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PHYSICO- CHEMICAL CHARACTERIZATION OF LEAD BASED INDIAN TRADITIONAL MEDICINE- *NAGA BHASMA*

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ABSTRACT

Naga bhasma, a lead based herbo-metallic preparation has been used in treating various ailments such as diabetes mellitus, diarrhea, skin and spleen diseases. The major elements of *naga bhasma* are lead and sulphur, while trace elements include calcium, potassium and phosphorous. A detailed study on the physico-chemical characteristics of *naga bhasma* is required to allay fears on its toxicity owing to the presence of heavy metals. However, as per *Ayurveda*, the toxic character of metallic ingredients is eliminated due to scrupulous purification and calcination steps. But, with different manufacturers adopting various procedures for preparation, the characterization of the bhasma using modern analytical tools, apart from classical tests like *varitara* and *niruttha* becomes essential. *Naga bhasma* samples from three different batches from the same manufacturer were procured and analyzed. *Niruttha* test confirmed the absence of free metal in the *bhasma*. Thermogravimetry analysis showed that *naga bhasma* sample was thermally stable until 900º C, indicating the absence of free organic molecules. The FTIR spectra revealed that all the samples contained organic moieties probably in the form of a complex. Particle size and surface area analysis of the *naga bhasma* samples indicated the presence of micron-sized particles. Elemental analysis indicated the presence of arsenic impurity in the samples. Electron microscopy studies revealed that *bhasma* contained particles in micron & sub-micron ranges. Energy dispersive X-ray Analysis too showed the presence of arsenic along with Lead. XRD showed the lead oxide phase in all three samples.

Keywords: *Naga bhasma*, Lead, Total ash, Physico-chemical characterization, X- ray diffraction, Elemental analysis

INTRODUCTION

Ayurveda, a traditional Indian System of Medicine, is believed to be in existence from time immemorial. There are evidences for the usage of drugs derived from minerals, vegetable and animal products[1]. Metal-based drugs were prepared by transmutation of base metals into noble ones along with the use of plant extracts meant to eradicate the toxic effects of metal[2, 3]. In ancient times, lead was used as pill and as well as liquid jade[4]. When lead is subjected to a programmed heat treatment with herbal ingredients in a controlled atmosphere, reaction between the herbal and lead leads to the formations of a herbo-metallic preparation, called *Naga bhasma*[5]. This herbo-metallic preparation has been used in treating diabetes, diarrhea, spleen and skin disorders[6]. *Naga bhasma* has shown testis regenerative potential on partially degenerated testes[7]. Clinical studies have proved the anti-diabetic activity of *Naga bhasma*[8].

The preparation of *Naga bhasma* starts with purification steps in which the metal is quenched in thrice sequentially in sesame oil, butter milk, cow's urine, sour rice gruel and horse gram decoction[9]. The purification steps are called *shodhana*[10]. This is aimed at rendering the metal to a form capable of reacting with herbal ingredients to be added during the later stages of preparation. This purified lead is stirred well under heating with tamarind and peepal bark powder to reduce the metal to the powder form in a process called *Jarana*[11]. The powder form of lead is ground well with Arsenic disulfide and sour rice gruel until a doughy mass is obtained which is made into round shaped discs. These discs are sun dried well before being subjected to 50 cycles of *Arddha gajaputa* and 10 cycles of *puta*. The analysis of commercially procured *naga bhasma* samples from different manufacturers revealed the presence of oxide, sulfate, and carbonate and arsenate forms of lead as a mixture[12]. Singh et al. (2010) found that *naga bhasma* contained lead sulfide in crystalline form along with the organic contents with the incorporation of various nutrient elements from the herbs during preparation. Histopathology study on rats revealed that prepared drug was totally safe at a dose of 6mg/100g/day[6]. Pravin et al.(2009) prepared *naga bhasma* by following two different protocols and identified the drug to be present as lead sulfide with particles in the size range of 57.4-120µm[11]. *Naga bhasma* causes cognitive dysfunctions and affects neurochemical parameters at high dosages[13]. With respect to identification of chemical nature of *bhasma*, a core-shell model has been proposed recently for *lauha bhasma* through physico-chemical characterization[14]. In the present work, in addition to the physico- chemical characterization through conventional studies for studying the quality of *bhasma* such as floatability test *(varitara)*, metal irreversiblity test *(niruttha)*, detailed information on structural and chemical characteristics of *naga bhasma* has also been revealed by modern analytical instruments like thermogravimetric Analyzer, Fourier transform infrared spectrometer (FTIR), X-ray fluorescence spectrophotometer (XRF), particle size analyzer, surface area analyzer, scanning electron microscope with energy dispersive analysis of X-rays and X- ray diffractometer.

