

Evaluation of Heavy Metals in Naswar Famous Brands and Their Implications on Human Health

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Abstract— Background: Naswar consumption is widespread in Pakistan, posing significant health risks due to the presence of various toxic substances. The heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), iron (Fe), lead (Pb), and nickel (Ni) are of particular concern due to their potential to cause severe health issues. **Objective:** The objective of this study was to evaluate the concentrations of heavy metals in various brands of Naswar marketed in Pakistan and to assess their implications for public health. **Methods:** Five different brands of Naswar commonly used in Pakistan were Tara, Hot, Cool, M. Mukhi and Naswar selected for analysis. The samples were collected from local markets and prepared by oven drying for eight days. Each one-gram sample was digested using a mixture of sulfuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) through a wet digestion method. **Results:** The toxic metals under study were Cadmium (Cd), Chromium (Cr), Copper (Cu), Cobalt (Co), Manganese (Mn), Zinc (Zn), Iron (Fe), Lead (Pb) and Nickel (Ni) in various brands of each Naswar products present in Pakistan. The mean values of heavy metals in various brands of Naswar products was recorded as Cd (0.3 to 0.4 mg/kg), Cr (4.7 to 6.5 mg/kg), Cu (15 to 17 mg/kg), Co (0.40 to 0.43 mg/kg), Mn (42 to 45 mg/kg), Zn (36.5 to 40.2 mg/kg), Fe (36.5 to 40.2 mg/kg), Pb (1.2 to 1.7 mg/kg) and Ni (3.9 to 4.4 mg/kg). The results of the heavy metals (Cd vs Fe, Co vs Fe, Co vs Ni, Mn vs Zn, Mn vs Fe, Zn vs Ni and Fe vs Pb) showed that they have strong relationship between them and they support to each other in the environment while the other heavy metals within the samples have weak association and they are independently accumulated in the tobacco products due to soil impurities and other environmental pollution factors. **Conclusion:** This study is also responsible to create awareness among people about the toxicity of the Naswar products which are harmful for human health.

Keywords— Heavy Metal, Human Health, Hazards, Pakistan.

I. INTRODUCTION

The use of smokeless tobacco products is a growing problem as concern to public health, with an approximately 360 million users globally [1], with over 90% of those users living in South Asia. There were several diseases associated with tobacco products [2, 3], naswar products are becoming more and more popular in South Asian countries [4]. The improper guideless of tobacco control legislation and regulations is the primary reason for this rise in use [5].

One of the main causes of these legal flaws is ignorance of the harmful effect of naswar products. In contrast to smoking tobacco products like cigarettes, there is less scientific evidence about the toxicity of naswar products, which are commonly consumed in developing countries. As a result, the general public believes that these products are safe or less hazardous for human health [6]. Significant national variations in smokeless tobacco product kinds, formulations, and associated complicated health issues [7].

However, research from all around the world indicates that smokeless tobacco products is a major risk factor for heart diseases, poor childbirth, and upper respiratory tract cancer. It also causes to 0.65 million deaths annually [8]. About 8% of Pakistanis are expected to use smokeless tobacco, with Paan, Gutkha, and Naswar being the most popular tobacco products [9]. Sun-dried tobacco leaves are combined with slaked lime,

ash, and flavorings in local cottage industries used this method to manufacture the Naswar products [10]. The finished product is then packaged and marketed in tiny poly-ethylene bags.

Long-term naswar use has been often associated with mouth cancer; according to pooled estimations, regular naswar users have a tenfold increased risk of oral cancer compared to non-users [11]. Additionally, regular users of Naswar have been reported to have a range of cardiovascular diseases and other oral diseases [12, 13]. Because of the increased demand and popularity in recent years, the scientific studies evaluating the composition of naswar were carried out at least ten years ago [10] and do not accurately reflect the advancements in preparation techniques and product composition [7].

Furthermore, different parts of Pakistan have varied naswar product compositions. Furthermore, the quantity of tobacco, ash, and slaked lime, as well as different kinds of additives utilized in the different naswar brands. Therefore, it is essential to perform a compositional study of the naswar products already on the market in order to evaluate the risks related to their toxicity. The goal of the current study is to thoroughly evaluate the levels of heavy metal and hazardous metal contamination in the most popular naswar brands in Pakistan's Punjab province.

