ORIGINAL ARTICLE

To Evaluate the Concentration of Heavy Metals in Different Shisha Products Present in Pakistan and Associated Risk Factors on Public Health

Usman Ahmad[1], Altaf Hussain[2], Rahmat Wali[3], Abdul Mateen[4]

[1,3,4]Department of Biology, PMAS Arid Agriculture University Rawalpindi – 46300, Pakistan;[2]Department of Botany, University of Sargodha, Sargodha – 40100, Pakistan

Corresponding Author: Usman Ahmad, Department of Biology, PMAS Arid Agriculture University Rawalpindi – 46300, Pakistan

Email: usmanahmad239@yahoo.com

Received: 15-05-2024
Revision : 20-08-2024
Accepted: 27-09-2024

ABSTRACT

Background: Tobacco Shisha 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: To evaluate the concentrations of heavy metals in various brands of tobacco Shisha present in Pakistan and to assess their implications for public health.

Methods: Five different brands of tobacco cigarettes commonly used in Pakistan— AF. Apple, AF. Gum, AF. Cherry, AF. Banana and AF. Rose —were 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 (H_2SO_4) and hydrogen peroxide (H_2O_2) through a wet digestion method. The digested samples were then analyzed using Atomic Absorption Spectrophotometry (AAS-5000, Perkin-Elmer Corp., 1980) to determine the concentrations of heavy metals. Statistical analysis was conducted using SPSS version 25, and significance was assessed using T-Test at a 0.05 significance level. The standard reference used for all heavy metal detections were Standard Reference Material (SRM 1570) from National Institute of Standard and Technology. The results were deviated in the range ±2% of the reported value.

Results: The mean concentrations of heavy metals in the tobacco samples were as follows: Cd (0.23 to 0.27 ±0.018), Cr (4.63 to 4.78 ± 0.071), Cu (14 to 17 ± 1.30), Co (0.35 to 0.40 ± 0.024), Mn (43 to 46.8 ± 1.48), Zn (35.1 to 40.2 ± 2.55), Fe (34.8 to 37.9 ± 1.44), Pb (1.20 to 1.37 ± 0.061) and Ni (4.00 to 4.39 ± 0.166).

Conclusion: This study provides the relevant data of tobacco shisha products for the all concerned departments. Highlighting that Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) are toxic pollutants that negatively impact their health, alongside other harmful chemicals in shisha.

Keywords: *Flame AAS, Toxic metals, Human Health, Correlation, a significance (T-Test), Pakistan*

INTRODUCTION

Even though tobacco products are detrimental to human health, they are freely and legally available on the market. Smoking contains a variety of chemicals that are breathed into the respiratory system and have an impact on various internal organs. There were 400 recognized carcinogenic chemicals in tobacco smoke in 1959 today; there are over 4000 harmful compound.[1]

Through skin contact, ingestion of food, drinking water, and the air we breathe, harmful metals can enter the body. Unnoticed and undiagnosed, they may be the cause of harm to the pancreas, liver, kidneys, bones, and central nervous system. Sodium retention, which causes high blood pressure, heart disease, and mental retardation, is caused by heavy metals.[2] In addition to major illnesses and mortality, heavy metal ions can induce aging.[3]

The Middle East has not done a thorough investigation on the amounts of heavy metals in tobacco products used in shish kabob. During the growing season, tobacco leaves are a rich source of harmful heavy metals. Both passive smoking and the usage of tobacco products have a direct negative impact on the health of smokers and contribute to the buildup of heavy metals in the environment.[4]

The World Health Organization stated that the amount of smoke inhaled from a single cigarette is 100–200 times less after an hour-long hookah smoking session. Tobacco smoke contains toxic components such carcinogens, heavy metals, and carbon monoxide.[5] The traditional shisha has no filters, which is responsible for introducing a huge amount of hazardous heavy metals into the lungs.[6]

Heavy metal concentrations in tobacco products are determined by a variety of factors, including soil type and pH, pesticide and fertilizer use, and so on. Metals includeing iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) are essential micronutrients for plant growth and yield.[7]

The bioavailability of metals to plants is largely determined by soil variables; different species or varieties growing in the same soil may absorb metals at different rates.[8]

Other metals, like lead, cadmium, and nickel, on the other hand, are not necessary for plant growth but can have detrimental effects on the environment and human health.[9] Moreover, several hazardous metals are known carcinogens. Group 1 human carcinogens include cadmium (Cd), chromium (Cr), arsenic (As), beryllium (Be), and nickel (Ni).[10] The molasses and glycerin in shisha dilute the amount of hazardous ingredients per smoked piece. It has been shown that the number of harmful metals those smokers inhale that reaches their respiratory systems is the most crucial indicator. The objectives of present study are to determine the toxicities in various brands of Pakistani shisha and to determine the correlation of heavy metals are present in various brands of shisha.

