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Detection of Heavy Metals In Bhasma (Traditional Medicines) By EDXRF Spectroscopy

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Abstract

Medicines are developed to maintain human health by preventing and treating various health related problems. India has six recognized system in this category; Sidha, Yoga,Unani, naturopathy & homeopathy. Ayurveda is one of the well-established systems of medicine in India and its popularity is also increasing among other countries. Therefore there is an increasing need to audit the quality of ayurvedic preparations like Bhasmas. In the present study Qualitative analysis of Bhasma (Traditional Medicines) has done by Color test and utilizing non destructive EDXRF spectroscopy. Color test (preliminary examination) were performed to check the presence of lead and arsenic in Bhasma samples whereas, EDXRF used for confirming the presence of lead and arsenic. Forensic toxicologist should be aware about the toxicity from consumption of ayurvedic preparations during viscera examination in cases of poisoning. Qualitative analysis has been done in the present study however, further quantitative analysis can also be performed to quantify the amount of heavy metals present in the samples.
Keywords: Ayurveda, Ayurvedic preparations, Bhasma, Toxicity, Lead toxicity, Arsenic toxicity, EDXRF, Rasa shastra.

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Introduction

Medicines are developed to maintain human health by preventing and treating various health related problems. Ayurveda involves the harmony of body with mind and is in use from our ancestral times .[1] In the traditional Indian medical system of Ayurveda, Bhasma which consists of two words *bha and sma* means *delusion and ever* respectively is a type of medicinal powder made through calcination of stones, gems, minerals or metals. The procedure of preparation of ayurvedic medicine comes under "Rasa Shastra" which includes different steps which includes detoxifying of precursors, crushing them with herbal juices, making small pieces, drying, and heating. Minerals, metals and gems are processed under various steps for preparation of bhasma and this procedure may lasts from almost seven days upto three years. The final product of long-term preparation methods is residual product of metals and minerals. Ayurveda claims that toxic contents of metals get removed by the process called as shodhana (process for reduction of toxic elements). Several types of bhasma are manufactured and used in Ayurveda each of them having a specific indications, properties and healing characteristics Some of the commonly used Bhasmas are: vanga bhsma,naga bhasma, shankh bhasma. [2] Dghaim and his co workers determined that the heavy metals in herbal medicines has been used in the United Arab Emirates (UAE) Seven herbs, total 81 samples from local market of Dubai were collected and analyzed for the presence of Cd, Pb, Cu, Fe, Zn. For dissolution of sample microwave-assisted digestion was used. Examination of concentration of heavy metals was done using Atomic Absorption Spectroscopy (AAS). Concentration of metals varies in different herb samples. It was analyzed that unsafe levels of metals were present in herbs i.e. the concentration of metals exceeded the permissible limits of World Health Organization. [3] Now, a day's herbal medicines consisting bhasma are manufactured on large scales, due to which manufacturers have various issues such as availability of better-quality raw material, standards, proper standardization methodology of single drugs formulation, etc. and this poor standardization leads to toxic effects due to their consumption.[4] Bhasmas which were made up of different metals gets accumulated in the body because their rate of excretion is less than that of the rate of administration. Some of these traditional remedies may also contain toxic amount of lead and other heavy metals like mercury and arsenic.[5]

Accumulation of toxic metals occurs because during the preparation of bhasma various herbal

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plants are used along with minerals, some toxic metals were absorbed by the plants from the environment as various industrial waste are dumped into water from where these toxic substances reach upto the earth surface or soil and finally taken up by the plants from soil and becomes the source of toxicity this cycle repeats every time when industrial wastes get dumped into the water.

Lead toxicity one of the Heavy metal toxicities is a multisystem disease which effects on the human body. Long term exposure of lead will lead to decreased performance that measure functions of the nervous system anemia, Along with an increase in blood pressure, severe damage to the brain and kidneys and ultimately it leads to death.[6]. Lead affects the action of enzymes required for synthesis of hemoglobin and cytochrome production and interacts with sulfhydryl groups. Person affected with lead poisoning shows various signs and symptoms such as; dry throat, thirst, burning abdominal pain, anaemia, headache, insomnia, paraesthesia, coma and death may occur. Lead poisoning is treated by gastric lavage with one percent solution of sodium or magnesium sulphate. Ayurvedic medicines are also known to contain traces of arsenic which reaches inside the body by means of oral, inhalation or parenteral administration. As the arsenic enters the human body it interferes with the glycolysis and causes toxicity by uncoupling mitochondrial oxidative phosphorylation. But it has been observed that arsenic poisoning is much less common than lead poisoning.[7] There were many cases where death takes place mainly due to consumption of Bhasma. So, the present study has been focused on Qualitative analysis of Bhasma for analyzing presence of toxic metals like lead and arsenic in bhasma.[8]

