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RESEARCH ARTICLE

ESTIMATION OF METALS LIKE Zn, Ni, Cd, AND Fe FROM "TOBACCO" (1) AND "PANMASALA" (2) BY SPECTRAL ANALYSIS: A REVIEW

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AAS, HPLC,
Digestion tube Air Condenser.

ABSTRACT

In the present article an attempt has been made to analyze large scale production of tobacco mixed gutkha and panmasala by several spectroscopic techniques. In the Asian countries like India the materials used by the peoples at the extreme level as their regular diet. Due to their regular consumption and addict numerous disadvantage has been come out in the light. Number of research article flaunting that in the materials like tobacco and panmasala (Raj Shrestha Ashwini Kumar *et al.*, 2012) such as rajngandha etc. trace amount of heavy metals and transition metals are present, presence of these metals has adverse effect on human health. This article summarizes recent efforts to estimate and amount t hazardous substances present in these materials (1, 2). In primary investigation the metals present in tobacco and panmasala (1, 2) were quantitatively estimated, then the metals were characterized by AAS. Some organic substances are estimated by using HPLC. Tobacco is a plant within the genus *Nicotiana* of the Solanaceae (nightshade) family. Harmful effects of tobacco consumption can derive from the thousands of different chemicals in the smoke, including cadmium nickel, arsenic, iron, zinc many other transition and heavy metals. The heavy metals were estimated by using AAS from both samples (1,2). HPLC data used for the characterization of samples and indicates presence of organic hazardous chemicals including polycyclic aromatic hydrocarbons (such as benzopyrene), formaldehyde, tobacco-specific nitrosamines (TSNAs), phenols, and many others (Supadminevi *et al.*, 2012). Tobacco also contains beta-Caroline alkaloids.

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INTRODUCTION

Rajnigandha is the world's largest selling Premium Panmasala brand and has redefined the Panmasala industry by selling benchmarks for other to follow. This non-tobacco panmasala is the rich blend of selected Ingredients like nut, catechu, lime, cardmomseeds, menthol, sandalwood and added flavours. In India Pan chewing has been an age old habit. Company that produces chewable Tobacco Products called Gutkha. Today, ManikchandGutka is the leading producer of gutkha in india.Gutkha has always been a controversial business but, now the man behind one of India's more famous gutkhabrands; manikchand seems to have bitten of more than he can chew. Investigators have been revealed that heavy metals are present in tobacco smoke and have long been associated with various diseases. Inhalation of tobacco and panmasala transports heavy metals in mainstream smoke trough the oral cavity to the lungs.

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From the lungs the heavy metals are transferred to the peripheral circulation and other body organs along with other smoke constituents including addictive nicotine. Elevated cadmium levels in lung, liver and kidney tissues, body fat, blood and urine, have been correlated with smoking history. Tobacco [*Nicotina tabacum* L] is an industrial plant which has the ability to accumulate metals. The accumulation of heavy metals in the tobacco plant is a consequence of a complex interaction between the soil, plant and animal environment. The fertilizers and pesticides which are used during the production of tobacco contain high concentrations of metals and represent primary factors in the pollution of agricultural soil, as well as plants. The research of some authors has shown that tobacco has greater tendency towards the absorption of lead and cadmium in relation to other heavy metals. The mobility of cadmium trough the plant in the comparison to lead is greater, so that the greatest amounts of cadmium accumulated in the leaves, then the root and lastly in the stem of the plant. Tobacco smoking is a worldwide problem with 1.3billion people currently smoking cigarettes and one person losing life every 6second due to tobacco related illness.

Heavy metals are present in tobacco smoke and have long been associated with various diseases. Inhalation transports heavy metals in mainstream smoke through the oral cavity to the lungs. From the lungs the heavy metals are transferred to the peripheral circulation and other body organs along with other smoke constituents including addictive nicotine. Elevated cadmium levels in lung, liver and kidney tissues, body fat, blood and urine, have been correlated with smoking history. Elevated lead levels in the blood and amniotic fluid and in the cord blood of newborn babies have also been associated with smoking. In addition to all these toxic heavy metals in the tobacco leaves, high contents of zinc can also be detected. Studies have shown that the increased intake of zinc into the body can lead to a deficiency of copper in the liver, the serum and the heart, and the decrease of the activity of copper metalloenzymes. In addition, the increased intake of zinc into the body can have a detrimental effect on the storage of iron and can lead to the occurrence of anemia.