METHODS

It was one year study conducted at University of Sargodha, Pakistan after the approval of ethical committee of the Sargodha University, Punjab. The Non-probability 'convenience sampling' technique was used in this study. Using the WHO sample size calculator, the sample size for this study was 90, based on previous study's (50%) population parameter, 95 % confidence interval, and margins uncertainty (5 percent).

$$N = \frac{p(1-p)\,z2}{E2}$$

All the local brands which are commonly used and available in local markets of Pakistan are considered in this study. All those brands which are imported and those tobacco paan products which are openly sell in local markets of Pakistan which are not consenting to take a part of the study.

The study was conducted to evaluate the concentrations of heavy metals in various brands of tobacco shisha marketed in Pakistan and their implications for public health. The brands analyzed included AF. Apple, AF. Gum, AF. Cherry, AF. Banana and AF. Rose. Samples of these brands were collected from local markets across different regions of Pakistan. Each brand was represented by multiple samples to ensure comprehensive analysis and reliable results. For sample preparation, one gram of each tobacco 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.[1]

The standard reference used for all heavy metal detections were Standard Reference Material (SRM 1570) from National Institute of Standard and Technology. The results were deviated in the range ±2% of the reported value.

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).

RESULTS

The statistical analysis of the collected data was performed using SPSS software, version 25. The results were expressed as mean values with standard deviations (SD). The significance of the differences between the heavy metal concentrations in the different cigarette brands was assessed using a T test at a 0.05 significance level. Additionally, correlation matrices were used to explore the relationships between the various heavy metals in the tobacco samples.

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 shisha found in Pakistan, their mean values were recorded in Table 1 and Figure 1.

The presences of different heavy metals of tobacco shisha is a source of toxic metals which was recorded in ranges as Cd (0.23 to 0.27 ±0.018), Cr (4.63 to 4.78 ± 0.071), Cu (14 to 17 ± 1.30), Co (0.35 to 0.40 ± 0.024), Mn (43 to 46.8 ± 1.48), Zn (35.1 to 40.2 ± 2.55), Fe (34.8 to 37.9 ± 1.44), Pb (1.20 to 1.37 ± 0.061) and Ni (4.00 to 4.39 ± 0.166) reported in Table 2.

The present study of heavy metals is compared with the reported values in different brands of the Dokha and shisha as shown in Table 3. It indicated that heavy metals are correlated with each other and responsible to accumulate in smoke of shisha which are harmful for human health.

Table 1: Fluctuation of Various Heavy Metals is Present in Different Brands of Shisha

Various Brands of tobacco products	Concentration of different heavy metals present in Shisha								
	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
AF. Apple	0.27 ± 0.018	4.63 ± 0.071	16.1 ± 1.30	0.35 ± 0.024	46.8 ± 1.48	35.8 ± 2.55	37 ± 1.44	1.27 ± 0.061	4.39 ± 0.166
AF. Gum	0.26 ± 0.018	4.8 ± 0.071	15.8 ± 1.30	0.35 ± 0.024	43 ± 1.48	40 ± 2.55	37.9 ± 1.44	1.30 ± 0.061	4.18 ± 0.166
AF. Cherry	0.24 ± 0.018	4.78 ± 0.071	14.1 ± 1.30	0.40 ± 0.024	45.5 ± 1.48	40.2 ± 2.55	37.5 ± 1.44	1.37 ± 0.061	4.00 ± 0.166
AF. Banana	0.23 ± 0.018	4.78 ± 0.071	14 ± 1.30	0.39 ± 0.024	43.8 ± 1.48	40.1 ± 2.55	35 ± 1.44	1.20 ± 0.061	4.10 ± 0.166
AF. Rose	0.27 ± 0.018	4.7 ± 0.071	17 ± 1.30	0.35 ± 0.024	45.2 ± 1.48	35.1 ± 2.55	34.8 ± 1.44	1.28 ± 0.061	3.98 ± 0.166
Mean Concentration (n=5) ± (S.D)									