MATERIALS AND METHOD

Naga bhasma of varying batches procured from an Ayurvedic manufacturing company and labeled as XB1, XB2 and XB3 were subjected to various physicochemical parameters. Modern analytical instruments like thermogravimetric analyzer, Fourier transform infrared spectrometer (FTIR), particle size analyzer, surface area analyzer, X-ray fluorescence spectrophotometer (XRF), scanning electron microscope with energy dispersive analysis of X-rays and X-ray diffractometer were employed to determine the decomposition temperature (weight loss), to study the presence or absence of organic moieties and complex formation, to determine particle size, to analysis the surface area and pore volume, to determine the elemental composition, to study the morphology, to determine the crystalline phase respectively.

Estimation of Total Ash

A suitable quantity of the sample was weighed accurately in a silica crucible. The sample was spread uniformly on the bottom of the crucible, incinerated, cooled and weighed. Difference between the empty crucible weight and crucible with incinerated ash gives the total ash value[15]

Estimation of Acid Insoluble Ash

The residue from total ash estimation was boiled with hydrochloric acid. The insoluble matter was washed with hot water, transferred to a crucible, dried and weighed. Difference between the empty crucible weight and crucible with incinerated ash gives the acid insoluble ash value[15]

Estimation of Water Soluble Ash

The residue from total ash estimation was boiled with distilled water. The insoluble matter was washed with hot water, transferred to a crucible, dried and weighed. This weight was subtracted from the total ash taken which gives the water soluble ash content[15]

Determination of Loss on Drying (LOD)

The accurately weighed sample was dried in an oven at 105°C, cooled and weighed[15]

Determination of Bulk Density

A known mass of the sample was carefully poured into a long measuring jar and the volume corresponding to top level of the sample was noted, from which the bulk density was calculated as the ratio of mass of the sample to the volume.

Determination of Tapped Density

A known mass of the sample was carefully poured into a long measuring jar and subjected to 100 tappings, as per United States Pharmacopoeia –II (USP–II) in a tap density tester (TD1025, LABINDIA). The volume corresponding to top level of the sample was noted, from which the tapped density was calculated as the ratio of mass of the sample to the volume.

Determination of Hausner Ratio

This was calculated as the ratio of tapped density to bulk density

Floatability Test *(varitara)*

A pinch of the *bhasma* was sprinkled on water taken in a beaker and the floating tendency of the *bhasma* was observed.

Metal Irreversibility Test *(niruttha)*

A known amount of silver was heated in a muffle furnace for 15 minutes with the commercial *naga bhasma* at various temperatures in a silica crucible. The silica crucible was cooled to room temperature and the weight was recorded.

Thermogravimetry

The thermo gravimetric analysis was carried out using TG-DTA (TA Instruments SDT Q600 US). About 5mg of sample was placed in alumina cup and heated up to 1000° C at the rate of 10° C per minute.

Spectroscopic Analysis

The Fourier Transform Infra Red (FTIR) spectra of the different batches of commercial *naga bhasma* samples were recorded between 4000–400 cm^{-1} in a FTIR spectrometer (Spectrum 100, Perkin Elmer, USA). The samples were mixed with KBr and pelletized for analysis.

Sub–Sieve Particle Size Distribution & Surface Area Analysis

The sub–sieve particle size distribution was determined using laser diffraction technique (Bluewave Microtrac, Nikkiso, Japan). The samples were dispersed in water for analysis. The surface area, pore volume and the adsorption isotherms were recorded for the commercial *naga bhasma* samples using BET technique (ASAP 2020, Micromeritics, USA).

