Determination of toxic metals in raw and processed tobacco leaves samples by using Atomic Absorption Spectroscopy

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Abstract

Heavy metals, including Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Lead (Pb), and Cobalt (Co), are of significant concern in tobacco leaves and tobacco products due to their potential health hazards upon consumption. In India, tobacco is a fundamental agricultural commodity with significant social and economic impact. However, there is no information in the literature on how much heavy metal is present in tobacco leaves from India. This study focuses on the analysis of these heavy metals in tobacco leaves and various tobacco products to assess their concentrations and potential health risks associated with tobacco use. Samples are prepared using appropriate digestion methods to extract heavy metals from the matrix, followed by quantification through calibration with certified reference materials followed by Atomic Absorption spectrometry. The range of metal recoveries in the samples of spiked tobacco was 73 -102 % and RSD is below 15%. The result shows that comparatively higher concentration of heavy metals in tobacco samples collected from Kanpur market.

The findings contribute to understanding the sources and distribution of heavy metals in tobacco products, as well as their potential health implications for consumers.

Key words: Tobacco leaves, Heavy metals, AAS

1. Introduction

In India, tobacco is a fundamental agricultural commodity with significant social and economic impact.states like Andhra Pradesh, Gujarat, Karnataka, and Maharashtra being prominent tobacco-growing regions[1]. India produces both flue-cured and non-flue-cured tobaccos.Over 750 million kilogrammes of tobacco leaf are produced annually from the tobacco crop grown on 0.45 million hectares (0.27% of the net cultivated area) in India, which is the world's second-largest producer and exporter of tobacco leaf, after Brazil and China. About 300 million kilogrammes of flue-cured Virginia (FCV) tobacco are produced from 0.20 million hectares of land, while 450 million kilogrammes of non-FCV tobacco are produced from 0.25 million hectares[2]. The United States produces a significant amount of

International Journal of Architecture, Engineering and Construction ISSN (online) 1911-1118
Vol 12, No 2, June 2023 (UGC CARE 1) ISSN (print): 1911-110X

tobacco, mainly in states like North Carolina, Kentucky, and Virginia.In 2023, just about 432.45 million pounds of tobacco were produced in the United States. It cultivates various types of tobacco, including flue-cured, Burley, and dark-fired tobaccos[3].Native tobacco, has long been used for pipe smoking, chewing, and snuffing[4]. State-owned farms and farmers in the area around them both cultivate tobacco for commercial use. Tobacco production and processing throughout the nation are to be organised under the National Tobacco Enterprise[5, 6]. Chewing tobacco leaves and making cigarettes both require tobacco leaves. In instance, cadmium and other heavy metals naturally concentrate and accumulate at rather high quantities in leaves[7]. Tobacco leaves, widely known for their use in cigarette production, contain various chemical compounds, including toxic metals such as cadmium, lead, and nickel. These metals can accumulate in the leaves due to factors such as environmental pollution, agricultural practices, and processing techniques. The presence of these toxic metals poses serious health risks to consumers, as they can be absorbed by the body through smoking and lead to various adverse health effects, including respiratory issues, cardiovascular diseases, and cancer.

Both plants and people do not require cadmium. It accumulates in tobacco plants and is extremely poisonous. Smoking cigarettes causes the human body to absorb cadmium. The majority of heavy metals seriously harm people's health[8, 9]. Lead and cadmium are linked to brain illnesses and bone and renal ailments, respectively. Overexposure to Cu and Zn is linked to metabolic problems that can be fatal. Tobacco smoke is one of the primary sources of hazardous metals in our environment[10].

The amount of heavy metal build up in tobacco plants is the consequence of intricate interactions between the soil and plant. The kind of soil, pH level, water quality utilised for irrigation, chemical makeup of the metals, and type of tobacco plant all affect how much metal is accumulated in the soil[11]. Large quantities of fertilisers and insecticides are used by the growers to grow the tobacco plant. Fertilisers and pesticides frequently have high metal concentrations and have a significant role in polluting both plants and agricultural soil.The distribution and build-up of metals in tobacco leaves are a reflection of the soil and environment where the plant is grown in terms of mineral content[12, 13]. As a result, the actual metal content of tobacco varies greatly depending on its geographic origin, the usage of fertilisers with various chemical compositions, and other distinguishing characteristics like irrigation water. Heavy metal concentrations are significant in the phosphate fertilisers used in the growing of tobacco.