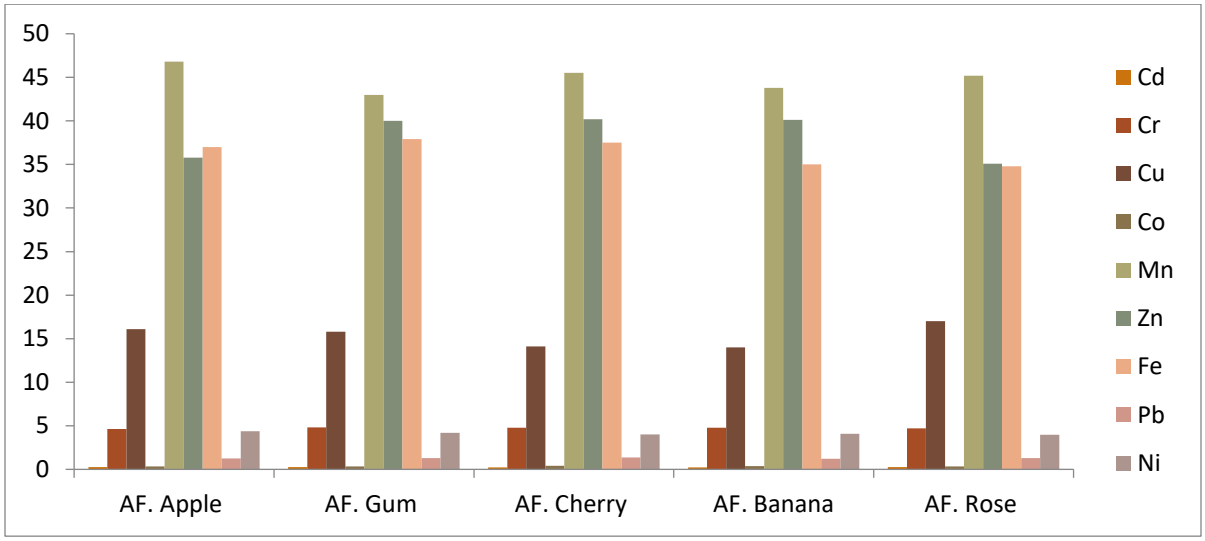

Figure 1: Fluctuation of Various Heavy Metals is Present in Different Brands of Shisha

Table 2: Minimum and Maximum Value of Heavy Metals is Present in Different Brands of Shisha

Heavy Metals in different brands of Shisha	Concentration of various heavy metals		
	Minimum Value	Maximum Value	Ranges
	mg/kg	mg/kg	mg/kg
Cd	0.23	0.27	0.23 to 0.27 ±0.018
Cr	4.63	4.78	4.63 to 4.78 ± 0.071
Cu	14	17	14 to 17 ± 1.30
Co	0.35	0.40	0.35 to 0.40 ± 0.024
Mn	43	46.8	43 to 46.8 ± 1.48
Zn	35.1	40.2	35.1 to 40.2 ± 2.55
Fe	34.8	37.9	34.8 to 37.9 ± 1.44
Pb	1.20	1.37	1.20 to 1.37 ± 0.061
Ni	4.00	4.39	4.00 to 4.39 ± 0.166
Mean Concentration (n=5) ± (S.D)			

Table 3: Comparison of Heavy Metals with Reported Values of Different Brands of Shisha

Parameters	Concentration of Various Heavy Metals			
	Present Study		Reported Values in different brands of Dokha and Shisha (Mahboub *et al.*, 2019)	
	Minimum Value	Maximum Value	Minimum value	Maximum value
	mg/kg	mg/kg	µg/g	µg/g
Cd	0.23	0.27	0.96	58.46
Cr	4.63	4.78	0.18	58.16
Cu	14	17	8.68	24.00
Co	0.35	0.40	0.11	25.05
Mn	43	46.8	10.52	159.24
Fe	34.8	37.9	20.73	654.70
Pb	1.20	1.37	5.56	468.62
Ni	4.00	4.39	2.04	59.00

The correlations between the heavy metals in the different brands of tobacco shisha are recorded in the table 4. The heavy metals are correlated with each other at $P < 0.05$ by T-Test (α significance at 0.05 by Two Tailed Test). The heavy metals in the different brands of tobacco shisha are shown in Table 4.

