 0.02	2.65 ± 0.7
2	China	4.68 ± 0.03	8.64 ± 0.09	2.43 ± 0.02	152 ± 2
3	Indonesia	1.22 ± 0.04	3.54 ± 0.2	0.039 ± 0.003	36.1 ± 4
4	China	28.5 ± 0.3	4.49 ± 0.03	0.443 ± 0.02	149 ± 3
5	Japan	0.469 ± 0.06	4.33 ± 0.1	0.738 ± 0.01	248 ± 2
6	China	4.73 ± 0.08	15.9 ± 0.3	4.39 ± 0.03	546 ± 9
7	USA	4.4 ± 0.05	10.8 ± 0.2	1.69 ± 0.1	256 ± 9
8	USA	5.66 ± 0.2	9.89 ± 0.4	5.9 ± 0.2	98.3 ± 4
9	China	2.18 ± 0.03	3.96 ± 0.08	0.046 ± 0.003	65.5 ± 2
10	Indonesia	3.37 ± 0.2	2.16 ± 0.05	0.651 ± 0.005	69.8 ± 3
11	Indonesia	19.4 ± 2	3.17 ± 0.3	1.01 ± 0.08	83.3 ± 10
12	China	2.76 ± 0.1	3.93 ± 0.2	0.055 ± 0.002	77.7 ± 6
13	China	1.26 ± 0.03	5.21 ± 0.08	0.118 ± 0.02	203 ± 10
14	Jordan	2.29 ± 0.2	4.2 ± 0.2	3.86 ± 0.04	7.59 ± 0.2
15	China	26.5 ± 0.1	4.47 ± 0.04	2.43 ± 0.004	116 ± 2
16	China	3.58 ± 0.07	9.42 ± 0.2	2.82 ± 0.08	12.5 ± 2
17	CRM1 USA	0.264 ± 0.01	0.069 ± 0.01	0.132 ± 0.006	244 ± 3
18	CRM2 USA	16.6 ± 0.1	2.4 ± 0.2	4.35 ± 0.08	2290 ± 200

Table 3. Total elemental analysis of homogenized hookah charcoal formulations. The results are the average of 3 replicates in μg g^{-1} (ppm) with ± 1 SD.

Assigned #	**Origin**	**Mg**	**Al**	**Mn**	**Cu**	**Zn**	**Ba**
1	Netherlands	471 ± 10	20.4 ± 1	259 ± 7	2.34 ± 0.01	8.64 ± 0.2	45 ± 1
2	China	2650 ± 20	1180 ± 10	289 ± 2	6.99 ± 0.1	39.2 ± 1	163 ± 1
3	Indonesia	264 ± 60	359 ± 30	7.81 ± 1	10.9 ± 0.5	6.1 ± 1	1.92 ± 0.1
4	China	1990 ± 6	1320 ± 30	48.9 ± 0.5	8.55 ± 0.2	23.4 ± 2	56.8 ± 1
5	Japan	854 ± 20	2550 ± 100	348 ± 4	10.1 ± 0.5	20.8 ± 1	62.5 ± 1
6	China	2980 ± 50	4420 ± 100	757 ± 10	43.6 ± 1	242 ± 5	88.9 ± 3
7	USA	1530 ± 30	2360 ± 60	388 ± 4	14 ± 1	85 ± 2	37.7 ± 3
8	USA	871 ± 20	596 ± 30	137 ± 4	7.28 ± 0.2	32.6 ± 1	15.5 ± 1
9	China	352 ± 7	289 ± 10	11.1 ± 1	17.8 ± 0.3	11.7 ± 1	1.17 ± 0.1
10	Indonesia	735 ± 6	360 ± 20	15.3 ± 0.4	9.28 ± 0.3	17.9 ± 2	1.65 ± 0.1
11	Indonesia	958 ± 100	1160 ± 100	26.1 ± 4	6.57 ± 0.3	11800 ± 2000	77200 ± 7000
12	China	500 ± 5	347 ± 9	10.4 ± 0.3	16.8 ± 0.03	7.43 ± 0.4	10.5 ± 2
13	China	426 ± 70	1940 ± 100	69.1 ± 2	11.9 ± 0.6	10.2 ± 1	15.8 ± 1
14	Jordan	1360 ± 60	56.9 ± 2	4.56 ± 0.1	1.48 ± 0.01	5.13 ± 0.5	16 ± 1
15	China	1510 ± 30	863 ± 20	39.5 ± 1	6.15 ± 0.2	19.4 ± 1	48.2 ± 1
16	China	371 ± 4	703 ± 90	133 ± 2	3.55 ± 0.01	24.2 ± 3	21.1 ± 1.8
17	CRM1 USA	92.9 ± 3	836 ± 20	2.64 ± 0.1	8.93 ± 0.7	16.2 ± 1	16.3 ± 1
18	CRM2 USA	7540 ± 500	15900 ± 4000	307 ± 9	25.3 ± 1	98 ± 7	22.7 ± 4

Table 4. Total elemental analysis of finely ground hookah charcoal formulations. The results are the average of 3 replicates in ng g^{-1} (ppb) with ± 1 SD. Highlights show samples of interest.