The accurate determination of toxic metals in tobacco leaves is crucial for regulatory compliance, quality control, and consumer safety. Atomic Absorption Spectroscopy (AAS) is a widely used analytical technique for quantifying trace metal concentrations in various matrices, including biological samples, environmental samples, and agricultural products[14]. AAS offers high sensitivity, selectivity, and precision, making it ideal for the analysis of toxic metals in tobacco leaves.the concentrations of cadmium, lead, arsenic, and nickel in both raw and processed tobacco leaves samples using AAS[15]. Raw tobacco leaves represent the untreated form of the plant, while processed tobacco leaves undergo various manufacturing processes such as drying, curing, and fermentation.Sample preparation techniques such as acid digestion or microwave digestion are employed to solubilize the metals from the matrix and enhance their detection by AAS. Calibration standards are prepared from certified reference materials to quantify the concentrations of toxic metals in the samples accurately. The determination of toxic metals in tobacco leaves is essential for ensuring product safety and protecting public health. By utilizing AAS, accurate and reliable data can be obtained on metal concentrations in raw and processed tobacco leaves, thereby supporting efforts to mitigate health risks associated with tobacco consumption.

The study aims to compare the levels of toxic metals between raw and processed tobacco leaves to assess the impact of processing techniques on metal accumulation. Additionally, the findings will contribute to understanding the potential health risks associated with the consumption of tobacco products and provide valuable data for regulatory authorities and public health agencies.

2. Materials and methods

2.1 Instrument and apparatus

Whole Analysis was performed by using AAS (atomic absorption spectrophotometer) in flame mode. The digested samples were analysed by the AAS equipped with a vapour generation assembly (Alalytik jena ZEEnit 700). Acetylene gas used for production of flame to ionize metals.

2.2 Chemical and Reagents

All reagents were from analytical reagent grade. Double deionised water (Milli-Q) were used for all kinds of dilutions. HNO_3, H_2O_2 and HCL were purchased from Merck. All glassware and plastic ware were cleaned by soaking in dilute HNO_3 (1/9, v/v) and were properly rinsed with double distilled water prior to use. The element stock solutions used for calibration were produced by diluting stock solution of 1000 mg/l. Standard solutions and dilution were prepared using distilled-deionized water.

2.4 Sample collection

Tobacco leaf and packed tobacco samples were collected form three different cities (Lucknow, Ayodhya and Kanpur) of Uttar Pradesh. Seven different brand samples were collected from each city and different type of tobacco leaf samples were also collected from local market. All tobacco samples were collected in polyethylene bag and transported to the laboratorywhere the samples were stored at 4 ^{o}Ctill analysis.

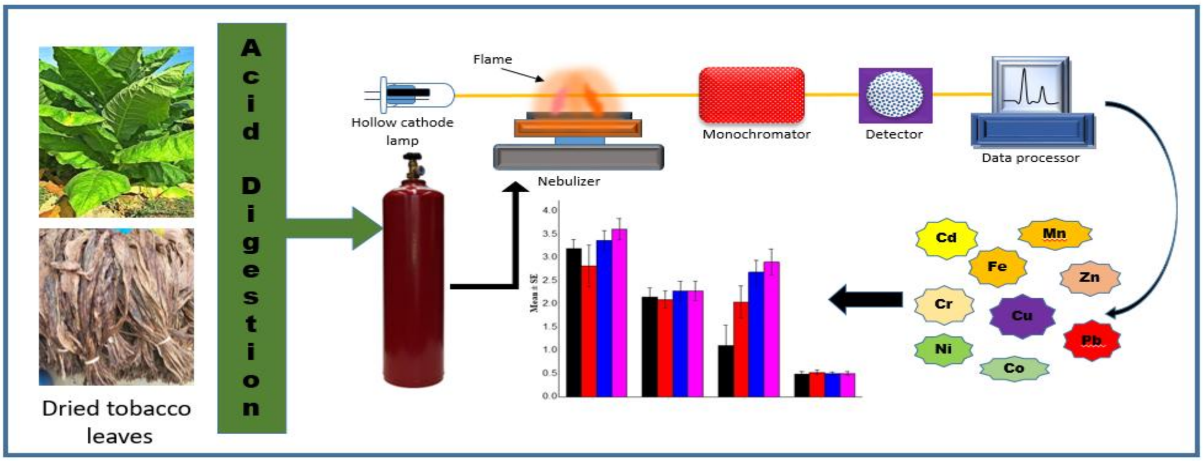

Figure:- Analysis of metals in Tobacco leaves samples

2.5 Samples preparation

1 g of each samples were digested in 10 ml of freshly prepared 1:1 Nitric acid andPerchloric acid. Beaker was covered with a watch glass till initial reaction subsided in about 2 hours. The beaker was placed on hot plate and temperature gradually allowed to rise to 150^{o} C and the content boiled genteelly for about 2 hours.