Material and Method

Sample collection: Five random samples of different bhasma (vanga, naga, tamra, sip and shankh bhasma) were collected from (Hamirpur) Himachal Pradesh and (Phagwara and Jalandhar) of Punjab region.

Sample Analysis: samples were qualitatively analyzed for the presence of lead and arsenic by performing color tests (preliminary examination) and EDXRF spectroscopy was used for confirmation of lead and arsenic in the samples.

Sample preparation for color tests: 0.5gm of bhasma powder was taken in a test tube and 1-

2ml of distilled water was added.

Color test to check the presence of lead

Test 1: Sample (Bhasma) after the addition of sodium hydroxide results in white precipitate, which get dissolved in an excessive sodium hydroxide and yields black ppt. when sodium sulphide was further added into the solution.

Test 2: Sample was taken in a test tube and treated with acetic acid and potassium chromate which results in formation of yellow precipitates. These yellow precipitates were not dissolved in ammonia but get dissolved when sodium hydroxide was further added.

Color test to check the presence of arsenic

Test 3: sample was taken in a test tube and silver nitrate was added white precipitates were appeared, addition of ammonia has been done to one portion of suspension and nitric acid to another portion, it was observed that solution got dissolved in either one of the reagents.

Test 4: few drops of sample taken in a test tube and Copper (II) sulphate was added to the sample solution which results in formation of green color precipitates. Precipitates were then separated and heated after addition of sodium hydroxide.

Sample preparation for EDXRF spectroscopic analysis: sample holder was covered with Mylar sheet and then bhasma powder was added on to the sample holder for analysis.

Samples were analyzed using instrument Shimadzu Energy dispersive x-ray fluorescence spectrometers manufactured in Japan having specifications as model no. EDX 7000 and serial no. Q24545500882 which was Nitrogen free and x-ray tube was used as an excitation source and a solid-state detector to number and in concentrations ranging from a few parts per million to 100 percent. The instrument contains the x-ray generating elements, sample chamber, detector, and detector electronics, embedded microprocessor controller and associated power supplies. Instrument was calibrated to ensure for proper working procedures. Sample was loaded into the sample holder covered with the Mylar sheet and were kept under the XRF for scanning about 5-7 minutes The personal computer (PC) having PCEDX navy software and includes a second Ethernet Interface board and other standard PC elements was connected with dedicated Ethernet cable for transfer of spectral data as a result.

Result and discussion:

In the present study, 5 samples of different bhasma were qualitatively analysed and it was found that color test showed the positive presence of lead in three samples (naga, vanga, and tamra) howeverno sample shows the positive presence of arsenic. Further analysis for confirmation of lead and arsenic in bhasma was performed by utilizing EDXRF.

It was found that lead was present in three samples (naga, vanga, tamra) whereas presence of arsenic was not reported in any of the sample.

Naga Bhasma (Sample -1): Test 1, in which when sample was treated with sodium hydroxide (dil.) brown precipitates appears which turns black in color after adding sodium sulphide (dil). so, Appearance of black color indicates the positive presence of toxic element lead in the sample as shown in figure 1.

Positive presence of the lead was also shown by color test 2 in which acetic acid was added to the sample solution which turns to the brown color appears and on the addition of potassium chromate brown color changed to the yellow precipitates which were not dissolved in ammonia solution figure 2.

When Naga bhasma which is made up of lead as the main constituent was analyzed using EDXRF as confirmatory test strongest peak identified was of lead (Graph 1) which lies between 10.020.0 KeV, along with lead different elements present were: Calcium, Chlorine, Antimony, Iron, copper. In the previous studies of naga bhasma the strongest peak identified using X-ray diffraction was of Lead Germanium oxide & other peaks were of Rubidium, Thallium, Lead Phosphate. Various characteristics peaks were identified which corresponds to the electron configuration of electrons with in the atoms. It was analyzed with the help of ICP-AES that there is percentage of lead decreases as processing of bhasma proceeds raw sample (99.4%) and end product (58.4%). Size of naga bhasma was identified using Scanning electron microscope (ranges 1mm to 20mm) [9]

Vanga Bhasma (Sample-2): Test 1, in which when sample solution in a test tube was treated with

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dilute sodium hydroxide white precipitates appears these white precipitates turns black on addition of dilute sodium sulphide. Black color appeared due to the presence of lead in sample

solution. After 2-3 minutes pink color appeared on the upper portion of the solution. Black color indicates the positive presence of lead in the sample as depicted in figure 3.