Assigned #	Origin	V	Cr	Co	Ni	As	Cd	Pb	U
1	Netherlands	27.1 ± 2	569 ± 30	89.6 ± 2	1060 ± 90	14.8 ± 3	6.5 ± 1	632 ± 10	0.65 ± 0.2
2	China	1890 ± 30	1700 ± 20	717 ± 9	1520 ± 30	458 ± 30	126 ± 4	5550 ± 200	114 ± 2
3	Indonesia	422 ± 40	1180 ± 480	167 ± 40	357 ± 2	82.1 ± 6	6 ± 0.5	620 ± 40	15.8 ± 1
4	China	2340 ± 10	2790 ± 40	664 ± 5	2120 ± 40	1120 ± 20	29.1 ± 5	1830 ± 30	81.9 ± 2
5	Japan	849 ± 10	5980 ± 40	811 ± 9	2370 ± 10	1970 ± 20	194 ± 9	5390 ± 30	59.5 ± 4
6	China	7100 ± 200	8320 ± 300	3800 ± 90	7390 ± 200	10300 ± 500	2100 ± 40	55600 ± 1000	485 ± 30
7	USA	2800 ± 80	3550 ± 80	989 ± 30	2190 ± 100	1130 ± 50	903 ± 2	12700 ± 100	172 ± 5
8	USA	1550 ± 30	2450 ± 400	415 ± 20	958 ± 50	567 ± 30	693 ± 100	8290 ± 1000	155 ± 10
9	China	365 ± 20	722 ± 20	263 ± 8	755 ± 10	115 ± 10	8.29 ± 0.8	981 ± 100	22.6 ± 1
10	Indonesia	487 ± 160	1270 ± 300	221 ± 90	675 ± 30	141 ± 1	5.31 ± 0.9	645 ± 170	26.3 ± 1
11	Indonesia	848 ± 90	1750 ± 200	219 ± 30	768 ± 80	347 ± 30	9.82 ± 2.7	769 ± 90	44.7 ± 4
12	China	444 ± 20	1760 ± 100	207 ± 9	1250 ± 80	127 ± 2	3.99 ± 0.6	360 ± 40	19.1 ± 1
13	China	3640 ± 200	1440 ± 200	836 ± 60	668 ± 40	352 ± 40	18.4 ± 1	617 ± 40	28.7 ± 2
14	Jordan	213 ± 6	161 ± 2	124 ± 20	519 ± 80	23.3 ± 4	3.33 ± 1.4	95.2 ± 9	10.3 ± 2
15	China	1580 ± 20	1860 ± 70	513 ± 5	1590 ± 50	810 ± 40	26.5 ± 3	1770 ± 100	69.3 ± 2
16	China	135 ± 10	309 ± 10	124 ± 45	274 ± 10	27.4 ± 4	103 ± 4	619 ± 100	4.25 ± 0.4
17	CRM1 USA	5940 ± 30	3820 ± 50	10000 ± 400	14000 ± 200	13000 ± 300	82.8 ± 20	3300 ± 100	233 ± 6
18	CRM2 USA	42600 ± 5000	36100 ± 6000	4850 ± 500	10100 ± 2000	12300 ± 200	222 ± 6	55200 ± 6000	2230 ± 9

Table 5. Total arsenic concentrations of the charcoal samples and CRM-coal-A1, results are average of three replicates with ± 1 SD.

Sample	**concentration/ mg Kg^{-1}**
#1	1.31 ± 0.03
#2	0.71 ± 0.03
#3a	11. 2 ± 0.59
#3b	15.1 ± 1.12
#4	1.38 ± 0.14
#5	2.59 ± 0.13
CRM -Coal -A1	16.5 ± 1.53

Table 6. Effects of H_3PO_4 and HNO_3 concentration on extraction efficiency, results are average of two replicates with ± 1 SD.

