Physicochemical Analysis of Different Cigarettes Brands Available in Pakistan

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Abstract
The exposure to nicotine, sugar and trace elements in tobacco are associated with health risks including inflammation, sensitization and carcinogenesis. The aim of this study was to investigate the physicochemical properties of tobacco used in the cigarette brands available in Pakistan. Physical properties such as moisture, volatile matters, ash, pH, conductivity, and total reducing sugar were measured using standard procedures. Heavy metals, toxic metals, and nicotine were determined using atomic absorption spectrophotometer, flame photometry, and liquid chromatography, respectively. Local, and local branded samples were found to be rich with iron and magnesium and safe in terms of lower copper content when compared with the branded samples and other reported data of different countries of the world. Significant differences (p < 0.05) have been observed in the values of ash, conductivity, iron, zinc, copper, nickel, cobalt, magnesium, lithium, sodium, and potassium in local, local branded and branded samples. The samples of different brands were discriminated and characterized on the basis of metal contents using statistical techniques such as multivariate analysis, and analysis of means. The properties of the local samples were found close to that of the local branded samples showing the same origin of tobacco leaves. On the basis of conducted study, it is concluded that more strict regulations are needed for import and export of cigarettes.

Keywords: Tobacco; Cigarette; Trace metals; Nicotine; Moisture; Volatile matter; Multivariate analysis

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Introduction

Pakistan is a country of about 180 million people. Per capita income of the country is 1245 $ in the year 2011-12. The tobacco industry contributes approximately the US $1 billion to the national economy. According to the Federal Board of Revenue (FBR), in the year 2007-08 the total cigarette market of the country was 78 billion sticks, of which 63 billion cigarettes were local legitimate brands while the remaining 15 billion cigarettes were smuggled. A number of steps has been taken at government level to discourage smoking. In September 2010, federal health ministry announced that a cigarette manufacturer should print pictorial health warning on a cigarette pack. But, still the smoking trend is an uprising. It is estimated that while at least 27.40% of males and 4.40% of females smoke in Pakistan, particularly in youth. The local market is flooded with a number of brands, imported comparatively expensive to cheap local branded and new brands are continuously entering into the market.

Tobacco smoke is a complex mixture of aerosol containing thousands of distinct organic and inorganic chemicals that are originally present or produced during heating, burning and chemical "cracking" of the cigarette. These include particulates (tar) of sticky solids, gases such as

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carbon monoxide, and volatiles. Most importantly, the smoke contains the addictive drug "nicotine" which is an alkaloid obtained from the leaves of tobacco plant, *Nicotina Tobacum Solanaceae*. It is highly toxic, carcinogenic and a powerful insecticide. Nicotine is recognized as the main harmful compound of tobacco. Chronic exposure to nicotine may cause coronary disease, peptic ulcer, reproductive disturbance, hypertension, fetal illness and birth defects [1]. Research has revealed that a cigarette contains about 40% tobacco, and 60% of non-tobacco contents [2]. Several attempts were also made to estimate and quantify the presence of organic constituents, from which polycyclic aromatic hydrocarbons present in cigarette smoke cause bone loss [3].

The presence of toxic metals in tobacco smoke is another reported class of carcinogen [4, 5]. Several heavy metals found in tobacco smoke, such as Cd, Cr, Pb, Zn, Ni, Cu, and Mg cause severe toxic effects in smokers [6-10]. It is reported that Cu and other metals were increased significantly in a cataractous human lens due to cigarette smoking [11]. Agha and Gokmen exhibited significant correlations between the number of daily and yearly cigarettes smoked with cadmium concentration in blood [12].

The distribution of toxic metals in different components of Pakistani and imported cigarettes were conducted by Kazi and coworkers using electrothermal atomic absorption spectrometry [6]. They determined toxic metals (Al, Cd, Ni and Pb) in filler tobacco, filter (before and after smoking) and ash. They found that the concentration of Al was in the range of 333–546 μg cigarette^{-1}, and a major amount of Al (97.3 % to 99.0 % of the total content) was left in ash of the cigarette. A reverse trend was observed for Cd (1.66–2.96 μg/cigarette) where only 15.00–31.30% of the total content was left in the ash. Ajab and her team evaluated the trace metals in tobacco of local and imported cigarette brands smoked in Pakistan by using atomic absorption spectrophotometer through microwave digestion [6, 8]. The results showed high concentrations of Mn, Cd and Zn (84.78, 0.525 and 14.34 μg/g) in imported cigarettes respectively. Certain elevated levels were observed for Co, Pb and Cu (3.34, 14.16 and 7.88 μg/g) in local brand

cigarettes. The consumption of tobacco products and the number of smokers have been increasing steadily in the country, so continuous improvement in the safety precaution, health concerned awareness needs steadily follow up of the toxicity of the cigarettes to make awareness and its impact not only on smokers but the society as well. The aim of this study is to conduct a more detailed assessment of toxicants in the cigarettes of various brands including, local, local branded and branded, available in the Pakistani market. Physicochemical parameters like pH, conductivity, ash content, volatile matters, moisture and metals were studied. Assessment was also based on the analysis of organic constituents, namely nicotine and reducing sugars. Comparison was made between the results of different brands to determine their relative toxicity level. The obtained data has been treated statistically using multivariate approach.