II. MATERIAL AND METHODS

The study was conducted to evaluate the concentrations of heavy metals in various brands of tobacco Naswar marketed in Pakistan and their implications for public health. The brands analyzed Tara, Hot, Cool, M. Mukhi (K2) and Naswar were included. Samples of these brands were collected from local markets across different regions of Sargodha, Pakistan as shown in figure 1. Each brand was represented by multiple samples to ensure comprehensive analysis and reliable results.

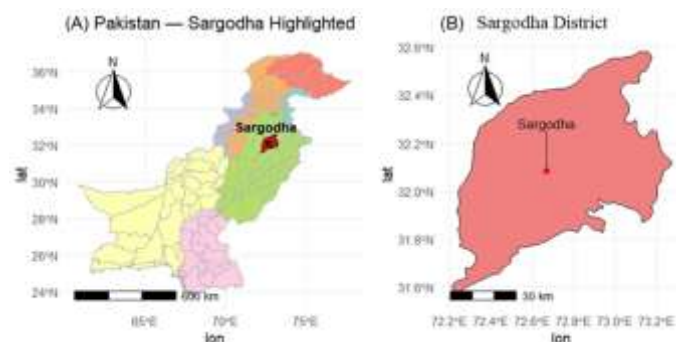


Fig. 1: Study area map for the collection of different brands of Naswar in Sargodha Pakistan

Study design

For sample preparation, one gram of each naswar sample was initially oven-dried for eight days to remove moisture. The dried samples were then subjected to wet digestion using a mixture of 4 ml of sulfuric acid (H_2SO_4) and 8 ml of hydrogen peroxide (H_2O_2). The digestion process was carried out in a digestion chamber for approximately 30 minutes. Following the cessation of fume production, an additional 2 ml of H_2O_2 was added, and the samples were reheated until they became colorless. The digested samples were then diluted to a final volume of 25 ml with distilled water and stored in labeled plastic bottles for subsequent analysis.

The mineral analysis was performed using an Atomic Absorption Spectrophotometer (AAS-5000, Perkin-Elmer Corp., 1980) and a Double Beam Spectrophotometer (U-2900/2910). The concentrations of cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), iron (Fe), lead (Pb), and nickel (Ni) were determined in the tobacco samples. The detection limits for these metals were as follows: Cd (0.8 mg/kg), Cr (3 mg/kg), Cu (15 mg/kg), Co (9 mg/kg), Mn (1.5 mg/kg), Zn (1.5 mg/kg), Fe (5 mg/kg), Pb (15 mg/kg), and Ni (6 mg/kg).

National Institute of Standard and Technology's Standard Reference Material (SRM 1570) served as the benchmark for

all heavy metal detections. The results were deviated in the range $\pm 2\%$ of the reported value.

Study setting

University of Sargodha, Pakistan

Study duration

One-year Duration

Inclusion Criteria

All the local brands which are commonly used and available in local markets of Pakistan are considered in this study.

Exclusion Criteria

All those brands which are imported Naswar products which are openly sell in local markets of Pakistan which are not consenting to take a part of the study.

Analysis plan

The statistical analysis of the collected data was performed using SPSS software, version 25 and R software. The results were expressed as mean values. The significance of the differences between the heavy metal concentrations in the different Naswar brands was assessed using One Way ANOVA Test at a 0.05 significance level. Additionally, Least Significant Difference (LSD) Test was used after the One-Way ANOVA Test at 0.05 used to explore the relationships between the various heavy metals in the Naswar samples. The LSD formula to calculate the LSD value as

$$LSD = t_{v, \alpha} \sqrt{S^2 \times \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

t_v = Degree of freedom within the sample

α = Significance level (0.05)

S^2 = Mean square difference within the sample

n = number of samples

III. RESULT

The toxic metals under study are Cadmium (Cd), Chromium (Cr), Copper (Cu), Cobalt (Co), Manganese (Mn), Zinc (Zn), Iron (Fe), Lead (Pb) and Nickel (Ni) present in various brands of naswar found in Pakistan, their mean values were recorded in table 1 and figure 2.

The presences of different heavy metals of tobacco naswar is a source of toxic metals which was recorded in ranges as Cd (0.3 to 0.4 mg/kg), Cr (4.7 to 6.5 mg/kg), Cu (15 to 17 mg/kg), Co (0.40 to 0.43 mg/kg), Mn (42 to 45 mg/kg), Zn (36.5 to 40.2 mg/kg), Fe (36.5 to 40.2 mg/kg), Pb (1.2 to 1.7 mg/kg) and Ni (3.9 to 4.4 mg/kg) reported in table 2.