Acid	**Acid concentration/ mol/L**	**Concentration of total arsenic/ mg·L^{-1}**	**Concentration of extracted arsenic/ mg·L^{-1}**	**Extraction efficiency**
H_3PO_4	0.0	11.2 ± 0.59	3.10 ± 0.02	27.7%
	0.2		9.40 ± 0.47	84.0%
	0.6		10.7 ± 0.13	95.6%
	1.0		10.9 ± 0.19	97.4%
	1.2		11.0 ± 0.72	98.4%
	1.4		12.3 ± 1.80	110%
	1.6		11.5 ± 0.00	102%
HNO_3	0.0		3.10 ± 0.02	27.7%
	0.2		6.54 ± 0.27	58.4%
	0.6		10.0 ± 0.21	89.4%
	1.0		10.3 ± 0.18	92.0%
	1.2		10.6 ± 0.17	94.8%
	1.4		12.2 ± 1.36	109%
	1.6		12.2 ± 0.01	109%

Table 7. Effect of concentration of ascorbic acid in 1.4 mol L^{-1} HNO$_3$ as antioxidant on the spike recovery; samples spiked with 10 mg L^{-1} of As^{3+} and As^{5+} . Results are average of three replicates with ± 1 SD; a: not detectable; b: not quantifiable.

Concentration of ascorbic acid (mol/L)	**Species**	**Concentration without spiking/ mg·L^{-1}**	**Concentration with spiking of 10 mg·L^{-1}/ mg·L^{-1}**	**Spike recovery**
0	As^{3+}	-^{a}	-^{a}	0.00 %
	As^{5+}	10.6 ± 0.57	28.6 ± 1.17	180 %
0.05	As^{3+}	-^{a}	-^{a}	0.00 %
	As^{5+}	12.4 ± 1.48	32.4 ± 0.75	200 %
0.1	As^{3+}	-^{a}	-^{b}	0.00 %
	As^{5+}	11.6 ± 0.43	32.0 ± 0.72	203 %
0.2	As^{3+}	-^{a}	-^{b}	0.00 %
	As^{5+}	11.4 ± 0.89	31.2 ± 1.41	197 %
0.3	As^{3+}	-^{a}	-^{b}	0.00 %
	As^{5+}	11.5 ± 0.48	33.4 ± 4.13	219 %

Table 8. Effect of concentration of ascorbic acid in 1.2 mol L^{-1} H_3PO_4 as antioxidant on the spike recovery; samples spiked with 10 mg L^{-1} of As^{3+} and As^{5+} . Results are average of three replicates with ± 1 SD.

Concentration of ascorbic acid (mol/L)	**Species**	**Concentration without spiking/ mg·L^{-1}**	**Concentration with spiking of 10 mg·L^{-1}/ mg·L^{-1}**	**Spike recovery**
0	As^{3+}	0.55 ± 0.03	1.36 ± 0.18	8.10%
	As^{5+}	11.0 ± 0.24	27.1 ± 4.74	161%
0.05	As^{3+}	1.54 ± 0.15	7.28 ± 0.12	57.5%
	As^{5+}	9.11 ± 0.66	23.4 ± 0.97	143%
0.1	As^{3+}	1.82 ± 0.12	8.92 ± 0.35	71.0%
	As^{5+}	8.64 ± 0.43	21.1 ± 0.11	124%
0.2	As^{3+}	1.97 ± 0.03	10.1 ± 0.46	81.2%
	As^{5+}	8.74 ± 0.82	20.2 ± 0.80	114%
0.3	As^{3+}	2.02 ± 0.08	9.58 ± 0.35	75.6%
	As^{5+}	8.26 ± 0.45	20.8 ± 0.67	126%

Table 9. LODs, LOQs and retention times of As^{3+}, DMA, MMA and As^{5+} in charcoal samples.

	LOD/ $\mu g \cdot kg^{-1}$	**LOQ/ $\mu g \cdot kg^{-1}$**	**Retention time/ min**
As^{3+}	31	100	2.57 ± 0.01
DMA	25	85	3.43 ± 0.03
MMA	23	78	4.50 ± 0.03
As^{5+}	65	200	12.50 ± 0.03

Table 10. Concentrations of As^{3+} and As^{5+}, total arsenic concentration, extraction efficiency and column recovery in samples #1 – #5 and CRM-Coal-A1; results are average of three replicates with ± 1 SD.

Sample	**c(As^{3+})/ mg·kg^{-1}**	**c(As^{5+})/ mg·kg^{-1}**	**c(As_{total})/ mg·kg^{-1}**	**Extraction efficiency**	**Column recovery**
#1	0.29 ± 0.08	0.80 ± 0.04	1.09 ± 0.11	82.8%	78.9%
#2	0.16 ± 0.00	0.46 ± 0.01	0.62 ± 0.01	87.6%	79.1%
#3b	2.42 ± 0.16	8.36 ± 0.34	10.8 ± 0.49	71.5%	84.9%
#4	0.08 ± 0.00	0.88 ± 0.03	0.96 ± 0.03	69.7%	80.7%
#5	0.48 ± 0.03	1.63 ± 0.06	2.11 ± 0.09	81.3%	80.1%
CRM-Coal-A1	0.66 ± 0.01	9.28 ± 0.47	9.95 ± 0.47	60.3%	82.8%