Materials and Methods
Sampling

Total 42 brands of cigarettes (8 local, 13 local branded, and 21 branded) commonly smoked in Pakistan were purchased from local markets of Karachi, the biggest city of Pakistan (around 20 million population). The origins of the samples were from different production areas of the world. A composite sample of each brand was made by mixing of 20 cigarettes taken randomly from four different batches (5 cigarettes from each pack of the same brand with different batch number). The weight of tobacco samples was measured ranging from 595–685 mg per cigarette.

Proximate analysis

Moisture, volatile matter, and ash content of the tobacco samples were estimated by thermogravimetric analyzer (TGA-2000, Navas Instruments, Spain) using ASTM method D 5142–90. Weight loss at 105 °C was used to measure moisture in nitrogen environment until a constant weight is obtained. Volatile matter was estimated by measuring the loss in weight of the sample heated at 950 °C for 7.0 minutes in a rigidly controlled nitrogen environment. Ash was determined by measuring the mass of the residue remaining after complete combustion of samples at 750 °C in the presence of oxygen.

pH, conductivity, and total reducing sugar measurements

The pH, conductivity, and total reducing sugar (TRS) was measured using the methods described by Elson *et al*. [13]. For pH and conductivity measurements, 2.00 g±0.05 g sample was boiled with 20 ml distilled water in a 50 ml Erlenmeyer flask. After cooling, sample was stirred on a mechanical shaker for 30 minutes. The flasks were kept in dark for an additional hour and swirled manually at the time of removal from the dark and decanted the supernatant into a 10 ml polystyrene beaker. The pH and conductivity of the samples were measured by pH meter (Orion 520A) and conductivity meter (Jenway 4320) respectively.

Sample assay for TRS was made by boiling 500-1000 mg of sample with 0.80-1.00 g charcoal powder, 20 ml of 20 % sorbitol solution and 30-40 ml of 0.05 M HCl in a conical flask. After cooling, the solution was transferred into a 100 ml volumetric flask and adjusted to volume with 0.05 M HCl. Solution was filtered into 100 ml conical flask through Whatman No. 44 filter paper. After discarding initial 5 ml of the filtrate, 2 ml was adjusted to volume with 0.05 M HCl in a 25 ml volumetric flask. 2 ml from each sample was transferred into separate test tubes along with blank. 15 ml of potassium ferricyanide alkaline solution was added in each and pre-heated to 95±1 °C for 20 minutes and then cooled. The baseline of spectrophotometer was adjusted using 0.05 M HCl solution at 420 nm. The absorbance of each standard solution and test solutions were recorded along with the blank one using Uv-vis spectrophotometer 160 (Shimadzu).

Nicotine

A reversed phase ion-pair liquid chromatographic method was used for the determination of nicotine in commercial tobacco products [14]. Sample assay was made by shaking 0.40-0.80 g of grinded tobacco with 40-60 ml distilled water in 100 ml volumetric flask for 1 hour. The volume was adjusted with distilled water and filtered in a conical flask through Whatman filter paper No. 44. Initial 10 ml of filtrate was discarded. 10 ml from the remaining filtrate was diluted with distilled water and adjusted to volume in a 25 ml volumetric flask. Samples were filtered in a HPLC vials through 0.45-micron filter paper using swinage.

The HPLC (Shimadzu, class 10A Vp) was equipped with pump, Nucleosil column 150×4.6 mm, C18. The mobile phase used a mixture of methanol and buffer pH 7.0. UV maximum absorption for nicotine was observed at 262 nm. Flow rate was set at 1.0 ml min^{-1}. Aliquots of 20 μl were injected into chromatograph. Two replicates of each type of cigarette extract were auto injected in HPLC. Results with <5% relative standard deviation were considered as acceptable.

Metal analysis

Mineral metals (Na, K and Li) and trace metals (Fe, Zn, Mg, Mn, Cu, Co, Cr and Ni) were analyzed by flame photometer (Corning 410) and atomic absorption spectrophotometer (PE-2380), respectively. Sample assay was prepared by digesting a 0.40-0.80 g sample in a mixture of nitric acid and perchloric acid (10:4 v/v). The mixture was heated onto a water bath with constant shaking for one hour. After cooling, it was filtered and transferred into a 25 ml volumetric flask and the volume was adjusted up to the mark with deionized water. The samples were analyzed in triplicate. The accuracy of the results was controlled measuring two certified standard reference materials (Spectronic CombiCheck 30 and CombiCheck 40, Darmstadt, Germany) in a series after every seven samples. The reference values of recommended daily dietary intake and toxic intake of each metal were used for comparison [15].

Statistical analysis

Single factor analysis of variance (ANOVA) was performed with Excel software (Microsoft, USA). Differences were considered statistically significant when $p < 0.05$. Multivariate analysis comprising of principal component analysis (PCA), cluster analysis and correlations study was performed using the XLSTAT function of Microsoft excel and Minitab 14.