The type and number of chemical constituents varies in its different formulations. Thousands of different chemicals have been detected in tobacco smoke. 60-70% of them are proven carcinogens, the only chemical which causes addiction in tobacco products is nicotine, however the tobacco plant is well known to absorb trace elements from the soil and to accumulate them in its leaves on a large scale. Quantity of these trace elements in tobacco depends upon genotype, type of water, soil and their respective pH, stalk position, fertilizers, pesticides applied. Betel leaf is perishable and preparation of BQ is somewhat complex or requires visits to shops selling *pan/BQ*. With the emergence of commercial *pan masala* and *gutkha* about three decades ago, not only did the Indian market witness massive growth in the sales of smokeless tobacco and areca nut products, but also a huge worldwide export market developed. In the USA, Europe, the Middle East, Australia and many other countries. *Panmasala* is basically a preparation of areca nut, catechu, cardamom, lime and a number of natural and artificial perfuming and flavoring materials (Daniel N. Willis *et al.*, 2014; Rajesh Kumar and Yadav, 2014; Gupta Bhawna, 2013; Yadav and Chadha, 2002; Aruna and Rajesh Vikrant Ranjan Mohanty, 2010; Dibyendu Bhowmick and Chandradipa Ghosh, 2013).

MATERIALS AND METHODS

All the materials used in this study are A.R. grade supplied by S.D. Fine Chemicals Mumbai. Materials were used in commercial purity without further purification.

The method describes the identification of metals in tobacco and panmasala² brand by quantitative analysis of the materials.

Procedure and experimental work

Preparation of solution of tobacco

Take 10 mg of tobacco sample in a test tube and add to it about half test tube of water and boil it. The sample is sparingly soluble in water then filter it, collect the filtrate and residue and analyze them separately to find the trace of metals like Fe, Ni, Zn, Cd etc.

Identification of zinc in tobacco

Zinc is identified by characteristic white ppt. Take about 2 ml of tobacco solution in test tube. Add excess of NH_4Cl solution. Add NH_4OH solution. Hydroxide insoluble in water in presence of NH_4Cl , white ppt appears.



Zinc



Cadmium

Identification of Cadmium in tobacco

Cadmium is identified by the characteristic yellow precipitate of CdS .

Take about 2 ml tobacco solution in test tube. Add few drops of Dil. HCL solution. Heat the solution and pass H_2S gas. Light yellow ppt appears.

Identification of Nickel in tobacco

Take about 2 ml tobacco solution in test tube, add excess of NH_4Cl solution, add the NH_4OH solution and pass the H_2S gas. Black precipitate appeared.



Nickel



Iron

Identification of Iron in tobacco

Iron identified by the characteristics red ppt. of $Fe(OH)_3$. Take about 2 ml tobacco solution in test tube. Add excess of NH_4Cl solution Add the NH_4OH solution red precipitate appeared.

ANALYSIS OF METALS THROUGH ATOMIC ABSORPTION SPECTROSCOPY

This spectroscopic technique is suitable for estimation of heavy metals like Cd, As etc. and traces of transition metals like Zn, Fe, Ni etc. from tobacco sample.

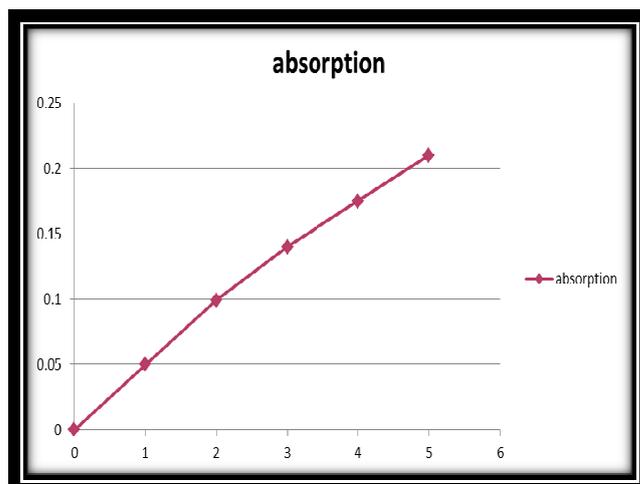
Sample preparation for AAS

Weight about 0.5 gm of Tobacco sample approximately. Transferred this whole sample in a round bottom flask, add 10 ml of deionised water then add 15 ml concentrated HNO_3 and 0.5 ml solution of conc. H_2SO_4 add and kept it for overnight at room temperature. Next day, sample dried in oven until it becomes black and cherry coloured, after getting cherry- red colour, remove from oven add perchloric acid and nitric acid in