The digested samples were allowed to cool, filtered and transferred to 25 ml volumetric flask and make up to marked with de-ionized water.The reagent combination was digested using the same digestion technique to prepare the blank solutions, which were then diluted to 25 ml with deionized water. The digested were kept in glass test tube with stopper and later the heavy metal concentrations were determined by using an atomic absorption spectrophotometer the actual concentration were calculated by using the formula.

Actual concentration of metal in sample = PPMR X Dilution factor

PPMR = AAS reading of Digest

$$\text{Dilution factor} = \frac{\text{Volume of digest used}}{\text{Weight of sample digested}}$$

3. Quantification and validation of method

Seven-point calibration curves were generated using the standard solutions 2.5ppm, 5ppm, 10 ppm, 20ppm, 50 ppm 100 ppm, 200 ppm in the flame AAS. The sample solutions were analysed into the instrument AAS immediately after calibration, and the metal concentrations were measured directly. Each sample was repeatedly analysed three times. The constituents in each of the six digested blanks were determined using the same analytical technique. For the recovery of the heavy metals 10 ppm of standard were spiked in tobacco leaves sample. All the samples were digested and analysed on instrument with the previously optimized procedure.

The digests of spiked samples for both raw tobacco leaves and processed tobacco samples were examined in order to gauge the effectiveness of the optimised approach. The range of metal recoveries in the samples of spiked tobacco was 73 -102 %. For studies of biological samples like plants, these results fall within the permitted range. Results are given in table -1

Table-1 Recovery and RSD value of heavy metals in tobacco leaves samples

S.No	Heavy metal	Spiking level (ppm)	Recovery	RSD
1.	Iron (Fe)	10 ppm	82.05 %	3
2.	Manganese (Mn)	10 ppm	87.62 %	7
3.	Zinc (Zn)	10 ppm	102.16 %	4
4.	Cupper (Cu)	10 ppm	73.24 %	9
5.	Nickel (Ni)	10 ppm	93.65 %	6
6.	Chromium (Cr)	10 ppm	91.75 %	7
7.	Cadmium (Cd)	10 ppm	98.64 %	3
8.	Lead (Pb)	10 ppm	79.85 %	11
9.	Cobalt (Co)	10 ppm	90.38 %	13

4. Results

Table 2 shows the details of metal contents in tobacco leaves and tobacco products available in major cities of Uttar Pradesh. The mean iron (Fe) content was 129.01±15.94 (range 105.08 to 155.10) in tobacco leaf samples, 167.68±12.75 (range 150.42 to 185.71) in tobacco products from Ayodhya, 206.24±15.69 (range 185.01 to 228.42) in tobacco products from Lucknow and 253.68 ±19.29 (range 227.56 to 280.95) in tobacco products from Kanpur.

The mean manganese (Mn) content was 33.99±5.56 (range 27.24 to 41.66) in tobacco leaf samples, 47.34±6.62 (range 38.74 to 54.74) in tobacco products from Ayodhya, 53.01±7.42 (range 43.38 to 61.30) in tobacco products from Lucknow and 51.96±7.27 (range 42.52 to 60.08) in tobacco products from Kanpur.

The mean zinc (Zn) content was 83.38±5.84 (range76.25 to92.38) in tobacco leaf samples, 88.30±6.18 (range80.75 to97.83) in tobacco products from Ayodhya, 95.89±6.71 (range87.69 to106.24) in tobacco products from Lucknow and 110.28±7.72 (range100.85 to122.18) in tobacco products from Kanpur.

The mean copper content (Cu) 29.18±2.04 (range 26.69 to 32.33) in tobacco leaf samples, 32.10±2.25 (range 29.36 to 35.57 in tobacco products from Ayodhya, 31.78±2.22 (range 29.06 to 35.21 in tobacco products from Lucknow and 35.60±2.49 (range 32.55 to 39.44 in tobacco products from Kanpur.

The mean nickel (Ni) content was 10.65±2.98 (range 7.03 to 15.07) in tobacco leaf samples, 10.33±2.89 (range 6.82 to 14.62 in tobacco products from Ayodhya, 11.26±3.15 (range 7.44 to 15.94 in tobacco products from Lucknow and 11.61±3.25 (range 7.67 to 16.43 in tobacco products from Kanpur.

Chromium (Cr) 2.95±0.53 (range 2.05 to 3.75) in tobacco leaf samples, 2.58±1.19 (range 0.00 to 3.63 in tobacco products from Ayodhya, 3.12±0.56 (range 2.17 to 3.96 in tobacco products from Lucknow and 3.37±0.61 (range 2.34 to 4.28 in tobacco products from Kanpur.