Results and Discussion

The physicochemical characteristics of local, local branded and branded cigarettes are shown in the (Table 1). Proximate analyses (moisture, volatile matter, and ash content) are the important quality parameters of tobacco. The moisture content in local, local branded and branded cigarettes are ranged as 8.65–11.63%, 8.42–11.43%, and 8.28–13.09% respectively. Generally tobacco is stored up to 3 years for making a good quality cigarette. During the period, it acquires an appropriate value of moisture and its taste has been improved significantly. The suitable amount of moisture in tobacco should be from 10–13% for the manufacturing of a cigarette. Lower moisture leads to unfavorable condition for cigarette making, while high moisture leads to moldy tobacco. The average moisture content in tobacco was estimated as 9.68, 9.34, and 10.44% for local, local branded and branded samples respectively. An appropriate amount of moisture content in 50% branded cigarettes compared to the other two brands reflects the careful monitoring and treatment of the crop in proper timing during the development period. Local and local branded samples with lower moisture content may add humectants such as glycerol and propylene glycol in tobacco which not only increase the moisture holding-capacity but consequently aid in processing of tobacco [16].

A volatile matter percentage is indeed the best criterion to measure the combined sensory and physiological strength of tobacco. It assesses the organoleptic characteristics of tobacco, responsible for its flavor [17]. Moreover, most of the volatile organic species are known toxicants in cigarette smoke [18]. A large variation in the percentages of volatile matter has been observed in the samples. It was estimated in the range of 65.18– 69.91%, 65.15–72.97%, and 65.62– 76.43% for local, local branded and branded samples respectively. A minimum amount of volatile matter has been observed in the tobacco sample of Melburn (65.15 %). Pine Lights (Super Slim) Low Tar shows maximum amount of volatile matter (76.43 %).
Ash content is a measure of the inorganic and mineral constituents of tobacco. The average value of ash content in the local, local branded and branded samples were estimated as 13.27, 11.83, and 11.49% respectively. The ash content of local cigarettes is higher than the other two brands. It is attributed to the cultivation of a crop in mineral rich soil [19, 20]. Conductivity estimated as 8.13, 6.48, and 6.87 $\mu S/cm^1$ in an aqueous extract of local, local branded and branded specimens. Conductivity of the local samples was significantly higher than the other two brands. The tobacco samples of all the studied brands were found to have the acidic nature (pH 4.9 – 5.4).

TRS was measured as 3.38–4.02 mg/g (an average of 3.67 mg/g), 3.59–4.79 mg/g (average 3.94 mg/g), and 3.13 - 5.87 mg/g (average 3.73 mg/g) in local, local branded, and branded samples respectively. The Bay Side was found to the lowest amount of TRS (3.13 mg/g) and ESSE Lights has the highest one (5.87 mg/g).

Nicotine, the most hazardous component of tobacco was estimated as 13.20–16.66 mg/g, 11.81–18.15 mg/g, and 10.56–19.18 mg/g for the local, local branded and branded samples. It is important to noteworthy that Mild Seven Charcoal Filter showed the lowest amount of this toxic component (10.58 mg/g) and Benson & Hedges (Special Filter) showed the maximum amount (19.18 mg/g) among studied samples. Nicotine content in a local cigarette was found in the range of 13.20–16.66 mg/g with a mean value of 14.54 mg/g. A significant difference (p = 0.70) was observed in the local branded samples (range 11.81–18.15 mg/g, average 13.84 mg/g) and branded samples (range 10.56–19.18 mg/g, average 14.08 mg g^{-1}). The amount of nicotine in the samples was found to be many folds higher than the accepted range (0.6 mg/cigarette). Benson & Hedges (Special Filter) shows the highest amount of 19.18 mg/g. Nicotine in the cigarettes marketed in Pakistan was almost double as shown from the data reported in Jordan cigarettes (Imported) and four times greater than Jordan local brands [21]. It shows that the samples marketed in Pakistan are more injurious to health compared to Jordan cigarettes as far as nicotine content is concerned.

Table 1. Physicochemical Properties of tobacco samples in different brands of cigarettes.