X- ray fluorescence spectroscopy

Aluminium cups were filled with 2 grams of boric acid and then 1 gram of each sample was used to finely cover the boric acid. The aluminium cups were pelletized using a hydraulic press at 25 tons to obtain 34 mm diameter pellets. Samples were analyzed using XRF spectrometer (Bruker S8 Tiger). A 4KW, Rh anode X-ray tube was used to generate X-rays.

Morphological Analysis

The surface morphology of the commercial samples of *naga bhasma* was qualitatively assessed using a cold field emission scanning electron microscope (JSM 6701F, JEOL, Japan).

Elemental Analysis by Energy Dispersive Analysis of X–Rays (EDAX)

The samples were mounted on a brass stub and sputter coated with gold and introduced into the specimen chamber of the cold field emission scanning electron microscope (JSM–6701F, JEOL, Japan) under ultra high vacuum for EDAX analysis.

X- ray diffractometry

Three Commercial batches of *naga bhasma* were characterized using a X-ray diffractormeter (D8 Focus, Bruker) equipped with a photo scintillation detector. Angular range (2θ=10-60°), rate 0.01°/sec.

RESULTS AND DISCUSSION

Physiological role of elemental lead has been under debate since time immemorial. While traditional *Ayurveda* proposed that imbalance of lead in human system causes anemia and gastrointestinal disturbances due to poor secretion of gastric juices, modern medical literature do not report significant physiological effects of elemental lead[16]. Interestingly, when lead is formulated in the form of *naga bhasma* (lead complexes with herbal constituents), it is known to exert significant physiological influences alleviating urinary disorders as well acting as appetizer, immunomodulator and aphrodisiac[5]. It is also indicated in the treatment of diabetes, rheumatoid arthritis, fever, anemia, cough, abdominal syndrome, bowel irritation, piles, diarrhea, and ulcer. *Naga bhasma* (30-125 mg/day) is prescribed with adjuvants like honey, sugar candy, sesame oil, rice and wheat to treat various diseases[3, 5]. It is cautioned in classic texts that improperly incinerated lead causes adverse effects like diabetes, jaundice, emaciation, anemia, skin disorders, and oedema [11]. Heating and quenching the metal in various plant and animal media (sesame oil, buttermilk, cow's urine, decoction of horse gram, sour rice gruel) removes the inorganic impurities and incorporating beneficial organic moieties into the metal which render them suitable for further process of preparation of *bhasma* (grinding with plant drug and repeated calcination)[17]. The preparation protocol for *bhasma* varies from manufacturer to manufacturer, there are many *Ayurvedic* texts describing different methods and raw materials for preparing *naga bhasma*[11, 12]. It is important to understand the structure and composition of various constituents present in the *bhasma* which suppresses its toxic effects and inserting therapeutic effects to the metal. It has been hypothesized that repeated calcination of metal with suitable raw material change the inherent quality of the metal, which render them non-toxic and suitable for the treatment of chronic ailments[18]. The conventional tests like *Varitara*, *Rekhapurna*, *Niruttha* etc., performed to check the quality of *bhasma* are not quite reliable. Characterization of *naga bhasma* using modern analytical tools became inevitable.

Naga bhasma from different batches procured from the same manufacturer was first subjected to Ayurvedic tests as prescribed by AYUSH[15]. Initially, total ash of the samples was determined to check proper incineration of the metal. This is an important parameter as improperly incinerated lead has been reported to introduce deleterious effects like diabetes, jaundice, emaciation, anemia, skin disorders, and oedema[11]. Accordingly the samples under investigation showed proper incineration of lead as evident (Table 1) from the total ash value (> 95%), negligible moisture content (0 % loss on drying), lower solubility in acid (25-30%) and lower sulfur content (9-11%). The results are comparable to the reported values [11]. The observation is supplemented further by performing *niruttha* test, during which a *bhasma* preparation containing free metal would form an alloy with silver thereby reducing the weight of silver. Lead-silver alloys are known to form at a temperature of 304 - 579 ° C[18]. In the present case, weight loss occurs in the temperature range of 600 ° C -800 ° C suggesting the absence of metallic lead (Table 2).