2:1 ratio(20 ml perchloric acid and 10 ml of nitric acid),add this solution until they becomes yellow. After getting yellow color, take 10 ml of sample for analysis of atomic absorption spectroscopy (Raj Shrestha Ashwini Kumar *et al.*, 2012; Supadminidevi *et al.*, 2012; Bhis, 2000; Nair *et al.*, 2012; Daniel *et al.*, 2014) .

Detection of Iron in Tobacco Byatomic Absorption Spectroscopy

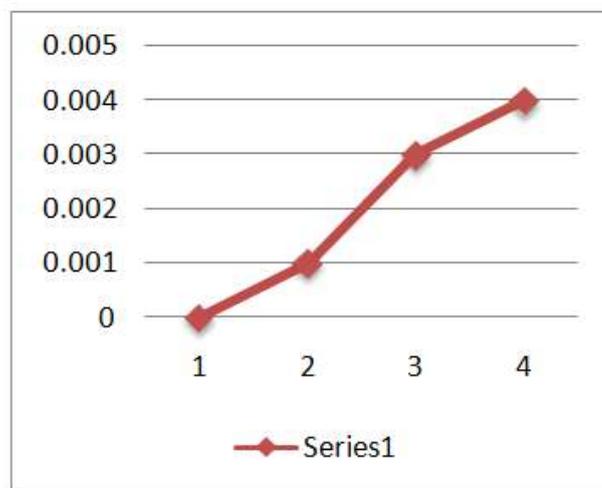
Concentration of tobacco sample in ppm	Absorbance
1.0 ppm	0.050
2.0 ppm	0.099
3.0 ppm	0.140
4.0 ppm	0.175
5.0 ppm	0.210



Graph of Concentration Vs Absorbance of Iron

Detection of Zinc in Tobacco by Atomic Absorption Spectroscopy

Concentration of tobacco sample in ppm	Absorbance
1.0 ppm	0.01
2.0 ppm	0.012
3.0 ppm	0.034
4.0 ppm	0.049