Type	Sample Codes	Sample Names	TRS (mg/g)	Nicotine (mg/g)	pH	Conductivity (µS/cm)	Moisture (%)	Volatile Matter (%)	Ash (%)
Local	L1	K2	3.84±0.11	15.35±0.71	5.30±0.02	9.64±0.01	9.14±0.22	68.35±1.21	14.67±1.07
	L2	Royal	3.41±0.22	13.92±0.44	5.10±0.03	8.61±0.02	8.95±0.24	67.11±1.22	13.54±0.89
	L3	No. 6	3.51±0.14	13.19±0.82	5.00±0.04	8.26±0.02	9.51±0.32	69.25±1.20	12.68±0.84
	L4	Embassy Filter	3.38±0.11	16.66±0.43	5.10±0.02	7.23±0.02	9.25±0.33	69.29±1.45	12.27±0.44
	L5	Wonder	3.68±0.32	15.49±0.74	5.10±0.02	7.56±0.02	8.65±0.46	66.79±1.21	15.66±0.27
	L6	Kisan	3.67±0.17	14.44±0.92	5.20±0.03	8.11±0.01	11.58±0.25	65.18±1.98	13.13±0.54
	L7	Weldon	3.85±0.11	13.44±0.83	5.20±0.04	8.06±0.03	11.63±0.24	66.78±1.41	11.35±0.87
	L8	Sakoon	4.02±0.15	13.82±0.62	5.10±0.02	7.54±0.03	8.74±0.32	69.91±1.85	12.86±0.88
Local Branded	LB1	Melburn	3.93±0.24	13.00±0.83	5.10±0.03	7.7±0.03	11.43±0.44	65.15±1.88	11.88±0.94
	LB2	Morven Gold	3.74±0.13	13.04±0.82	5.10±0.04	5.61±0.03	8.66±0.23	67.58±1.07	12.10±0.54
	LB3	Diplomat	3.87±0.23	13.47±0.72	5.10±0.02	6.38±0.04	9.31±0.32	67.37±1.21	12.24±0.85
	LB4	Gold Leaf	3.89±0.13	16.98±0.82	5.10±0.03	6.27±0.04	10.01±0.41	70.19±1.81	8.88±0.54
	LB5	Capstan International	4.79±0.20	14.23±0.93	5.10±0.04	5.58±0.04	8.92±0.32	72.93±1.74	11.17±0.53
	LB6	Capstan Filter	3.74±0.24	11.81±0.84	5.20±0.05	5.24±0.04	9.12±0.42	71.85±1.21	11.41±0.54
	LB7	Wills Navy Cut	4.51±0.24	13.56±0.71	5.20±0.04	6.26±0.03	8.52±0.25	68.46±1.11	13.09±0.54
	LB8	Gold Flake	3.85±0.32	11.85±0.75	5.10±0.03	7.02±0.03	9.28±0.24	68.89±1.01	11.26±0.56
	LB9	Gold Steek	3.66±0.22	18.15±0.73	5.20±0.02	6.5±0.04	9.44±0.32	67.99±1.04	12.06±0.52
	LB10	Euro	3.96±0.17	13.90±0.81	5.40±0.04	7.63±0.03	7.68±0.41	68.69±1.46	13.94±0.64
	LB11	Red & White	3.92±0.17	13.20±0.72	5.10±0.04	6.48±0.03	8.43±0.53	72.97±1.84	12.24±0.54
	LB12	Princeton	3.72±0.14	13.16±0.74	5.10±0.04	6.71±0.03	11.38±0.56	66.71±1.46	11.87±0.46
	LB13	Park Lane	3.59±0.12	13.53±0.81	4.90±0.03	6.85±0.04	9.34±0.32	69.81±1.34	11.60±0.48

Type	Sample Codes	Sample Names	TRS (mg/g)	Nicotine (mg/g)	pH	Conductivity (µS/cm)	Moisture (%)	Volatile Matter (%)	Ash (%)
Imported	I1	Marlboro (Lights)	3.73±0.22	12.95±0.82	5.10±0.03	5.72±0.04	9.08±0.41	68.59±1.54	11.54±0.58
	I2	Marlboro (Menthol)	3.44±0.24	13.23±0.72	5.20±0.03	6.46±0.04	9.83±0.22	68.62±1.56	11.40±0.97
	I3	Marlboro (Filter)	3.3±0.11	16.78±0.83	5.10±0.04	6.52±0.03	11.39±0.25	68.21±1.65	12.23±0.98
	I4	Silk Cut (Mild)	3.61±0.13	16.19±0.81	5.10±0.04	7.42±0.03	8.49±0.34	70.72±1.66	11.45±0.88
	I5	Silk Cut (Ultra Mild)	3.81±0.12	14.39±0.81	5.30±0.05	6.32±0.03	11.88±0.42	66.46±1.50	10.92±0.87
	I6	Silk Cut (Extra Mild)	3.79±0.14	13.83±0.71	5.10±0.04	6.31±0.04	12.08±0.52	66.75±1.04	11.90±0.84
	I7	Mild Seven	3.59±0.24	10.56±0.52	5.10±0.04	7.20±0.04	11.82±0.51	66.53±1.85	11.96±0.49
	I8	Mild Seven (Lights)	3.61±0.21	14.17±0.73	5.10±0.03	7.21±0.04	9.09±0.53	70.77±1.09	12.64±0.54
	I9	Hi Light (Special Mild)	3.55±0.12	11.99±0.63	5.00±0.03	8.4±0.04	12.11±0.36	67.71±1.47	11.57±0.54
	I10	Benson & Hedges	3.3±0.12	19.18±0.72	5.20±0.03	7.83±0.03	12.31±0.45	68.96±1.84	9.89±0.44
	I11	Benson & Hedges (Lights)	3.73±0.12	15.93±0.69	5.30±0.03	5.55±0.03	8.88±0.44	73.74±1.24	10.75±0.32
	I12	Dunhill (International)	3.32±0.21	13.14±0.88	5.00±0.04	8.13±0.04	8.72±0.31	69.37±1.35	12.01±0.98
	I13	Dunhill (Lights)	3.62±0.23	13.90±0.72	5.40±0.04	6.53±0.04	8.48±0.29	68.98±1.51	11.46±0.88
	I14	Gold Leaf	3.51±0.14	10.85±0.81	5.30±0.03	7.11±0.03	12.17±0.48	65.62±1.42	12.17±0.74
	I15	555	3.59±0.12	16.63±0.74	4.90±0.03	7.10±0.03	12.68±0.37	69.58±1.64	9.87±0.85
	I16	Pine (Lights Super Slim)	5.72±0.2	14.55±0.73	4.90±0.04	4.53±0.04	8.28±0.35	76.43±1.66	9.93±0.88
	I17	Pine (Lights)	3.36±0.12	12.39±0.82	5.00±0.05	6.32±0.02	9.33±0.38	73.28±1.43	10.87±0.87
	I18	ESSE Lights	5.87±0.13	14.11±0.83	5.00±0.02	4.96±0.02	13.09±0.45	71.63±1.08	11.10±0.84
	I19	Davidoff Classic	3.26±0.13	14.90±0.52	5.10±0.02	8.18±0.08	8.49±0.48	68.05±1.08	12.24±0.84
	I20	Bay Side	3.13±0.22	12.39±0.75	4.90±0.01	7.29±0.08	12.29±0.35	68.59±1.04	10.63±0.99
	I21	Captain Black (Cigar)	3.57±0.21	13.71±0.84	5.30±0.03	9.28±0.04	8.91±0.35	68.67±1.04	14.85±0.87