After ensuring the absence of free metallic lead by metal irreversibility test (*niruttha)* and ash analysis, the physical qualities of the *bhasmas* like, floatability *(varitara)*, appearance, density and flow property of the samples were analyzed. As summarized in Table 3, samples from all the batches had similar appearance (non-

lustrous grey powder) and exhibited partial floatability in water (Figure 1). XB3 sample, however, seems to be denser in terms of bulk density as well as tapped density and exhibits lower Hausner ratio compared to XB1 and XB2. Having understood the extent of incineration and physical quality, we preceded further for particle size and surface area measurements the results of which are shown in Table 4. Particle size distribution (obtained by laser light scattering method) was found to be similar for all the three samples while XB3 seems to have the smallest particle size. When these results are correlated with the bulk density values, smaller particles of XB3 seem to pack closely than larger particles of XB1 and XB2. This is further supported by surface area measurements (Brauner Emmett Teller method) which indicated the lowest value for XB3 probably due to its close-packed nature.

Figure 2 shows the thermogram of the *naga bhasma* samples, XB1, XB2 and XB3 in N_2 atmosphere. TGA data of XB1 and XB3 sample showing degradation profile above 800º C and XB2 sample showed no degradation profile even up to 1000º C indicating proper *bhasmikaran* (incineration). In other words it shows the absence of free metal or organic matter[19] in comparison with the reported melting point and decomposition temperature of herbal components of *naga bhasma*[12]. This is in accordance with ash content analysis discussed in the previous section as well as *niruttha test* results. The absence of organic matter is further evident from FTIR spectra of the samples shown in Figure 3. In addition the FTIR spectra indicate the presence of Lead-Oxygen bond at 500-650 cm^{-1}. Similar spectra have also been reported earlier for commercial *naga bhasma* sample[12].

While TGA indicates only total thermally decomposable matter in the sample, presence of specific heavy metal impurities in the *bhasma* samples require elemental analysis using techniques like ICP-MS, AAS and X-ray Fluorescence (XRF) Spectroscopy. Previous reports on commercial preparations of *naga bhasma* employed ICP-MS and AAS for elemental analysis and concluded that arsenic was absent in the final sample. However, the present study using XRF indicates the presence of arsenic impurity in all the three samples as shown in Table 5. One of the case study reports revealed that some of the Ayurvedic medicines contain lead, arsenic and mercury higher than their permissible limits caused abdominal pain, vomiting[20]. Nevertheless, the absence of arsenic in previously reported commercial samples could either indicate better samples or could also be due to differences in the sensitivity of the characterization technique. The scanning electron micrographs (Fig. 4) of all commercially *naga bhasma* samples were showing that they form irregular aggregates of distorted nanoparticles of various sizes and shapes. In accordance with XRF results, Energy dispersive x-ray Analysis also shows the presence of arsenic. In addition other elements such as calcium, tin, molybdenum and potassium are observed. X- ray diffraction patterns (Fig. 5) of commercial batches of *naga bhasma* are shown in Fig. 5. XB1 sample shows diffraction peak at angle 2Θ=23.30, 24.57, 29.31, 31.78, 34.35, 37.87, 40.57, 41.29, 54.50, and 59.42° corresponding to the (001), (101), (111), (020), (120), (201), (121), (211), (031), (312) planes with reference to the JCPS File No. : 89-7387 confirming the presence of lead oxide (Pb_2O_3). XB2 sample matches with the JCPS File No. : 89-1947 confirming the presence of lead oxide (Pb_3O_4), showing diffraction peaks at angle 2= 22.54, 24.31, 25.36, 27.15, 28.63, 30.77, 40.93, 47.49, 53.82, 58.05, and 59.49° corresponding to the (210), (201), (211), (002), (220), (112), (400), (213), (431), (521), (432) planes. The diffraction pattern of XB3 is similar to that of XB1confirming the presence of lead oxide (Pb_2O_3). These results could be compared with the reported XRD patterns for similar samples[12]. Lead sulphide (PbS) has also been reported in some of the samples which are not observed in the present case[6].