Cadmium (Cd) 1.91±0.51 (range 1.40 to 3.00) in tobacco leaf samples, 1.85±0.49 (range 1.36 to 2.91 in tobacco products from Ayodhya, 2.04±0.54 (range 1.49 to 3.20 in tobacco products from Lucknow and 2.04±0.54 (range 1.49 to 3.20 in tobacco products from Kanpur.

The mean lead content (Pb) 0.86±1.17 (range 0.00 to 2.91) in tobacco leaf samples, 1.80±0.93 (range 0.00 to 2.83 in tobacco products from Ayodhya, 2.44±0.68 (range 1.61 to 3.46 in tobacco products from Lucknow and 2.66±0.74 (range 1.76 to 3.77 in tobacco products from Kanpur.

The mean cobalt content (Co) 0.24±0.16 (range 0.00 to 0.51) in tobacco leaf samples, 0.28±0.12 (range 0.19 to 0.52 in tobacco products from Ayodhya, 0.25±0.11 (range 0.17 to 0.47 in tobacco products from Lucknow and 0.26±0.11 (range 0.17 to 0.47 in tobacco products from Kanpur.

Table 2: Details of metal in tobacco leaf and tobacco products available in major cities of Uttar Pradesh

	Mean	**Median**	**Std. Deviation**	**Minimum**	**Maximum**	**Percentiles**	
						25%	**75%**
Tobacco Leaf Samples							
Iron (Fe)	129.01	130.04	15.94	105.08	155.10	117.97	139.50
Manganese (Mn)	33.99	34.00	5.56	27.24	41.66	27.48	40.05
Zinc (Zn)	83.38	82.81	5.84	76.25	92.38	76.27	87.17
Cupper (Cu)	29.18	28.98	2.04	26.69	32.33	26.69	30.51
Nickel (Ni)	10.65	9.14	2.98	7.03	15.07	8.52	13.31
Chromium (Cr)	2.95	2.99	0.53	2.05	3.75	2.65	3.24
Cadmium (Cd)	1.91	1.78	0.51	1.40	3.00	1.70	1.96
Lead (Pb)	0.86	0.00	1.17	0.00	2.91	0.00	1.77
Cobalt (Co)	0.24	0.18	0.16	0.00	0.51	0.18	0.34
Tobacco Products From Ayodhya							
Iron (Fe)	167.68	164.76	12.75	150.42	185.71	159.77	183.18
Manganese (Mn)	47.34	46.72	6.62	38.74	54.74	40.23	53.67
Zinc (Zn)	88.30	87.70	6.18	80.75	97.83	80.76	92.31

Cupper (Cu)	32.10	31.88	2.25	29.36	35.57	29.36	33.56
Nickel (Ni)	10.33	8.87	2.89	6.82	14.62	8.26	12.91
Chromium (Cr)	2.58	2.90	1.19	0.00	3.63	2.57	3.14
Cadmium (Cd)	1.85	1.72	0.49	1.36	2.91	1.64	1.90
Lead (Pb)	1.80	1.89	0.93	0.00	2.83	1.49	2.67
Cobalt (Co)	0.28	0.24	0.12	0.19	0.52	0.19	0.35
Tobacco Products From Lucknow							
Iron (Fe)	206.24	202.65	15.69	185.01	228.42	196.51	225.31
Manganese (Mn)	53.01	52.33	7.42	43.38	61.30	45.06	60.10
Zinc (Zn)	95.89	95.24	6.71	87.69	106.24	87.71	100.25
Cupper (Cu)	31.78	31.56	2.22	29.06	35.21	29.07	33.22
Nickel (Ni)	11.26	9.67	3.15	7.44	15.94	9.00	14.08
Chromium (Cr)	3.12	3.16	0.56	2.17	3.96	2.80	3.43
Cadmium (Cd)	2.04	1.89	0.54	1.49	3.20	1.81	2.09
Lead (Pb)	2.44	2.10	0.68	1.61	3.46	1.95	3.05
Cobalt (Co)	0.25	0.22	0.11	0.17	0.47	0.17	0.31
Tobacco Products From Kanpur							
Iron (Fe)	253.68	249.27	19.29	227.56	280.95	241.71	277.13
Manganese (Mn)	51.96	51.28	7.27	42.52	60.08	44.16	58.90
Zinc (Zn)	110.28	109.52	7.72	100.85	122.18	100.87	115.29
Cupper (Cu)	35.60	35.35	2.49	32.55	39.44	32.56	37.21
Nickel (Ni)	11.61	9.97	3.25	7.67	16.43	9.28	14.51
Chromium (Cr)	3.37	3.41	0.61	2.34	4.28	3.02	3.70
Cadmium (Cd)	2.04	1.89	0.54	1.49	3.20	1.81	2.09
Lead (Pb)	2.66	2.29	0.74	1.76	3.77	2.13	3.33
Cobalt (Co)	0.26	0.22	0.11	0.17	0.47	0.17	0.32

The mean iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and lead (Pb) contents were significantly lower ($p<0.05$) in the tobacco leaf samples than in the tobacco products from Ayodhya, Lucknow and Kanpur. In addition, the average iron, zinc (Zn), copper (Cu) and lead (Pb) content was higher in Kanpur, while manganese (Mn) was higher in Lucknow. The content of nickel (Ni), chromium (Cr), cadmium (Cd) and cobalt (Co) was significantly lower ($p<0.05$) in the tobacco leaf samples than in the tobacco products from Ayodhya, Lucknow and Kanpur (Table 2).