The results of mineral and trace metals are shown in the (Table 2). A large variation in metal contents has been observed in the tobacco cigarettes of different origins. Iron (Fe) is an essential element of red blood cells of human body. It is one of the least toxic trace elements. The recommended daily dietary and toxic intakes of Fe are 6-40, and 200 mg, respectively. Table 2 shows that the Fe content in local samples (599.22–915.75 μg/g) and local branded samples (407.12–752.04 μg/g) were considerably higher than the branded samples. Furthermore, it was also observed that the Fe content in a local and local branded sample was also substantially higher when compared to the reported data [22, 23]. This difference may be attributed to either the chemistry of soil or may be due to the favorable environmental conditions like pH, point of zero charge (pzc) which promotes the Fe uptake to the plant.

Zinc oxide impregnated charcoal is a constituent of cigarette filters which removes HCN and H_2S from tobacco smoke without affecting the flavor of tobacco [24]. The recommended daily dietary intake of zinc (Zn) is 5-40 mg, and the toxic intake of Zn is reported as 150-600 mg. Table 2 shows that the amounts of Zn were found higher in branded cigarettes (27.96-64.97 μg/g with a mean value of 38.16 μg/g) compare to the other brands. Significant difference (p = 0.0001) in the amount of Zn has been observed in local, local branded, and branded samples. Zn content of local and local branded samples are significantly less than the other data conducted in different countries like Germany (45.00 μg/g) [23], Korea and England (38.50 and 31.90 μg/g respectively) [25], Finland (49.7 μg/g) [26], Iran (18.10-42.20 μg/g) [27], Nigeria (24.59 μg/g) [28], and Jordan (34.30-107.30 μg/g) [29]. Magnesium (Mg) is an important element of muscles, bones, and blood of human body. It is non-toxic. The daily dietary intake of Mg is recommended as 250-380 mg. A significant difference (p = 9.71×10^{-5}) has been observed between the amount of magnesium in three brands. Its value is higher (average 54.17 μg/g) for local branded than local and branded cigarettes (53.67 and 42.26 54.17 μg/g, respectively). The results of Mg in Egyptian cigarettes are approximately 100 times higher than

the studied samples [30]. It may be due to chemistry of soil and the spray of pesticide during the propagation of tobacco plant and the use of wrapper containing Magnesium hydroxide [Mg $(OH)_2$].

Manganese (Mn) is an essential element of the cells human body. It is one of the least toxic trace elements. The daily dietary and toxic intakes of Mn are recommended as 0.4-10 mg, and 10-20 mg, respectively. It is evident from the Table 2 that Mn in branded samples (143.23 μg/g) was found to be three times higher than local and local branded tobacco (59.45 and 55.27 μg/g respectively). Optimum level of Mn in the local and local branded cigarettes reflect the quality of the tobacco leaf. These values are significantly less than the tobacco used in India [31], and Germany [32].

Nickel (Ni) is an essential element for limited biological activities. The daily dietary intake of Ni is recommended as 0.3-0.5 mg. However, its high dose (> 500 mg) is toxic and may be carcinogenic. The amount of Ni (3.94 μg/g) in the local branded tobacco is found lower than the tobacco used in France (4.82 μg/g) [30]. The Ni content in the tobacco of local (6.29 μg g^{-1}) and imported brands (7.39 μg/g) are lower than the tobacco used in Iran (10.00–30.00 μg/g) [27]. Copper (Cu) is an important element of muscles, bones, and blood cells of human body. The dietary intake of Cu is recommended as 0.50-6.00 mg/day. However, an intake of >250 mg/day is considered as toxic. Table 2 shows that the amount of Cu in tobacco of local and local branded samples was found to be 3.62 and 2.73 μg/g, respectively. Cu content in branded samples were estimated as 7.03–17.09 μg/g. These values are comparable with the data reported for India (18.00 μg/g) [22], Korea (7.70 μg/g) [23], England (13.00 μg/g) [25], Finland (15.60 μg/g) [26], Iran (5.18-17.60 μg/g) [27], Nigeria (5.98 μg/g) [28]; but significantly less than the tobacco samples used in Germany (21.70 μg/g) [23]. The high content of Cu in branded samples may be attributed to the excess use of copper based pesticides on tobacco crop during cultivation or the soil irrigated with copper contaminated water when the tobacco harvested

Table 2. Mineral Contents in the tobacco of different brands of cigarette samples.