TABLE 1. Fluctuation of various heavy metals is present in different brands of Naswar.

Various Brands of tobacco products	Concentration of different heavy metals present in Naswar products								
	Cd	Cr	Cu	Co	Mn	Zn	Fe	Pb	Ni
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Naswar	0.3	4.7	17	0.43	42	47	36.5	1.2	4.4
Casturi	0.4	5.5	17	0.41	43	58.8	42.2	1.5	4.2
Najma	0.35	6.5	16	0.42	42	47	40	1.8	4
Tara	0.35	6	15	0.4	45	50.8	40.2	1.7	4.1
K2 (M. Mukhi)	0.4	5	17	0.43	45	47.2	40.2	1.2	3.9

Mean Concentration ($n=5$) $\pm$ (S.D)

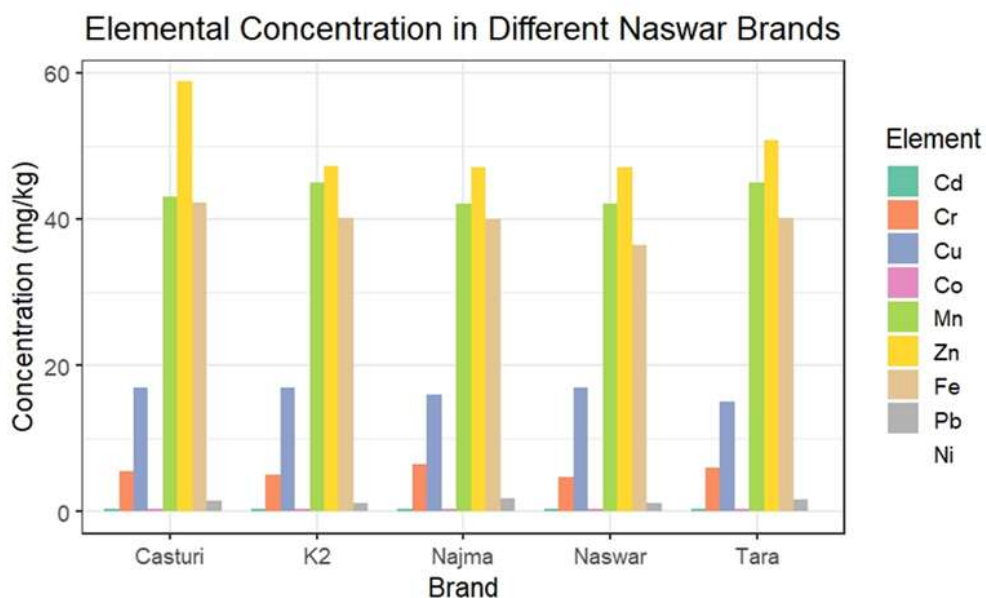


Fig. 2. Fluctuation of various heavy metals is present in different brands of Naswar.

TABLE 2. Minimum and Maximum value of heavy metals is present in different brands of Naswar.

Heavy Metals in different brands of Naswar	Concentration of various heavy metals		
	Minimum Value	Maximum Value	Ranges
	mg/kg	mg/kg	mg/kg
Cd	0.3	0.4	0.3 to 0.4
Cr	4.7	6.5	4.7 to 6.5
Cu	15	17	15 to 17
Co	0.40	0.43	0.40 to 0.43
Mn	42	45	42 to 45
Zn	47	50.8	47 to 50.8
Fe	36.5	40.2	36.5 to 40.2
Pb	1.2	1.7	1.2 to 1.7
Ni	3.9	4.4	3.9 to 4.4