Vanga bhasma showed positive presence for test 2 in which addition of acetic acid and potassium chromate turns the sample solution orange yellow color shown in figure 4.

Analysis of vanga bhasma using EDXRF which is an organometallic compound consist of tin oxide as an important constituent showed the lead peaks (Graph 2) between 10.00-12.0KeV. along with the peaks of other elements such as zinc, silicon, calcium, manganese, copper at different ranges. Earlier, in a study in which vanga bhasma was chemically analyzed, tin was found in the form of tin oxide at percentage level of 80%. Seventeen other elements were found when it was detected using X-ray fluorescence. Results of ICP-Ms showed the presence of Calcium, Silicon, Iron, Phosphorus, Aluminum and chlorine.[10]

Shankh Bhasma (Sample-3): Test 1, in which few drops sample treated with the sodium hydroxide solution formation of two layers takes place no white precipitates were present and after addition of dilute sodium sulphide solution turns white. Absence of black color in figure 5 indicates that lead was not present in the sample.

In Test 2, Appearance of Dark orange color on addition of acetic acid and potassium chromate shown in figure 6 showed the absence of lead whereas, after EDXRF analysis Shankh bhasma showed peak of calcium after analyzed by x ray fluorescence spectroscopy, other elements like iron, strontium, Sulphur, copper and nickel were analyzed. Peak of calcium lies between 0 to 12 KeV Lead was present in trace amount had small peaks within the range of 10- 20 KeV (graph 3).

XRD analysis of Shankh bhasma showed the presence of rhombohedral crystal form with 33 total peaks observed, almost similar major two peak values 29.401, 48.507 were noticed in the two samples of Shankh bhasma. The strong peaks were identified as $CaCO_3$ (calcium carbonate) as calcite form. Whereas, ICP analysis showed the presence of iron, potassium, sodium,

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magnesium, manganese, silica and phosphorus in trace amounts in almost all the samples.[11]

Sip Bhasma (Sample -4): Test 1, showed the absence of lead because on addition of sodium hydroxide(dilute) into sample solution no white precipitates were formed and black color was absent on addition of sodium sulphide in sample as shown in figure 7. In test 2, Yellow precipitate were absent as acetic acid is added to the sample solution and orange color appears on addition of potassium chromate shown in figure 8 that lead was not present in the sample.

Sip Bhasma showed 17 peaks and had strongest peak of calcium with various peaks representing different elements lead, iron, strontium, copper, nickel, zinc when sample of sip bhasma was analyzed using X ray fluorescence (graph 4). No work has been reported in literature on the analysis of sip bhasma.

Tamra Bhasma (Sample-5): due to black physical appearance (figure 9) of sample it was not possible to identify lead by performing color Test 1 for presence of lead.

Test 2, On the addition of acetic acid in sample solution black color appears on addition of potassium chromate this black color turns into yellow precipitates which indicates positive presence of lead as shown in figure 10.

Tamra bhasma (incinerated copper) when analyzed the peak of lead (graph 5) was found between 12-20 KeV, peaks of other elements were also present between 0-10 KeV these elements were iron, calcium, copper, manganese, barium. Tamra bhasma was analyzed at different levels that is raw, shodhita and somanathi tamra bhasma using Inductively Coupled Plasma, SEM and XRD. In raw tamra copper was more than in the somanathi bhasma. SEM was used to analyze the size of tamra bhasma which was more than 5.6 µm results of XRD shows different elements at different peaks and most of the peaks of Somanathi tamra bhasma resemble the Copper sulphide and copper oxide (CuS, CuO). [12]

In the present study, none of the samples shows positive color and confirmatory test for the presence of arsenic (Figure 11-20).