Table 1: showing the Ash (total, acid insoluble, water soluble, sulphated) and moisture content of commercial *naga bhasma* samples

Sample	Total	Acid	Water	Sulphated	Loss on
XB1	95.45±	64.65±	14.80±	9.60±	0.00
XB2	95.35±	72.32±	36.96±	9.50±	0.00
XB3	97.50±	66.42±	39.50±	10.86±	0.00

Table 2: Showing weight loss percentage during *Niruttha* test

	Weight Change (%)				
Temperature	400°C	500°C	600°C	700°C	800°C
Sample ID					
XB1	0.00	0.00	0.00	0.97	22.73
XB2	0.00	0.00	0.00	0.91	34.17
XB3	0.00	0.00	1.46	5.22	67.68

Table 3: Showing physical properties of *naga bhasma*

Sample	Appearance	Luster	Floatability on Water	Bulk	Tapped	Hausner
XB1	Grey powder	No	No	0.65	1.09	1.67
XB2	Grey	No	No	0.66	1.00	1.51
XB3	Grey	No	No	0.91	1.25	1.37

Table 4: Showing sub-sieve particle size distribution, BET surface area and pore volume of commercial *naga bhasma* samples

Sample	Sub–Sieve	BET Surface	Pore Volume
XB1	0.526–3.090	10.8300	6.44×10^{-2}
XB2	0.735–3.230	9.7240	5.85×10^{-2}
XB3	0.055–2.737	5.2713	22.14×10^{-2}

Table 5: Showing elemental composition of different batches of *naga bhasma*

Sample	Pb	Zn	Mn	Cu	As	Cd	Hg	Sn	Ag
XB1	59.05	-	-	0.09	4.47	-	-	-	-
XB2	59.40	-	-	0.01	4.47	-	-	8.31	-
XB3	53.00	0.15	0.01	0.54	6.29	-	0.06	2.70	-

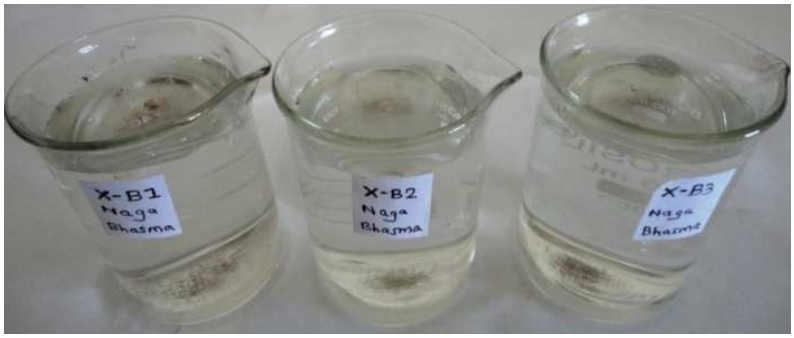
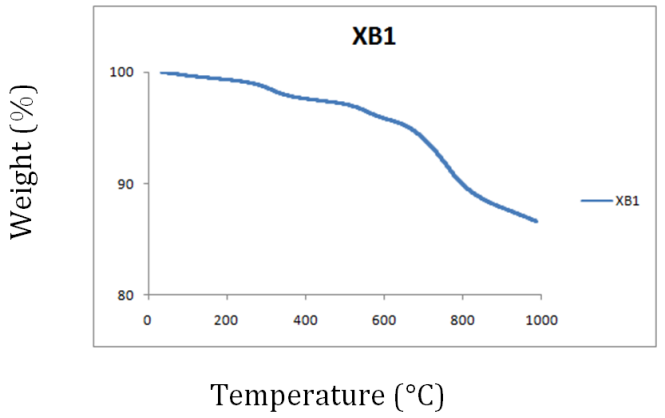
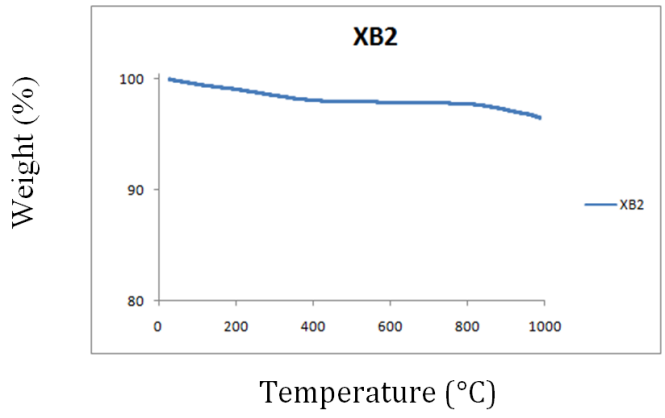