Type	Sample Codes	Fe (µg/g)	Zn (µg/g)	Mg (µg/g)	Mn (µg/g)	Cu (µg/g)	Co (µg/g)	Cr (µg/g)	Ni (µg/g)	Na (mg/g)	K (mg/g)	Li (mg/g)
Local	L1	599.22±3.04	32.50±0.25	53.02±0.14	65.38±0.05	4.00±0.01	4.41±0.01	1.78±0.01	5.55±0.01	4.32±0.02	25.75±0.11	14.33±0.11
	L2	897.53±2.13	28.18±0.16	52.84±0.17	52.07±0.09	4.21±0.01	4.97±0.01	2.37±0.02	7.87±0.02	4.99±0.02	16.09±0.11	12.66±0.21
	L3	696.42±1.49	19.09±0.09	56.54±0.08	67.19±0.09	5.11±0.01	7.29±0.02	1.00±0.01	5.45±0.01	2.66±0.02	15.43±0.12	12.33±0.13
	L4	737.21±3.74	23.07±0.14	64.49±0.09	49.36±0.13	1.59±0.02	3.53±0.01	1.54±0.01	9.90±0.02	3.32±0.01	12.11±0.13	12.33±0.11
	L5	818.19±2.84	26.65±0.13	52.08±0.09	65.71±0.12	5.97±0.02	3.35±0.01	1.40±0.01	7.64±0.01	3.32±0.01	16.08±0.13	13.00±0.13
	L6	915.75±3.47	31.87±0.04	48.91±0.15	59.37±0.14	2.77±0.01	0.47±0.01	0.44±0.01	4.57±0.01	2.32±0.01	19.08±0.12	12.00±0.14
	L7	817.53±3.41	29.40±0.09	47.32±0.17	58.03±0.15	2.54±0.02	3.07±0.01	0.48±0.01	4.24±0.01	2.32±0.01	17.75±0.13	12.00±0.14
	L8	815.59±2.75	26.78±0.15	54.17±0.23	58.46±0.14	2.75±0.02	3.16±0.01	0.70±0.01	5.09±0.01	1.99±0.01	15.76±0.12	11.00±0.12
	Range	599.22-915.75	19.09-32.50	47.32-64.49	49.36-67.19	1.59-5.97	0.47-7.29	0.44-2.37	4.24-9.90	1.99-3.16	12.10-25.75	10.99-14.33
	Average	787.18	27.19	53.67	59.45	3.62	3.78	1.21	6.29	3.16	17.25	12.45
Local Branded	LB1	451.55±3.21	19.18±0.11	48.12±0.34	35.68±0.02	3.08±0.02	1.53±0.01	0.73±0.08	3.74±0.01	3.33±0.01	16.41±0.11	12.33±0.12
	LB2	547.22±5.25	23.08±0.12	51.68±0.23	65.84±0.12	3.29±0.01	1.55±0.02	0.23±0.0	2.50±0.01	4.66±0.02	17.75±0.12	13.33±0.13
	LB3	539.39±1.23	26.38±0.23	53.09±0.22	54.59±0.11	1.60±0.01	1.27±0.01	0.64±0.02	5.99±0.01	5.99±0.01	19.09±0.11	11.00±0.11
	LB4	407.12±7.23	29.29±0.65	59.01±0.24	60.38±0.13	1.57±0.01	1.55±0.02	0.20±0.01	3.95±0.03	5.32±0.02	15.10±0.09	12.00±0.09
	LB5	544.84±6.41	26.35±0.21	61.12±0.16	63.80±0.14	6.28±0.01	2.10±0.02	0.24±0.01	3.16±0.10	4.66±0.01	17.10±0.09	10.66±0.05
	LB6	483.17±8.13	26.10±0.13	57.95±0.14	58.90±0.12	5.22±0.01	0.33±0.01	1.00±0.01	6.25±0.09	9.99±0.02	64.43±0.09	13.66±0.13
	LB7	668.77±9.12	30.01±0.24	61.27±0.15	48.00±0.13	5.03±0.02	1.06±0.01	1.32±0.02	5.45±0.08	9.66±0.04	53.10±0.11	13.33±0.17
	LB8	515.84±8.24	22.70±0.21	49.58±0.11	54.70±0.12	1.06±0.01	0.99±0.01	1.20±0.02	5.67±0.08	9.32±0.03	48.09±0.12	12.33±0.15
	LB9	726.15±7.34	31.68±0.12	49.41±0.24	53.03±0.13	3.38±0.01	3.32±0.02	1.09±0.03	4.54±0.02	9.32±0.03	52.09±0.13	12.00±0.14
	LB10	752.04±5.89	29.93±0.23	54.45±0.23	56.51±0.14	2.29±0.01	2.22±0.02	1.08±0.01	3.08±0.03	6.32±0.02	36.76±0.11	11.66±0.13
	LB11	750.76±9.43	37.11±0.22	61.96±0.33	59.89±0.13	0.22±0.02	6.00±0.02	1.48±0.02	4.50±0.01	6.32±0.02	45.44±0.09	10.66±0.14
	LB12	594.87±8.34	26.17±0.24	50.11±0.37	51.93±0.14	1.18±0.01	5.36±0.03	1.40±0.03	2.15±0.03	5.66±0.01	43.44±0.14	10.00±0.12
	LB13	561.06±4.53	24.89±0.21	46.48±0.11	55.20±0.21	1.31±0.01	3.86±0.01	1.67±0.03	1.87±0.02	5.66±0.03	41.41±0.13	9.33±0.09
	Range	407.12-752.04	19.18-37.11	46.48-61.96	35.68-65.84	0.22-6.28	0.33-6.00	0.20-1.67	1.87-6.25	3.33-9.99	15.10-64.43	9.33-13.66
	Average	580.21	27.14	54.17	55.27	2.73	2.39	0.94	3.94	6.63	36.17	11.71