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Conclusion:

It can be concluded from the present study that color test and EDXRF serves as efficient techniques for preliminary and confirmatory analysis respectively for analysis of Bhasma. EDXRF is a non-destructive, sensitive and time effective technique and requires minimal sample preparation. This study will helpful for a forensic toxicologist for various toxicological interpretations. However in present study focus was on qualitative analysis of Bhasma but it should be extended towards quantitative analysis also.

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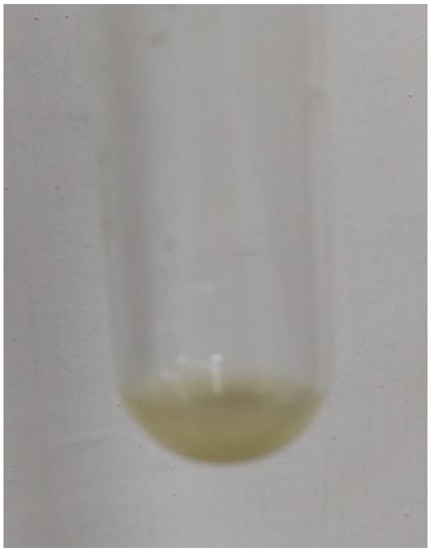

Fig.1 Presence of lead in Naga Bhasma (Sample1)

Fig.2 Presence of lead in Naga Bhasma (Sample1)

Fig.3 Presence of lead in Vanga Bhasma Bhasma (Sample2)

Fig.4Presence of lead in Vanga (Sample2)

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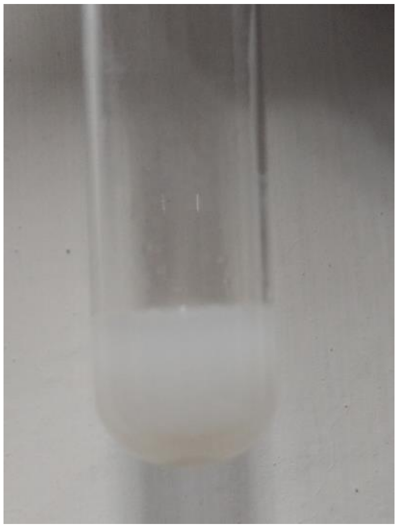

Fig.5 Absence of lead in Shankha Bhasma Bhasma (Sample3)

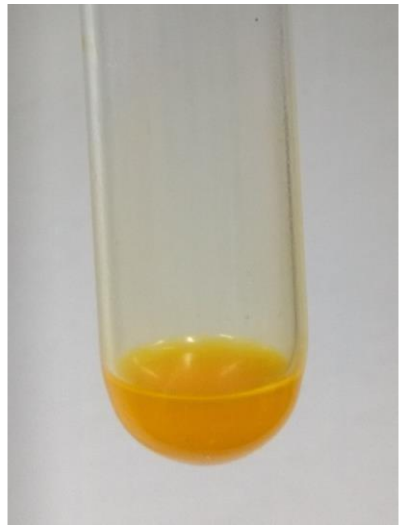

Fig.6 Absence of lead in Shankha (Sample3)

Fig.7 Absence of lead in Sip Bhasma Bhasma (Sample4)

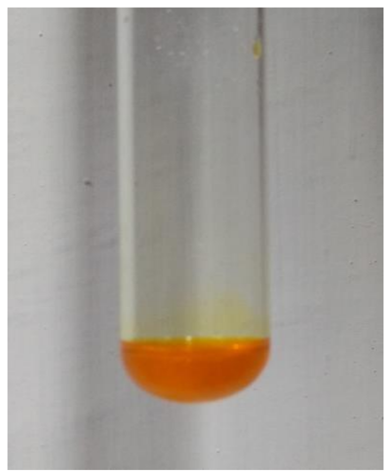

Fig.8 Absence of lead in Sip (Sample5)

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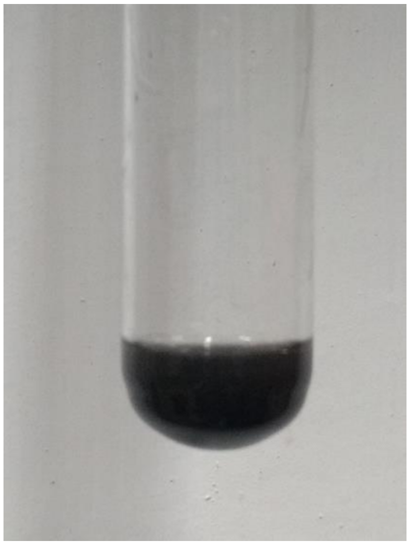