Table 4: Correlation between Heavy Metals in Different Brands of Shisha by Flame-AAS**.**

Correlation Matrix (P < 0.05)									
	Cd	***Cr***	***Cu***	***Co***	***Mn***	***Zn***	***Fe***	***Pb***	***Ni***
Cd	1								
Cr	-0.6847	1							
Cu	0.956296	-0.60028	1						
Co	-0.91748	0.530397	-0.92768	1					
Mn	0.386259	-0.83376	0.232009	-0.07011	1				
Zn	-0.81071	0.890428	-0.83374	0.676178*	-0.64211*	1			
Fe	0.106796	0.18495*	-0.1335*	-0.032	0.002094	0.389219	1		
Pb	0.139708	0.156748	-0.0125	0.154532	0.235826	0.139438	0.664244	1	
Ni	0.381054	-0.54679	0.198792	-0.46535	0.312222	-0.22691	0.385783	-0.26857	1
Significance of correlation between heavy metals in different brands at 0.05 by T Test*									

The heavy metals are shown positive correlation as well as negative correlation with each other reported in table 4. The positive correlation represents that there are strong relation between heavy metals present in different brands of tobacco shisha and negative correlation showed weak association between heavy metals present in different brands of tobacco shisha. These results are also supported by quantity of heavy metals in different types of tobacco in shisha and cigarette brands in Iraq.[11]

The significance of heavy metals is determined by T Test at 0.05 in various brands of tobacco shisha. For the sake of accuracy data were recorded as mean ± S.D, the statically significant value of sample is less than 0.05 ($P < 0.05$) while the P value of sample is greater than ($P < 0.05$) than there is not significant correction present between various brands of tobacco cigarette. The significance of heavy metals present between different brands of shisha is P value which is reported in Table 5.

Table 5: Significance of the Heavy Metals in the Shisha by T-Test (Two Tailed Test at 0.05)

Sr.No	Parameters	Correlation	Status	T-test (Two Tailed test α significance at 0.05)	Remarks P value (P < 0.05)
1	Cd vs Cr	-0.6847	Negative correlation	0.45	Non significant
2	Cd vs Cu	0.956296	Positive correlation	0.35	Non significant
3	Cd vs Co	-0.91748	Negative correlation	0.32	Non significant
4	Cd vs Mn	0.386259	Positive correlation	0.37	Non significant
5	Cd vs Zn	-0.81071	Negative correlation	0.23	Non significant
6	Cd vs Fe	0.106796	Positive correlation	0.03	Non significant
7	Cd vs Pb	0.139708	Positive correlation	0.35	Non significant
8	Cd vs Ni	0.381054	Positive correlation	0.10	Non significant
9	Cr vs Cu	-0.60028	Negative correlation	0.32	Non significant
10	Cr vs Co	0.530397	Positive correlation	0.36	Non significant
11	Cr vs Mn	-0.83376	Negative correlation	0.41	Non significant
12	Cr vs Zn	0.890428	Positive correlation	0.27	Non significant
13	Cr vs Fe	0.18495	Positive correlation	0.04	Significant
14	Cr vs Pb	0.156748	Positive correlation	0.37	Non significant
15	Cr vs Ni	-0.54679	Negative correlation	0.11	Non significant
16	Cu vs Co	-0.92768	Negative correlation	0.21	Non significant
17	Cu vs Mn	0.232009	Positive correlation	0.25	Non significant
18	Cu vs Zn	-0.83374	Negative correlation	0.15	Non significant
19	Cu vs Fe	-0.1335	Negative correlation	0.02	Significant
20	Cu vs Pb	-0.0125	Negative correlation	0.30	Non significant
21	Cu vs Ni	0.198792	Positive correlation	0.46	Non significant
22	Co vs Mn	-0.07011	Negative correlation	0.37	Non significant
23	Co vs Zn	0.676178	Positive correlation	0.04	Significant
24	Co vs Fe	-0.032	Negative correlation	0.42	Non significant
25	Co vs Pb	0.154532	Positive correlation	0.11	Non significant
26	Co vs Ni	-0.46535	Negative correlation	0.15	Non significant
27	Mn vs Zn	-0.64211	Negative correlation	0.04	Significant
28	Mn vs Fe	0.002094	Positive correlation	0.41	Non significant
29	Mn vs Pb	0.235826	Positive correlation	0.12	Non significant
30	Mn vs Ni	0.312222	Positive correlation	0.15	Non significant
31	Zn vs Fe	0.389219	Positive correlation	0.48	Non significant
32	Zn vs Pb	0.139438	Positive correlation	0.12	Non significant
33	Zn vs Ni	-0.22691	Negative correlation	0.11	Non significant
34	Fe vs Pb	0.664244	Positive correlation	0.35	Non significant
35	Fe vs Ni	0.385783	Positive correlation	0.12	Non significant
36	Pb vs Ni	-0.26857	Negative correlation	0.13	Non significant