Type	Sample Codes	Fe (µg/g)	Zn (µg/g)	Mg (µg/g)	Mn (µg/g)	Cu (µg/g)	Co (µg/g)	Cr (µg/g)	Ni (µg/g)	Na (mg/g)	K (mg/g)	Li (mg/g)
	I1	152.99±5.46	36.18±0.24	39.92±0.23	73.09±1.11	15.87±0.15	4.30±0.01	1.79±0.02	5.63±0.01	16.99±0.12	30.12±0.1	29.33±0.14
	I2	183.38±8.49	34.91±0.32	36.67±0.24	161.08±2.23	16.34±0.23	2.23±0.02	0.43±0.01	5.08±0.02	16.32±0.11	30.45±0.23	31.33±0.24
	I3	211.24±9.10	39.97±0.22	36.67±0.22	152.69±1.21	17.09±0.22	3.80±0.01	1.21±0.01	5.00±0.13	12.65±0.21	21.77±0.23	29.00±0.21
	I4	137.01±9.48	32.20±0.29	38.52±0.28	123.89±3.23	13.82±0.14	4.19±0.02	0.91±0.02	5.13±0.13	16.99±0.14	26.45±0.21	30.66±0.24
	I5	132.54±9.27	36.79±0.35	44.13±0.23	166.81±2.24	13.79±0.43	4.32±0.03	0.01±0.01	7.30±0.24	11.65±0.15	11.80±0.12	18.33±0.24
	I6	156.32±9.38	28.02±0.27	44.99±0.27	142.91±5.24	15.65±0.11	4.50±0.01	0.03±0.01	8.59±0.13	10.99±0.23	12.13±0.23	14.99±0.21
	I7	185.13±5.37	35.95±0.34	41.22±0.34	143.09±2.23	12.86±0.24	4.75±0.01	0.04±0.02	7.03±0.14	13.32±0.13	10.79±0.14	15.66±0.23
	I8	164.60±6.34	34.83±0.38	43.22±0.34	117.22±1.13	10.22±0.14	3.89±0.03	0.05±0.01	6.03±0.15	10.99±0.14	10.79±0.15	15.99±0.24
	I9	236.10±6.45	34.28±0.38	40.11±0.23	179.13±2.28	12.12±0.15	2.73±0.02	0.03±0.01	5.86±0.12	16.99±0.15	13.78±0.13	17.33±0.24
	I10	117.76±6.66	27.96±0.75	39.79±0.14	107.50±3.65	10.21±0.15	3.90±0.03	0.02±0.01	5.72±0.13	16.99±0.23	15.12±0.14	16.66±0.21
Branded	I11	126.98±8.43	30.48±0.77	46.35±0.23	172.61±4.56	9.94±0.16	2.95±0.01	0.02±0.01	5.73±0.42	17.99±0.14	17.13±0.12	16.66±0.11
	I12	147.31±8.27	37.64±0.37	34.63±0.14	163.81±2.45	15.66±0.14	2.92±0.01	0.04±0.01	6.26±0.51	23.32±0.15	31.44±0.13	21.33±0.13
	I13	116.13±8.15	31.49±0.11	39.82±0.28	183.88±1.25	12.59±0.25	3.40±0.01	0.03±0.01	5.30±0.24	18.66±0.24	12.12±0.14	18.66±0.13
	I14	137.73±8.37	32.85±0.24	40.18±0.18	151.66±5.34	11.68±0.48	2.97±0.02	0.03±0.01	5.87±0.23	16.65±0.23	14.45±0.12	16.66±0.14
	I15	86.86±9.45	32.52±0.34	39.56±0.18	133.08±2.34	8.90±0.71	3.08±0.03	0.03±0.01	5.23±0.23	15.32±0.41	15.12±0.14	16.00±0.15
	I16	104.24±9.73	56.07±0.11	66.90±0.26	184.64±3.45	11.27±0.19	4.48±0.03	0.02±0.01	6.50±0.21	9.31±0.24	9.20±0.13	10.99±0.14
	I17	124.73±9.81	46.04±0.11	36.28±0.28	134.41±2.46	7.61±0.28	3.89±0.03	1.33±0.01	10.26±0.24	16.99±0.15	15.77±0.14	11.66±0.13
	I18	186.80±9.84	64.97±0.23	71.42±0.14	223.95±6.12	13.61±0.12	6.99±0.03	3.75±0.01	13.84±0.12	15.64±0.16	13.54±0.13	8.66±0.14
	I19	189.32±9.88	37.21±0.27	37.35±0.13	92.22±1.11	7.66±0.23	3.65±0.01	1.94±0.01	11.61±0.13	18.32±0.16	16.44±0.12	10.66±0.14
	I20	165.34±9.24	36.80±0.32	36.35±0.23	110.13±1.11	7.03±0.35	3.44±0.01	1.11±0.01	11.84±0.14	19.32±0.51	14.43±0.11	18.66±0.23
	I21	290.31±8.34	54.12±0.37	33.35±0.24	90.05±2.23	10.01±0.12	3.21±0.01	0.85±0.01	11.37±0.15	18.99±0.23	15.76±0.14	22.00±0.33
	Range	86.86-290.31	27.96-64.97	33.35-71.42	73.09-223.95	7.03-17.09	2.23-6.99	0.01-3.75	5.00-13.84	9.31-23.32	9.20-31.44	8.66-31.33
	Average	159.66	38.16	42.26	143.23	12.09	3.79	0.65	7.39	15.92	17.08	18.63