Fig.9 Presence of lead cannot be Tamra Bhasma Bhasma due to its black appearance in nature (Sample5)

Fig 10. Presence of lead in Tamra (Sample5)

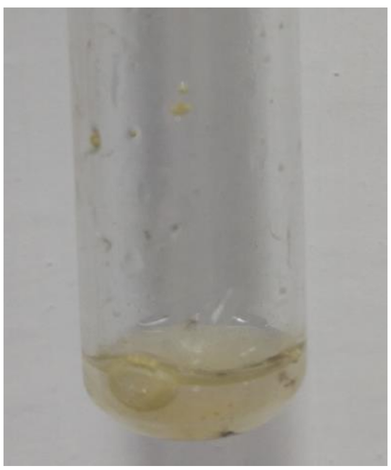

Fig11. Absence of Arsenic in Naga Bhasma Bhasma (Sample1)

Fig12. Absence of Arsenic in Naga (Sample 1)

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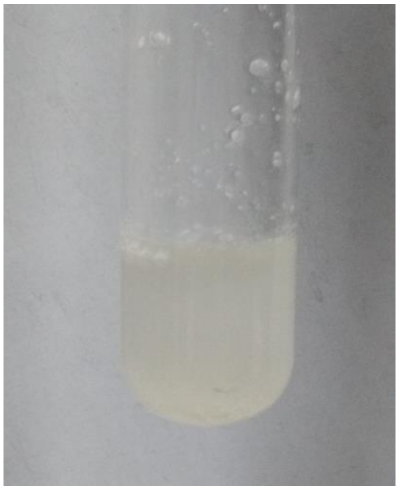

Fig.13 Absence of Arsenic in Vanga Bhasma Bhasma (Sample2)

Fig14. Absence of Arsenic in Vanga (Sample2)

Fig.15 Absence of Arsenic in Shanka Bhasma Bhasma (Sample3)

Fig.16 Absence of Arsenic in Shankha (Sample3)

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Fig17. Absence of Arsenic In Sip Bhasma Bhasma (Sample4)

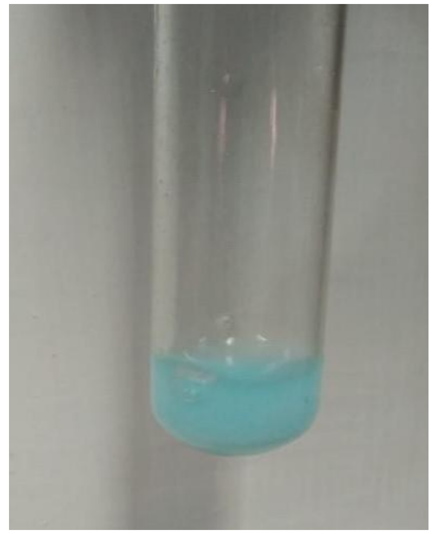

Fig.18 Absence of Arsenic in Sip (Sample4)

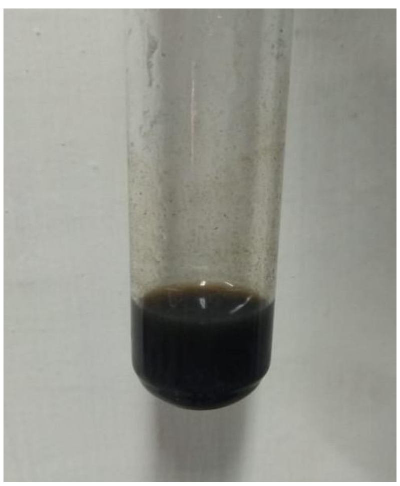

Fig.19 Absence of Arsenic in Tamra Bhasma Bhasma (Sample5)

Fig.20 Absence of Arsenic in Tamra (Sample5)