DISCUSSION

The outcome suggests that Fe had a noteworthy correlation with Cr and Cu. Similarly, Zn demonstrated the importance relationship with Mn and Co. The positive connection between Cr and Fe suggests a strong link and accumulation of heavy metals together these results are supported by reported values of traditional and flavored tobacco hookah in Iran.[12] The high link and accumulation of heavy metals with one another is indicated by the positive correlation between Zn and Co. Although there is a weak correlation and no relationship between Zn and Mn, they are not dependent on one another. The residual correlation between the heavy metals (Cd, Cr, Cu, Co, Mn, Zn, Fe, Pb, Ni) in various shisha brands is not statistically significant, implying that there is only a weak association between them and that they depend on a number of variables, including the pH and type of soil, the use of fertilizers and pesticides, among other things.[13, 14]

Cadmium is a very poisonous mineral that can have a negative impact on health. It can cause bone mineralization through renal failure or cause serious lung damage when levels of the mineral are elevated.[15] Various brands of shish have varying levels of calcium content, ranging from 0.23 mg/kg to 0.27 mg/kg ± 0.018 (S.D) is also supported by cadmium, nickel and lead content of tobacco and cigarette smoke.[16]Although cobalt is a necessary element, larger concentrations of it can be poisonous and have negative effects on the neurological system. In addition to being poisonous, nickel causes

lung fibrosis, kidney problems, and cardiovascular disorders and responsible for cancer.[17]

Another poisonous element that is bad for human health is chromium. It results in the development of ulcers that take months to heal and heal very slowly. Elevated levels of chromium may impede erythrocyte glutathione reed-ucates, hence decreasing the ability to convert met hemoglobin to hemoglobin. Moreover, the body becomes saturated with a very high concentration of iron that crosses the rate-limiting absorption stage. The brain, liver, and cardiac cells are all penetrated by these free irons. Additionally, lipid peroxidation brought on by free iron can seriously harm microsomes, mitochondria, and other cellular organelles, the potent human carcinogens, were greater than the recommended threshold set forth by WHO and FAO.[18] Various brands of shish contain varying amounts of Fe (34.8 mg/kg to 37.9 mg/kg ± 1.44 (S.D) this result is also supported by the analysis of heavy metals and PAHs in the waste resulting from hookah consumption.[19]

Lead toxicity, commonly referred to as lead poisoning, can be acute or persistent. Acute exposure might result in vertigo, hallucinations, lethargy, weariness, nausea, headaches, hypertension, stomach discomfort, and renal failure. Prolonged exposure to lead can cause birth deformities, mental retardation, psychosis, autism, allergies, dyslexia, hyperactivity, and weight loss, paralysis, weakening of the muscles, brain damage, kidney damage, and even death.[20]

Tobacco smoke 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, manufac-turing 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 Shisha items in the current study. Reducing the number of smokers and aiming for a healthy society are due to public awareness of heavy metals. International laws should be implemented in order to control the number of heavy metals present in tobacco products and to shield smokers from exposure to these metals. 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 shisha, iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) are toxic pollutants that have a severe impact on human health.

CONCLUSION

This information is crucial for health authorities and policymakers to develop strategies to mitigate the public health risks associated with tobacco shisha smoking. The findings also serve to raise awareness among smokers about the potential health hazards posed by the heavy metal content in shisha, thereby promoting informed choices and healthier lifestyles.

CONFLICT OF INTEREST / DISCLOSURE: Nil.

FUNDING SOURCE: Nil.

AUTHORS' CONTRIBUTION:

UA: Acquisition of data, manuscript writing, statistical analysis and data interpretation final approval of manuscript.

AH: Acquisition of data, data analysis and interpretation,

RW: Data analysis and interpretation, critical revisions

AM: Data analysis and interpretation, manuscript writing

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