Table 3: Mean changes of different metal in tobacco leaf and tobacco products available in major cities of Uttar Pradesh

	Tobacco Leaf Samples		Tobacco Products From Ayodhya		Tobacco Products From Lucknow		Tobacco Products From Kanpur		F	p-Value
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE		
Iron (Fe)	129.01	6.02	167.68	4.82	206.24	5.93	253.68	7.29	76.961	<0.001*
Manganese (Mn)	33.99	2.10	47.34	2.50	53.01	2.80	51.96	2.75	11.733	<0.001*
Zinc (Zn)	83.38	2.21	88.30	2.34	95.89	2.54	110.28	2.92	21.790	<0.001*
Cupper (Cu)	29.18	0.77	32.10	0.85	31.78	0.84	35.60	0.94	9.533	<0.001*
Nickel (Ni)	10.65	1.13	10.33	1.09	11.26	1.19	11.61	1.23	0.249	0.861
Chromium (Cr)	2.95	0.20	2.58	0.45	3.12	0.21	3.37	0.23	1.300	0.297
Cadmium (Cd)	1.91	0.19	1.85	0.19	2.04	0.21	2.04	0.21	0.219	0.882
Lead (Pb)	0.86	0.44	1.80	0.35	2.44	0.26	2.66	0.28	5.604	0.005*
Cobalt (Co)	0.24	0.06	0.28	0.05	0.25	0.04	0.26	0.04	0.130	0.941

*=Significant ($p<0.05$)

International Journal of Architecture, Engineering and Construction ISSN (online) 1911-1118
Vol 12, No 2, June 2023 (UGC CARE 1) ISSN (print): 1911-110X