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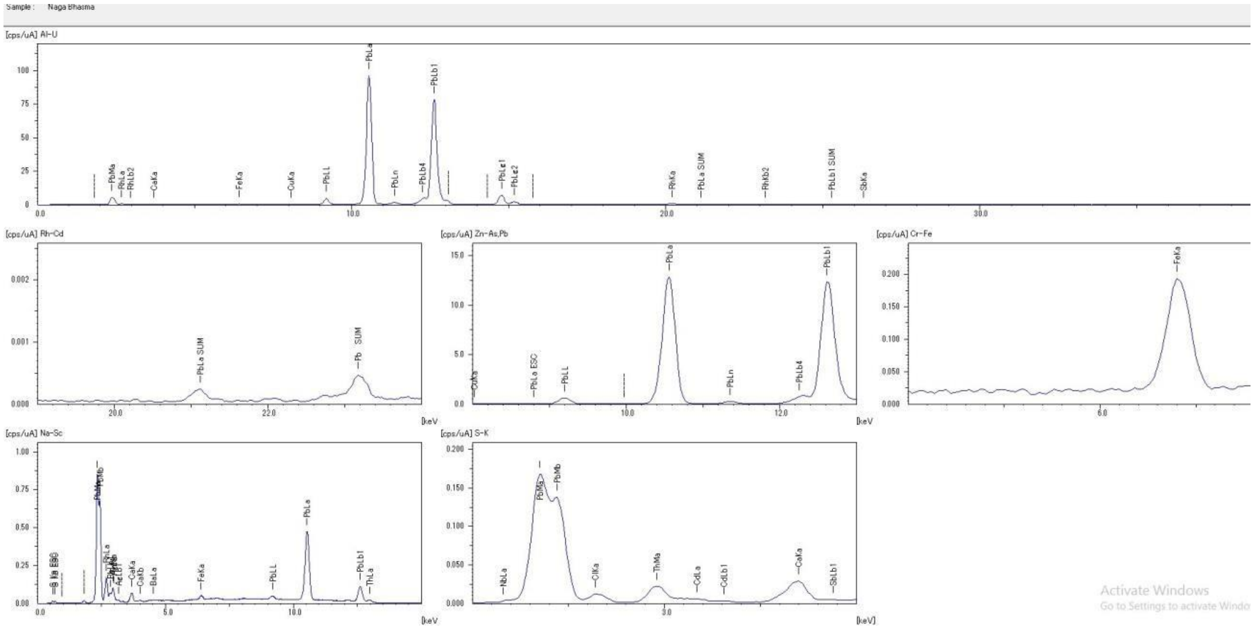
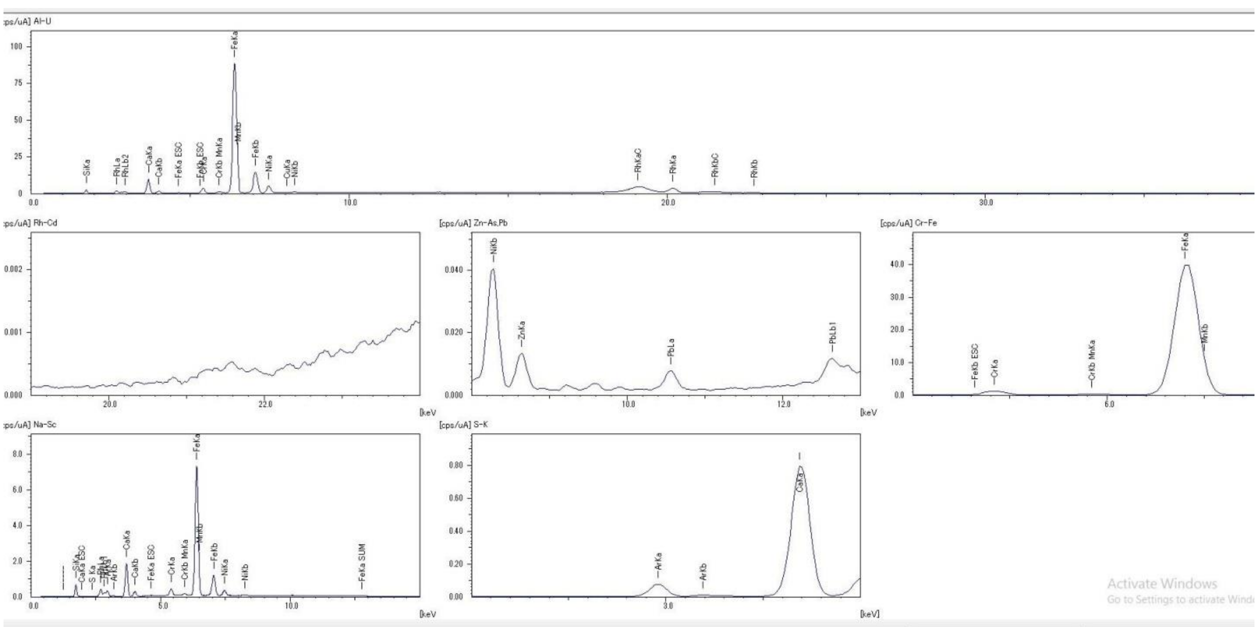