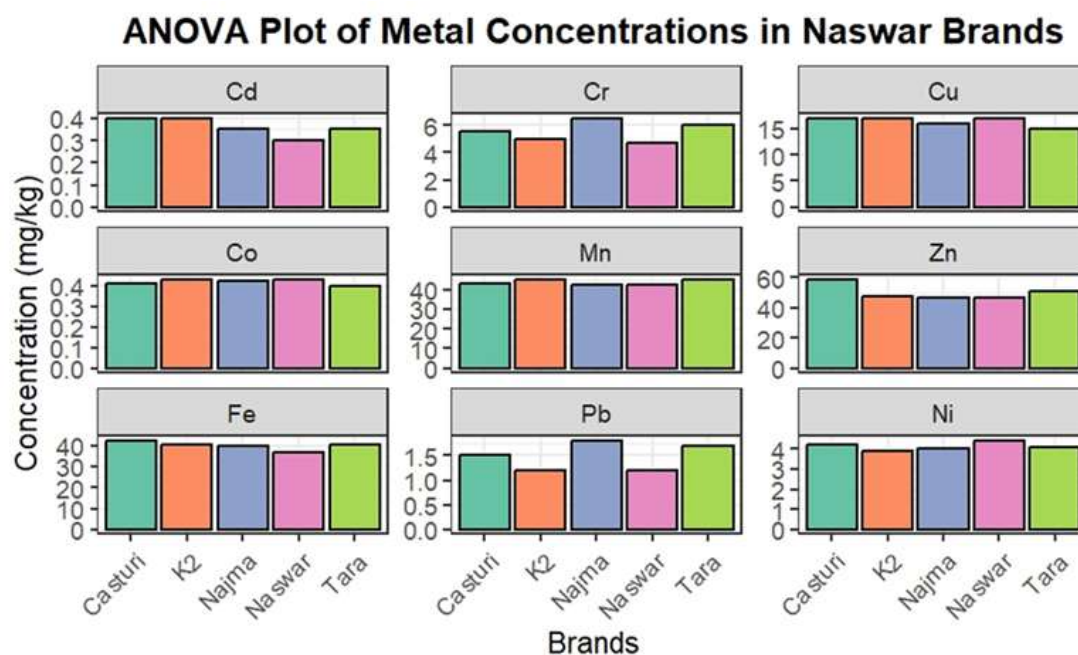
Mean Concentration (n=5) $\pm$ (S.D)

Fig. 3. ANOVA plot between the heavy metals present in Naswar products (n=5)

Furthermore, One way ANOVA Test the P value is 1.98E-35 at 0.05, which is less than the 0.05 thus alternate hypothesis is accepted and there are mean difference present between the different heavy metals in the Naswar products which were reported in figure 3. The correlation between the heavy metals are determined which is shown in figure 4.

The correlations between the heavy metals in the different brands of Naswar was determined by Least Significant Difference Test in table 3 and PCA Plot in figure 5. The heavy metals are shown positive correlation as well as negative correlation with each other. The positive correlation represents that there is strong relation between heavy metals present in different brands of Naswar and negative correlation showed weak association between heavy metals present in different brands of Naswar. The significance of heavy metals is determined by LSD Test at 0.05 in various brands of Naswar. If the mean difference in the samples are less than LSD value then it has become non-significant and if the LSD value within the samples are greater than LSD value then it has become significant.

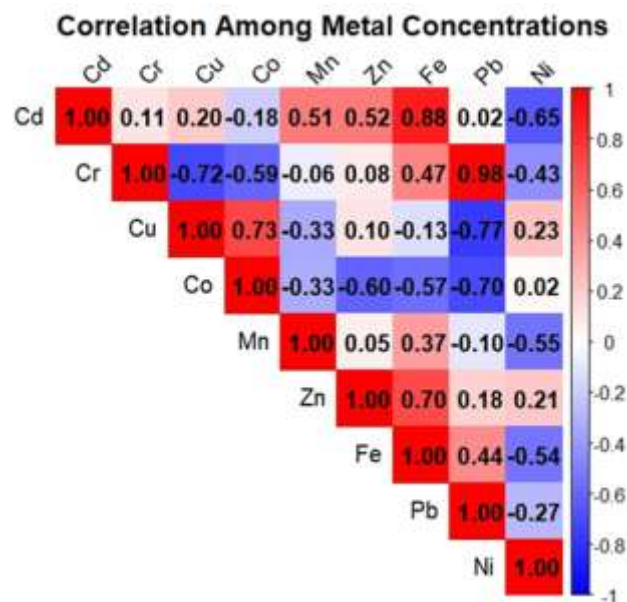


Fig. 4. Correlation plot between the heavy metals present in Naswar products (n=5)