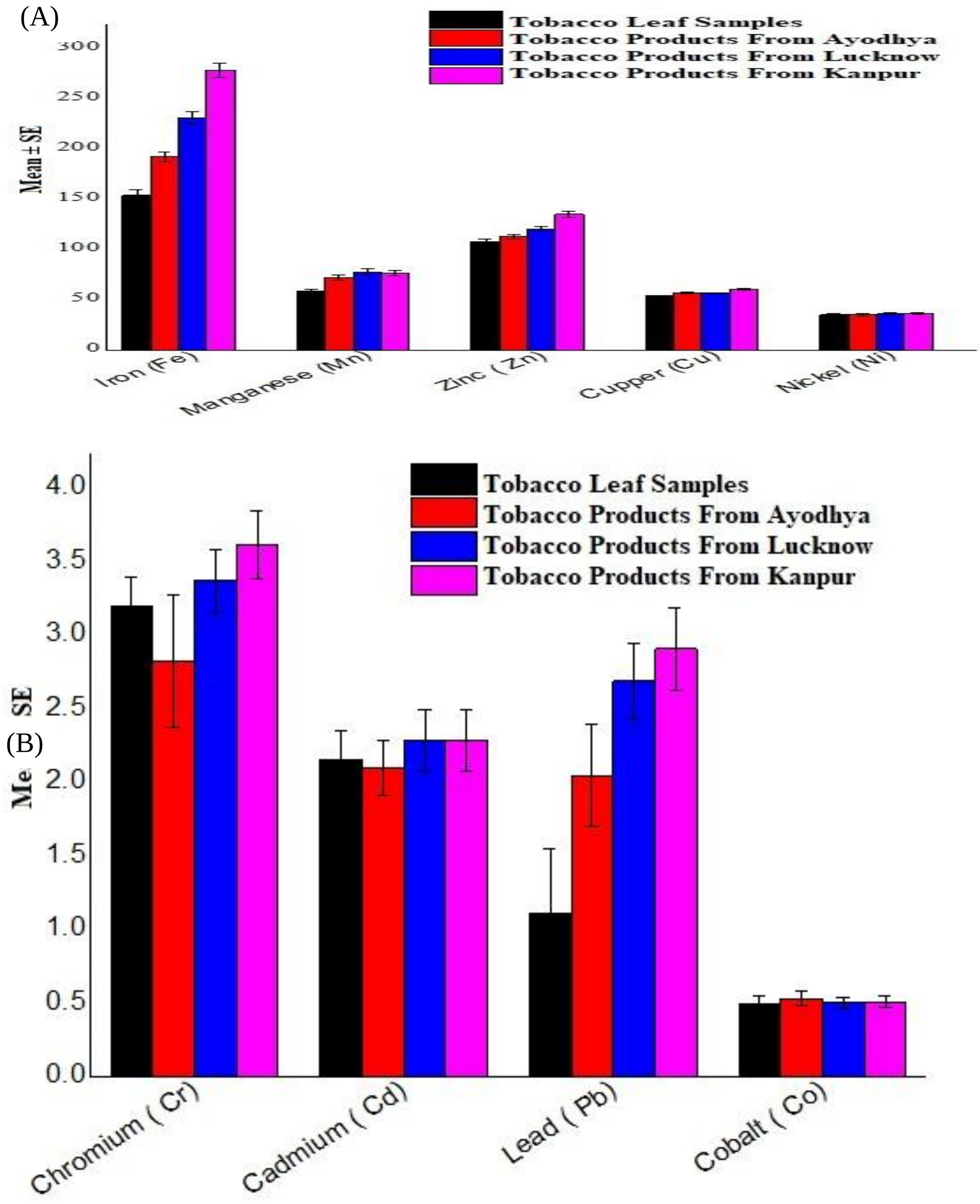

Figure-1 figure A and B shows the mean difference leaf samples and tobacco products for different major city of Uttar Pradesh

The mean contents of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and lead (Pb) were significantly lower in the tobacco leaf samples than in the tobacco products from Kanpur. The mean contents of iron (Fe) and manganese (Mn) were significantly lower in the tobacco leaf samples than in the tobacco products from Ayodhya, and the mean contents of iron (Fe), manganese (Mn), zinc (Zn) and lead (Pb) were significantly lower in the tobacco leaf samples than in the tobacco products from Lucknow (Table 3).

Table 4: Post-hoc test

	Tobacco Product (TP)											
	Leaf Samples vs TP Ayodhya		Leaf Samples vs TP Lucknow		Leaf Samples vs TP Kanpur		TP Ayodhya vs TP Lucknow		TP Ayodhya vs TP Kanpur		TP Lucknow vs TP Kanpur	
	Mean Diff.	p-Value	Mean Diff.	p-Value	Mean Diff.	p-Value	Mean Diff.	p-Value	Mean Diff.	p-Value	Mean Diff.	p-Value
Iron (Fe)	-38.67	0.001*	-77.23	<0.001*	-124.67	<0.001*	-38.56	0.001	-86.00	<0.001*	-47.44	<0.001*
Manganese (Mn)	-13.35	0.006*	-19.03	<0.001*	-17.97	<0.001*	-5.68	0.413	-4.62	0.585	1.06	0.991
Zinc (Zn)	-4.92	0.521	-12.51	0.009*	-26.90	<0.001*	-7.59	0.170	-21.98	<0.001*	-14.39	0.002*
Cupper (Cu)	-2.92	0.100	-2.60	0.166	-6.41	<0.001*	0.32	0.993	-3.49	0.037*	-3.82	0.020*
Nickel (Ni)	0.32	0.997	-0.61	0.982	-0.96	0.936	-0.93	0.940	-1.28	0.863	-0.35	0.997
Chromium (Cr)	0.37	0.801	-0.17	0.976	-0.42	0.744	-0.54	0.562	-0.79	0.247	-0.25	0.930
Cadmium (Cd)	0.06	0.997	-0.13	0.969	-0.13	0.969	-0.18	0.911	-0.18	0.911	0.00	1.000
Lead (Pb)	-0.94	0.234	-1.58	0.016*	-1.80	0.005*	-0.64	0.557	-0.86	0.306	-0.22	0.967
Cobalt (Co)	-0.04	0.928	-0.01	0.998	-0.02	0.994	0.03	0.974	0.02	0.984	0.00	1.000

*=Significant ($p<0.05$)

5. Discussion

It's well-documented that tobacco plants can absorb heavy metals from the soil, and these metals can accumulate in various parts of the plant, including the leaves. The specific levels of these metals can vary depending on various factors such as the region where the tobacco is grown, agricultural practices, processing methods, and the type of tobacco product. Regulatory bodies and research institutions may periodically conduct studies to assess the heavy metal content in tobacco products, including cigarettes, chewing tobacco, and others. Given the potential health concerns associated with tobacco use, metal toxicity in tobacco leaf samples is of serious concern. Due to soil and air pollution, heavy metals such as cadmium, lead and nickel can accumulate in tobacco plants and pose a major health risk to both smokers and non-smokers. These metals have been linked to a range of health problems, including respiratory diseases, cardiovascular disease and cancer. In order to assess the potential health risks and take precautions to reduce exposure to these dangerous compounds, it is essential to regularly monitor and analyze the levels of metals in tobacco leaf samples.