Graph1:EDXRF Spectrum of Naga Bhasma (Sample 1)



Graph 2: EDXRF Spectrum Of Vanga Bhasma (Sample2)

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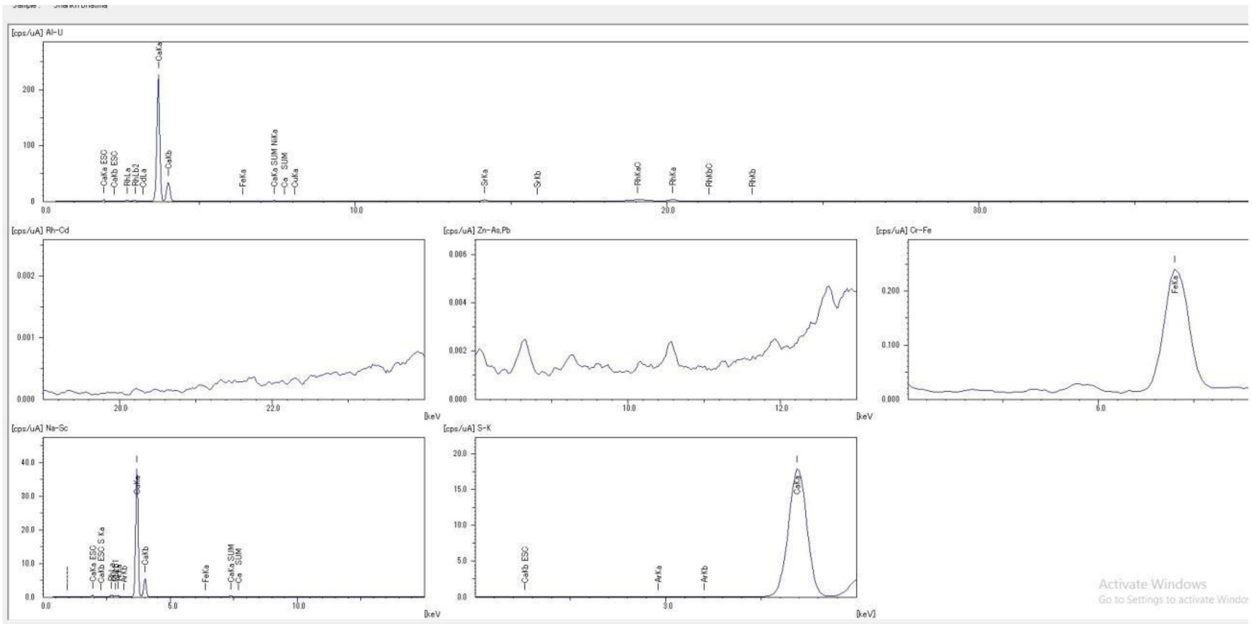
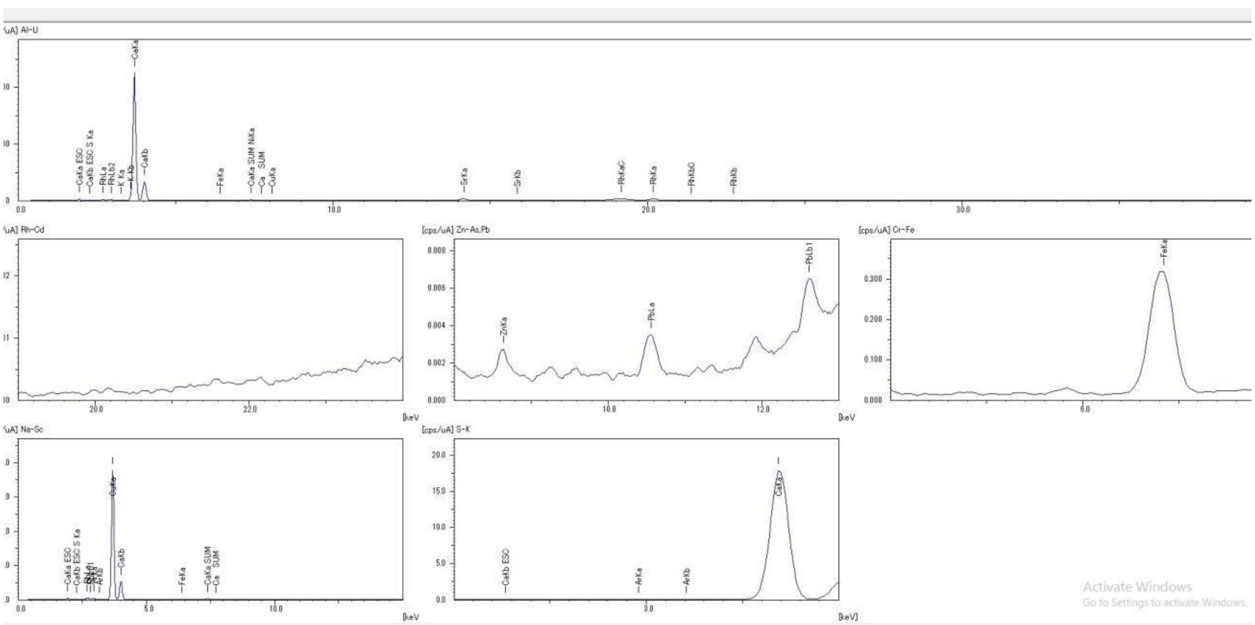