Fig. 1: *Varitara* (floatability) property of *naga bhasma*





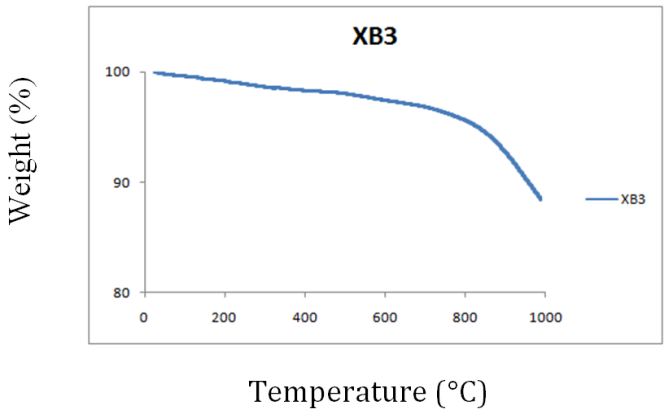


Fig. 2: Thermogram of *naga bhasma*

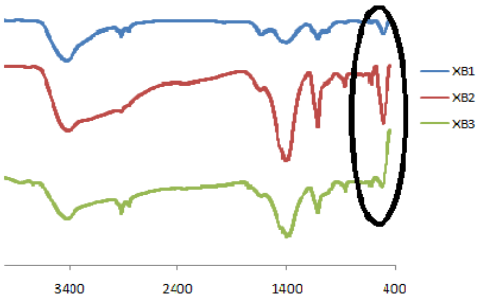


Fig. 3: FTIR spectra of commercial *naga bhasma* samples

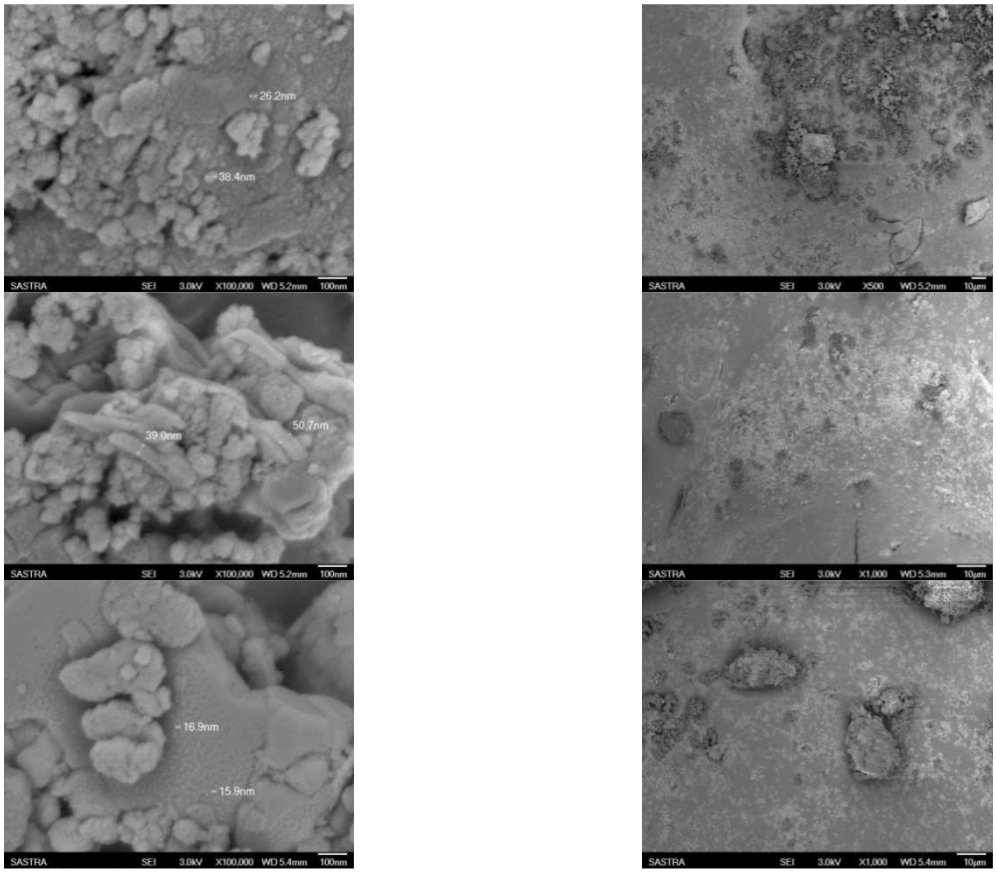
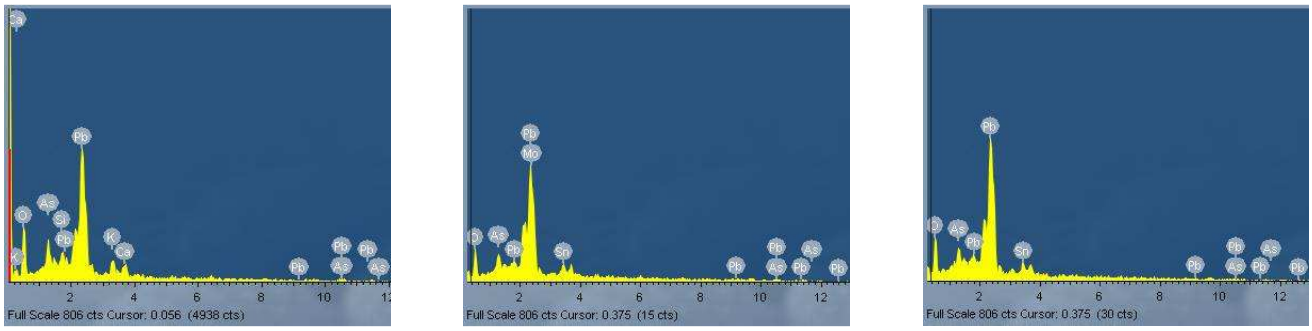