A worrying issue with the many chewing tobacco products available in India is the toxicity of the metals contained in the tobacco leaves. Heavy metals such as cadmium, lead and nickel are often found in large amounts in products such as gunkha, khaini and paan masala. These metals accumulate in the tobacco leaves as a result of processing chemicals and contaminated soils. Due to metal toxicity in these chewable tobacco products, consumers are at risk of respiratory problems, cardiovascular disease and cancer. To reduce the health risks associated with metal toxicity in the Indian chewing tobacco industry, strict quality control procedures and routine monitoring of metal levels in tobacco products are essential[16].

India ranks third in the world in terms of total tobacco consumption and offers one of the largest tobacco markets. There are two types of tobacco products used: smokeless (such as chewing tobacco and snuff) and smoked (such as cigarettes, biri and cigars). Although chewing tobacco and biri are the most popular forms of tobacco consumption in India, cigarettes are the most popular form of tobacco consumption worldwide. As a result, Indian tobacco consumption patterns differ from global trends. Due to the different patterns of tobacco consumption in India, a variety of Indian tobacco products, each with multiple brands, were considered in this study[1, 17]. While Cr shows a higher value for snuff, the results presented above indicate changes that are more important for chewing tobacco. Snuff generally contains more Pb, Cr, Fe and Cu than chewing tobacco; cigarettes contain more Pb, Zn and Cd; cigars contain more Cd, Fe, Zn and Cu than biri, and so on. The overall order of metal concentration in all product types in this study is Fe > Zn > Cu > Ni Cr > Pb Cd (the concentrations of Ni and Cr are almost the same, as are those of Pb and Cd). This corresponds to the order of metal concentrations in all soil types, suggesting that tobacco plants take up more metal when it is present in higher concentrations in the soil (Adamu et al., 1989, Verma et al. 2010). It is important to note that certain metals can become toxic if they are present in greater amounts than the human body requires[18]. In our study the mean Iron (Fe), Manganese (Mn), Zinc (Zn), Cupper (Cu), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Lead (Pb) and Cobalt (Co) were 129.01±15.94, 33.99±5.56, 83.38±5.84, 29.18±2.04, 10.65±2.98, 2.95±0.53, 1.91±0.51, 0.86±1.17 and 0.24±0.16 in in tobacco leaf samples, 167.68±12.75, 47.34±6.62, 88.30±6.18, 32.10±2.25, 10.33±2.89, 2.58±1.19, 1.85±0.49, 1.80±0.93 and 0.28±0.12 in tobacco products from Ayodhya, 206.24±15.69, 53.01±7.42, 95.89±6.71, 31.78±2.22,11.26±3.15, 3.12±0.56, 2.04±0.54, 2.44±0.68 and 0.25±0.11in tobacco products from Lucknow and 253.68±19.29, 51.96±7.27, 110.28±7.72, 35.60±2.49, 1.61±3.25, 3.37±0.61, 2.04±0.54, 2.66±0.74 and 0.26±0.11 in tobacco products from Kanpur.

Many researchers have reported the concentration of metals in cigarette tobacco as well as tobacco leaves. Moulin et al. (2006) analyzed 755 tobacco's leaves samples during 2001–2003 and found that cadmium concentrations in the samples ranged from 0 to 6.78 μg/g dry mass. The report also indicated that Cd contents of flue cured tobacco leaves as India (0.33 ± 0.13), France (1.46 ± 1.35) and processed one from USA (0.51 ± 0.05) μg/g dry mass. There are also other literatures which reported the contents of some metals such as Cu (14.9–21.1), Zn (51– 84), Ni (<1) 1 μg/g dry mass), in flue cured tobacco leaves 24–33 μg/g dry mass of Zn (Tso 1973); the concentration of nickel in cigarettes (2.32–4.20 μg/g dry mass) and in tobacco leaves (2.20–4.91 μg/g dry mass) (Stojanovic et al. 2004); the average concentration of Cd in both tobacco leaves and cigarettes in Mexican producedtobacco (4.41 ± 0.67 and 2.65 ± 0.99 µg/g dry mass respectively) (Saldivar et al. 1991). As compared the report of Murty et al. (1986) the concentration of Cd in present study flue cured tobacco was higher than flue cured tobacco of India, New Zealand and within the range of other countries (America, Germany, and Canada) flue cured tobacco concentration. Generally the level of Cd in present study was within the range of the literature values, which can range from 0 to 6.78 (Moulin et al. 2006). However, in comparison with the flue-cured leaves from India and France, Cd content in present study was found to be higher than that of Indians' and lower than Frances' flue cured leaves (Moulin et al. 2006).