TABLE 3. Significance of the heavy metals in the Naswar products by LSD at 0.05

Sr.No	Parameters	Mean Differences	Status	Least Significant Difference (α significance at 0.05)	Remarks P value ($P < 0.05$)
1	Cd vs Cr	-5.18	Negative correlation	14	Non-Significant
2	Cd vs Cu	-16.04	Negative correlation	14	Non-Significant
3	Cd vs Co	-10.86	Negative correlation	14	Non-Significant
4	Cd vs Mn	-0.058	Negative correlation	14	Non-Significant
5	Cd vs Zn	5.122	Positive correlation	14	Non-Significant
6	Cd vs Fe	15.982	Positive correlation	14	Significant
7	Cd vs Pb	-43.04	Negative correlation	14	Non-Significant
8	Cd vs Ni	-37.86	Negative correlation	14	Non-Significant
9	Cr vs Cu	-27	Negative correlation	14	Non-Significant
10	Cr vs Co	-42.982	Negative correlation	14	Non-Significant
11	Cr vs Mn	-49.8	Negative correlation	14	Non-Significant
12	Cr vs Zn	-44.62	Negative correlation	14	Non-Significant
13	Cr vs Fe	-33.76	Negative correlation	14	Non-Significant
14	Cr vs Pb	-49.742	Negative correlation	14	Non-Significant
15	Cr vs Ni	-6.76	Negative correlation	14	Non-Significant
16	Cu vs Co	-39.46	Negative correlation	14	Non-Significant
17	Cu vs Mn	-34.28	Negative correlation	14	Non-Significant
18	Cu vs Zn	-23.42	Negative correlation	14	Non-Significant
19	Cu vs Fe	-39.402	Negative correlation	14	Non-Significant
20	Cu vs Pb	3.58	Positive correlation	14	Non-Significant
21	Cu vs Ni	10.34	Positive correlation	14	Non-Significant
22	Co vs Mn	-1.12	Negative correlation	14	Non-Significant
23	Co vs Zn	4.06	Positive correlation	14	Non-Significant
24	Co vs Fe	14.92	Positive correlation	14	Significant
25	Co vs Pb	-1.062	Negative correlation	14	Non-Significant
26	Co vs Ni	41.92	Positive correlation	14	Significant
27	Mn vs Zn	48.68	Positive correlation	14	Significant
28	Mn vs Fe	38.34	Positive correlation	14	Significant
29	Mn vs Pb	-3.76	Negative correlation	14	Non-Significant
30	Mn vs Ni	1.42	Positive correlation	14	Non-Significant
31	Zn vs Fe	12.28	Positive correlation	14	Non-Significant
32	Zn vs Pb	-3.702	Negative correlation	14	Non-Significant
33	Zn vs Ni	46.04	Positive correlation	14	Significant
34	Fe vs Pb	46.04	Positive correlation	14	Significant
35	Fe vs Ni	35.7	Positive correlation	14	Significant
36	Pb vs Ni	-2.64	Negative correlation	14	Non-Significant