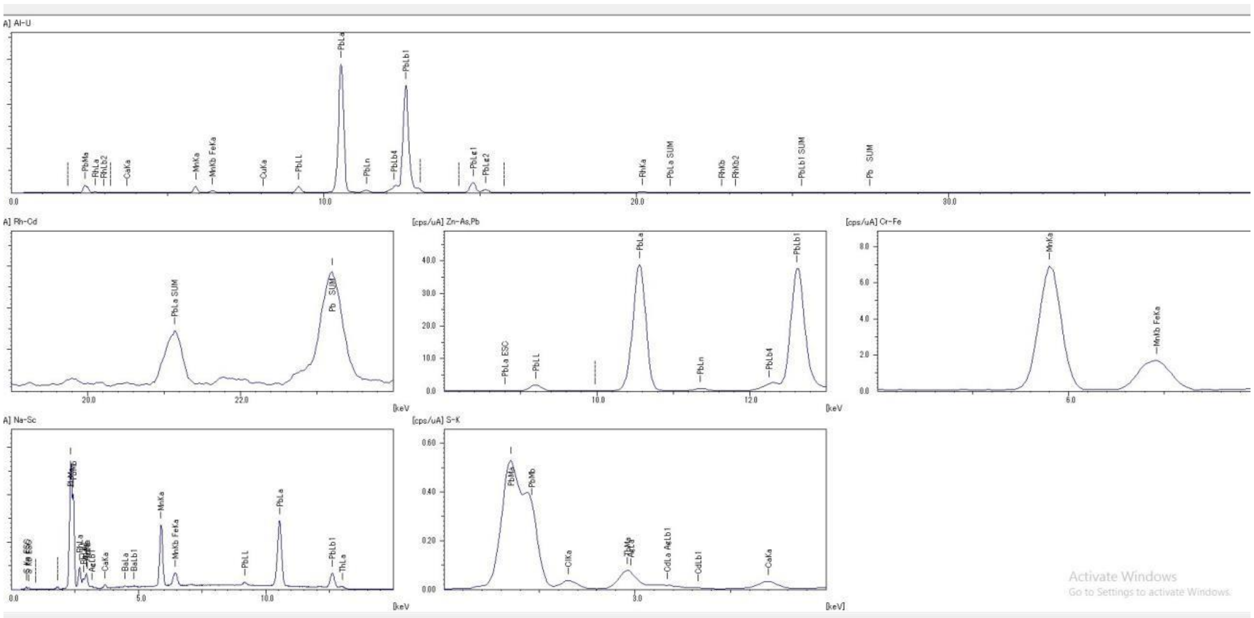


Graph 3: EDXRF Spectrum of Shankha Bhasma (Sample3)



Graph 4: EDXRF Spectrum of Sip Bhasma (Sample4)



Graph 5: EDXRF Spectrum of Tamra Bhasma (Sample 5)