Fig. 4: Scanning electron micrographs of commercial *naga bhasma* samples



XB1 **XB2** **XB3**

Fig. 5: EDAX spectrum of commercial *naga bhasma* samples

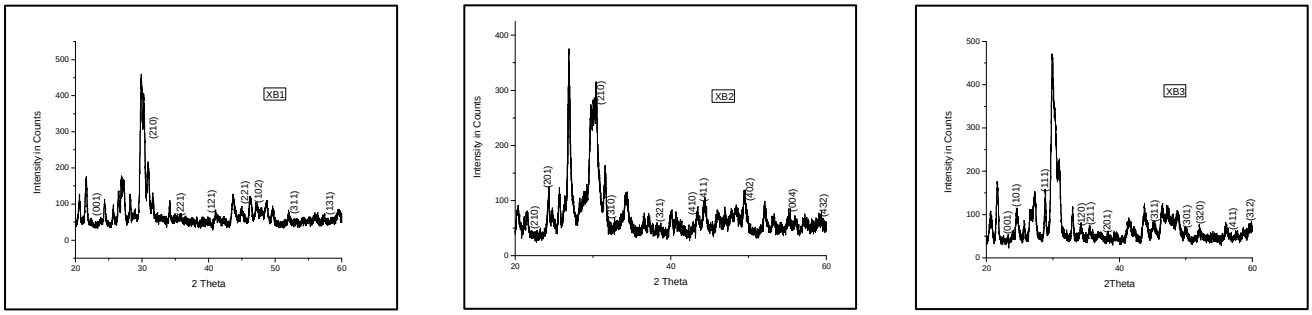


Fig. 6: XRD pattern of naga bhasma

CONCLUSION

The present study reveals the presence of batch to batch variation in *naga bhasma* samples prepared by the same manufacturer. This could be attributed to lack of gold standards for preparation of *bhasma*. The characterization of intermediates at every step of preparation and comparison with established benchmarks are required to address this issue. All the samples analyzed were found to be crystalline with Pb_2O_3 and Pb_2O_4 phases. Also all the samples were found to be containing co-ordination compounds.

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