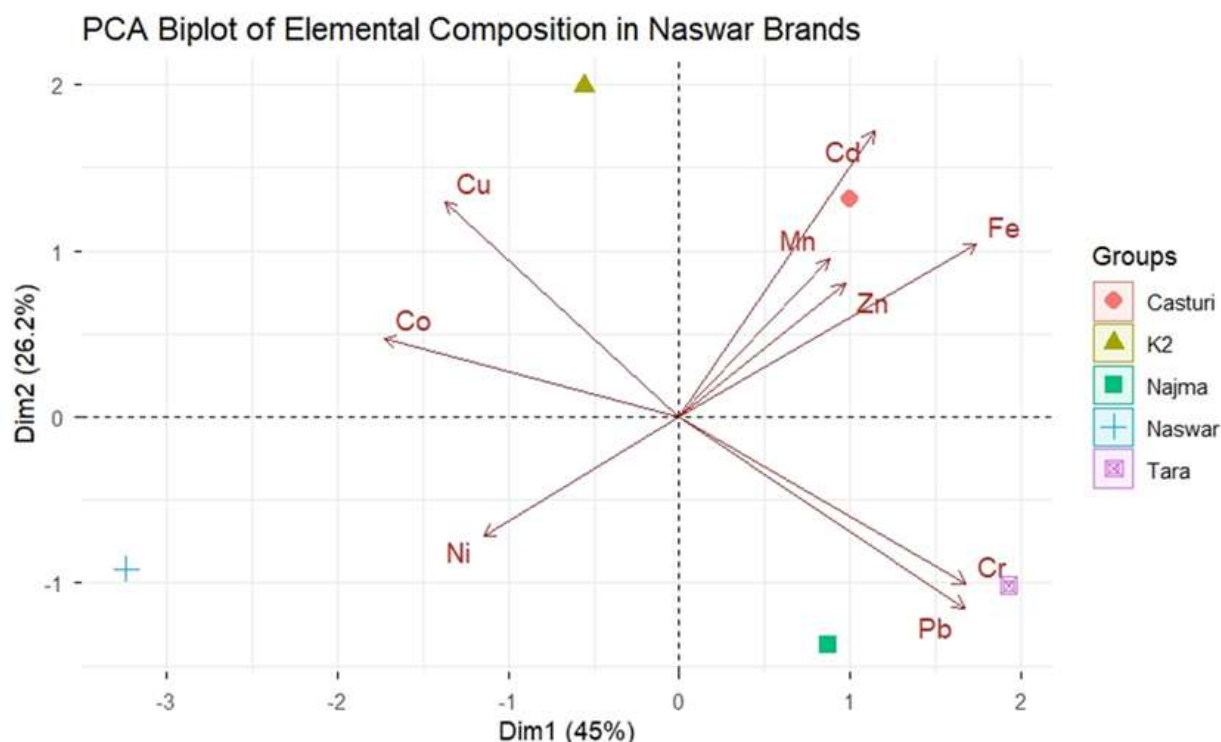


Fig. 5. PCA plot of the heavy metals in the Naswar products

IV. DISCUSSION

The results of the heavy metals (Cd vs Fe, Co vs Fe, Co vs Ni, Mn vs Zn, Mn vs Fe, Zn vs Ni and Fe vs Pb) showed that they have strong relationship between them and they support to each other in the environment while the other heavy metals within the samples have weak association and they are independently accumulated in the tobacco paan due to soil impurities and other environmental pollution factors. Highlighting that Cadmium (Cd), Cobalt (Co), Iron (Fe), Zinc (Zn), Manganese (Mn), Nickel (Ni) and Lead (Pb) are toxic pollutants that negatively impact their health, alongside other harmful chemicals in Naswar by Least Significant Differential Test (LSD).

There are certain limitations to the current study, despite the fact that it is the first and most thorough examination of the chemical composition of the most well-known naswar brands from all of Punjab's administrative regions. First, the study's naswar samples were chosen mostly on the basis of popularity; not all brands were included, which significantly reduced the study's findings' ability to be applied to other contexts. Second, we were unable to evaluate how changes in shelf life and storage affected the composition of naswar.

We have identified Nine distinct components in addition to the organic contents, six of which are carcinogenic to humans. These results also support earlier findings that tobacco products contain carcinogenic components [14]. Toxic heavy metals and other dangerous substances have previously been found in tobacco products worldwide by a number of investigations [15, 16, 17]. It's crucial to remember that the heavy metal content of each sample varied greatly from one another, indicating variations in the quantity and chemical

composition of the materials as well as in how the product was processed, wrapped, and stored. It is unknown if this is due to the usage of additives, pH, storage conditions, or direct contamination of tobacco leaves.

V. CONCLUSION

Tobacco products contains a number of hazardous, poisonous heavy metals that can harm both the environment and human health. Smokers are exposed to these metals. The soil, fertilizers, irrigation, manufacturing methods, types of additives, and other elements are directly linked to the origins of these heavy metals. Nine metals (Cd, Cr, Cu, Co, Mn, Zn, Fe, Pb, and Ni) were examined in a variety of Naswar items in the current study. For global health organizations like UNICEF and WHO, as well as health agencies like the Ministry of Health and the Ministry of Environment, this study offers fresh data. Furthermore, the results provide vital information for smokers in Pakistan by emphasizing that, in addition to other hazardous compounds found in Naswar; Cadmium (Cd), Cobalt (Co), Iron (Fe), Zinc (Zn), Manganese (Mn), Nickel (Ni) and Lead (Pb) are toxic pollutants that have a severe impact on human health.

Disclaimers

Altaf Hussain conducted the sample collection and laboratory analysis.

Author Contributions

Usman Ahmad and Altaf Hussain designed the study, performed the statistical analysis, and wrote the manuscript.

Muhammad Alam, Aiman Ali, Rehmat Wali and Irfan Mumtaz assisted with data interpretation and manuscript revision

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability

Data and supplements available on request to the

	corresponding author.
Funding	NA
Ethical Approval	Institutional Review Board (IRB) of University of Sargodha, Pakistan
Trial Registration	NA
Acknowledgments	Department of Botany at the University of Sargodha

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