In Regassa and Chandravanshi, 2016, the concentration of Ni was found to be within the range of literature value between 1 to 4.91 µg/g dry mass (Stojanovic et al. 2004; Tso 1973). The concentration of Cu is lower than the literature value which ranges from 14.9–21.14 µg/g. The level of Zn in the tobacco leaves in this study is also within the range of literature value, which ranges from 24 to 81 µg/g dry mass. Precise reported information was not obtained on the content of Cr in tobacco leaves from literatures. There are also different reports of metal contents in processed tobacco from different countries, some of these are, Zhang et al. (2005) and Nnorom et al. (2005) (Table 4)[19]. As presented in Table 4 the Cd concentration of present study is within the range of minimum concentration determined in Japan's cigarette (Zhang et al. 2005) and maximum concentration obtained in the France cigarette (Zhang et al. 2005) (Table 4)[20]. The concentration of Pb obtained by this method is less than all the literature report. Similarly, Ni in processed Shewa Robit and Billate is less than all other literature report. However, the Ni concentration determined in Nyala is within the range minimum report (2.32 µg/g) (Stojanovic et al. 2004) and maximum content of Germany's (9.11 µg/g dry mass) cigarette (Saldivar et al. 1991)[21, 22]. Comparative results given Table 4 revealed that the Zn concentration in present study is found to be higher than the literature value. In contrast to Zn, the amount of Cu is found to be lower than the literature values. The concentration of lead in present study is lower than its concentration in processed tobacco from other countries (Table 4). It has been demonstrated that most of the lead in green plant parts originate from deposition of air borne lead from automotive sources (Murty et al. 1986) and thus the lead content of tobacco leaves in this study can be expected to be low as such occurrences are minimum in Ethiopia[23]. Even the lead in the soil is not in soluble form to be available to plant as compared to other metals. In general, the concentrations of metals observed were more or less comparable with the reported literature values. However, relatively lower concentrations of Cu were observed in this study in comparison to the reported values.

Cadmium is the most studied metal in tobacco worldwide due to its toxic health effects (Nriagu, 1981; Järup et al., 1998). The observed variations could primarily be related to the chemistry of tobacco leaves and later to its processing. Tobacco is grown over wide geographical areas in India on varying soil types and hence the metal contents of soil itself are likely to vary (Bell et al., 1992; Pappas et al., 2006; and references therein). The metal uptake by tobacco plants is also function of soil pH and other related factors (Bell et al., 1992). It is well established that tobacco plants selectively enrich some of the heavy metals from soil and enrich them in leaves. Among different categories, biri, the cheapest of the smoking product, show minimum metal contents whereas the cigarette and cigar show higher metal contents. This indicates that processing of tobacco leaves enriches the metal content (Chepiga et al., 2000; Rustemeier et al., 2002; Kazi et al., 2009; and references therein)[24-26]. It is important to note that these different products are processed differently from the raw tobacco leaves and priced accordingly; in present case biri is the cheapest compared to cigarette and cigar in smoking category while chewing tobacco and snuff are the cheapest in non-smoking category. Moreover, different brands are exclusive product of different companies with their own processing type/formula. Such inner brand variations in metal content of tobacco products have been observed by others as well (Rickert and Kaiserman, 1994) [27].

6. Conclusion

The presence of heavy metals such as Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Lead (Pb), and Cobalt (Co) in tobacco leaves indicates potential contamination of the soil or air where the tobacco plants were grown. The accumulation of heavy metals in tobacco leaves suggests potential environmental contamination from various sources such as industrial activities, vehicular emissions, or agricultural practices. Heavy metals like Cadmium (Cd), Lead (Pb), and Nickel (Ni) are known to be toxic to human health even at low concentrations. The range of metal recoveries in the samples of spiked tobacco was 73 -102 % and RSD is below 15%. The result shows that higher concentration of heavy metals in tobacco samples collected from Kanpur market. Chronic exposure to these metals through smoking tobacco products can pose significant health risks, including respiratory problems, cardiovascular diseases, and even cancer.The levels of heavy metals detected in tobacco leaves may raise concerns regarding regulatory standards and guidelines for tobacco cultivation and manufacturing processes. Strict regulations may be necessary to mitigate the health risks associated with heavy metal exposure from tobacco consumption. Continuous monitoring of heavy metal concentrations in tobacco leaves is essential to assess the effectiveness of mitigation measures and ensure compliance with regulatory standards. Implementing measures such as soil remediation, proper waste management, and alternative farming practices can help reduce heavy metal contamination in tobacco plants.

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