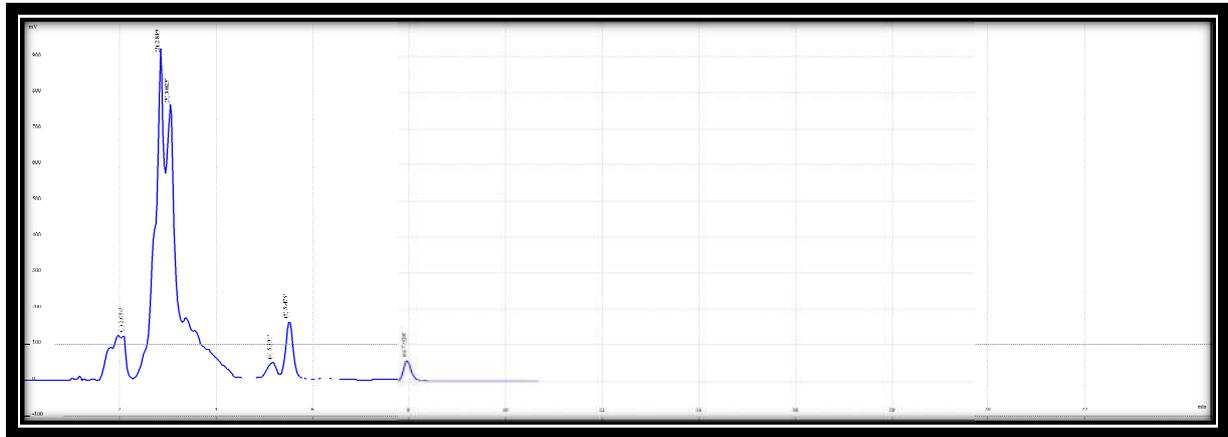


Graph of Absorption vs Concentration for Zinc

HPLC SPECTRA OF MANIKCHAND PANMASALA

Sample Name: Manikchand Panmasala

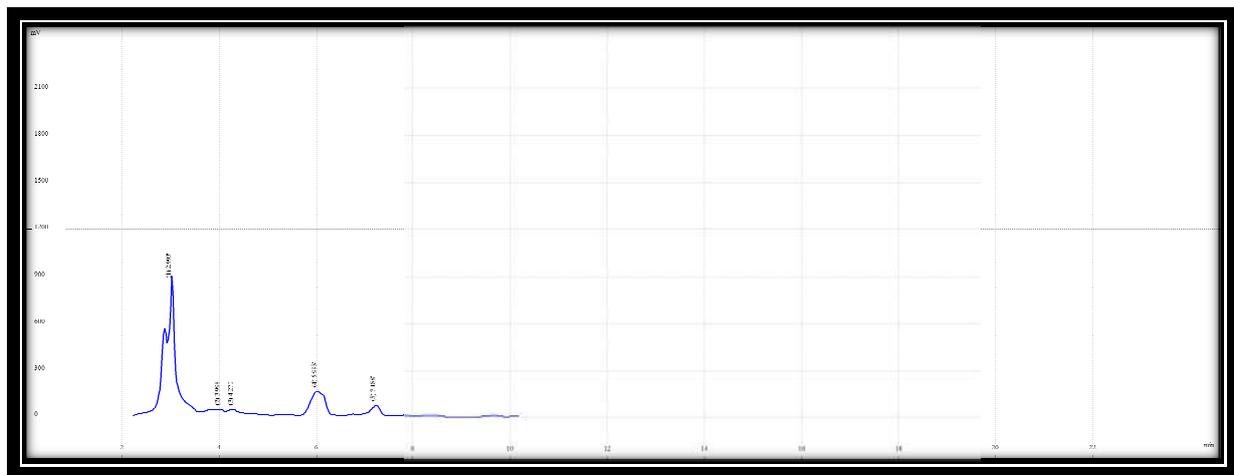
Wavelength: 254nm
 Mobile Phase: Acetonitrile:
 Water (70:30)
 Sample volume: 20µl
 Flow rate: 0.8ml/min
 Pressure : 9- 10MPa



Rank	Time	Conc.	Area	Resolution	T plate
01	2.054	3.0596	794857	3.04	2576
02	2.819	46.196	12001285	0.61	1143
03	3.023	36.450	9469250	0.98	1436
04	5.131	3.3495	870171	0.86	2086
05	5.475	8.1558	2118773	7.28	4283
06	7.928	2.7889	724528	0.00	9307

HPLC SPECTRA OF TOBACCO

Sample Name: TOBACCO
 Wavelength: 254nm
 Mobile Phase: Acetonitrile: Water (70:30)
 Sample volume: 20µl
 Flow rate: 0.8ml/min
 Pressure: 9-10MPa



Rank	Time	Conc.	Area	Resolution	T plate
01	2.993	66.6764	14386136	1.72	8732
02	3.998	6.1152	1319415	0.36	461
03	4.270	5.8562	1263527	2.61	544
04	5.993	16.0100	3454320	2.37	1790
05	7.188	5.3422	1125631	0.00	4571

Results for metals Fe,Zn by AAS

S.N.	Elements	Units	Result
1	Iron	mg/l	0.50 ppm
2	Zinc	mg/l	0.49 ppm

High performance liquid chromatography results analysis

Determination of contents in tobacco and panmasala by HPLC using Acetonitrile Take 1 gm tobacco and manikchand panmasala in separate RB add to this sample 50 ml acetonitrile. Use this dissolved sample for HPLC analysis.

RESULTS AND DISCUSSION

- The quantitative determination reveals that presence of metals in tobacco as well a panmasala .metals present were Fe, Ni,Zn,Cd.etc.
- By AAS technique the % of metals determined. (Fe,Ni,Zn,Cd)
- HPLC gives spectral data for the precence of several organic content like gallic acid etc.(peak 4 in the second graph)
- According to servey adverse effect have been found of chewing tobacco and panmasala (Aruna *et al.*, 2010; Dibyendu Bhowmick and Chandradipa Ghosh, 2013 and Devi and Rotti, 2012)

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