Cobalt (Co) is a constituent of the B12 vitamin. The daily dietary intake of Co is recommended in the range of 0.005-1.8 mg. However 500 mg or higher intake per day is considered toxic to bone tissues and carcinogenic. No significant difference was observed (p= 0.024) for Co content in all the brands studied. The values were estimated as 3.78, 2.39 and 3.79 μg/g for local, local branded and branded samples respectively. These values are considerably higher than the studies conducted in India [16], and Nigeria [28].

Chromium (Cr) is an important element for the insulin activity and DNA synthesis. Hexavalent Cr is carcinogenic. The daily dietary intake of Cr is recommended as 0.01-1.2 mg. The toxic intake of Cr is 200 mg/day or higher which affect the viability of cells of human body. A significant difference (p = 0.218) has been observed for Cr level in the three brands studied in this work. The average observed values of 1.21, 0.94, and 0.65 μg/g for local, local branded and branded class respectively were found to be higher than the data reported for the tobacco used in India (0.36–0.68 μg/g, with an average value of 0.48 μg/g) [22], and China (0.00–1.00 μg/g, with an average value of 0.55 μg/g) [32]. The higher values of Cr in the local and local branded samples may be due to uncontrolled Cr pollution, especially from the discharge of leather industrial effluent.

Sodium (Na), potassium (K), and lithium (Li) are essential to all living things. These metals contribute in muscles, bones and blood cells of a human body. Na is non-toxic metal and recommended as 2-15 g/day. The amount of Na in branded cigarettes was almost 2.4 and 5.0 times higher than a local branded and local cigarettes, respectively. The daily dietary intake of K is recommended in the range of 1400-7400 mg. However, 6g or higher amount of K is considered as toxic. K content in local branded tobacco was almost double as compared to the other two brands. Li is considered as stimulatory and antidepressant metal. The recommended intake of Li is 0.1-2.0 mg/day. However, an oral intake of 92-200 mg/day is considered toxic. The amount of Li in samples were found in order of the branded > local > local branded samples. No significant

differences for Na, K, and Li have been identified with p-value of 9.72×10^{-15}, 2.54×10^{-5} and 2.84×10^{-4} for local, local branded and branded samples, respectively.

Multivariate analysis

Principal component Analysis (PCA) was applied on the data to study variable – and sample – interrelationships simultaneously. No pattern recognition phase was identified on the exploratory PCA for physicochemical properties. (Fig. 1) consists of scree, score, and loading plots for PCA model, applied on metal contents. Three principal components (PCI, PCII, and PCIII) were found to be significant with the Eigen values of 4.94, 2.18, and 1.19 respectively. It is evident from a scree plot (Fig. 1a) and (Table 3) that 44.92 % of the total variability of PCI is mainly a function of Fe, Mn, Cu, and Na. Zn, Mg, Co, Cr, and Ni contribute in PCII, explained 19.82 % of the total variability. PCIII shows 10.80 % variation and is dominated by Cr, K, and Li.

The inter-sample grouping trends of the brands studied are represented in the biplot of PCI and PCII (Fig. 1b). A clear and distinct separation of imported samples were observed from local and local branded samples. A number of reasons may be proposed for the separation. Since the tobacco used in the studied brands were harvested from different tobacco fields of the world; therefore, the method of cultivation, soil chemistry of the particular region, a method of irrigation, a type of fertilizer applied, mode of processing and preservation *etc.* are some of the possible reasons [19]. However, the clusters of local and local branded samples are closer to each other. It is reasonable obvious because tobacco used in a local and local branded cigarette was harvested from the same geographical region of Pakistan (Khyber Pakhtunkhwa province).

The patterns of each brand of samples in the score plot were synchronized from the variable loadings. The loading plot explained the reason for the existence of the particular samples in the specific locations of the score plot. It shows that Cu, Na, and Li goes together, as a manifestation of the correlations, defining PCI and PCII for the majority of the imported samples.
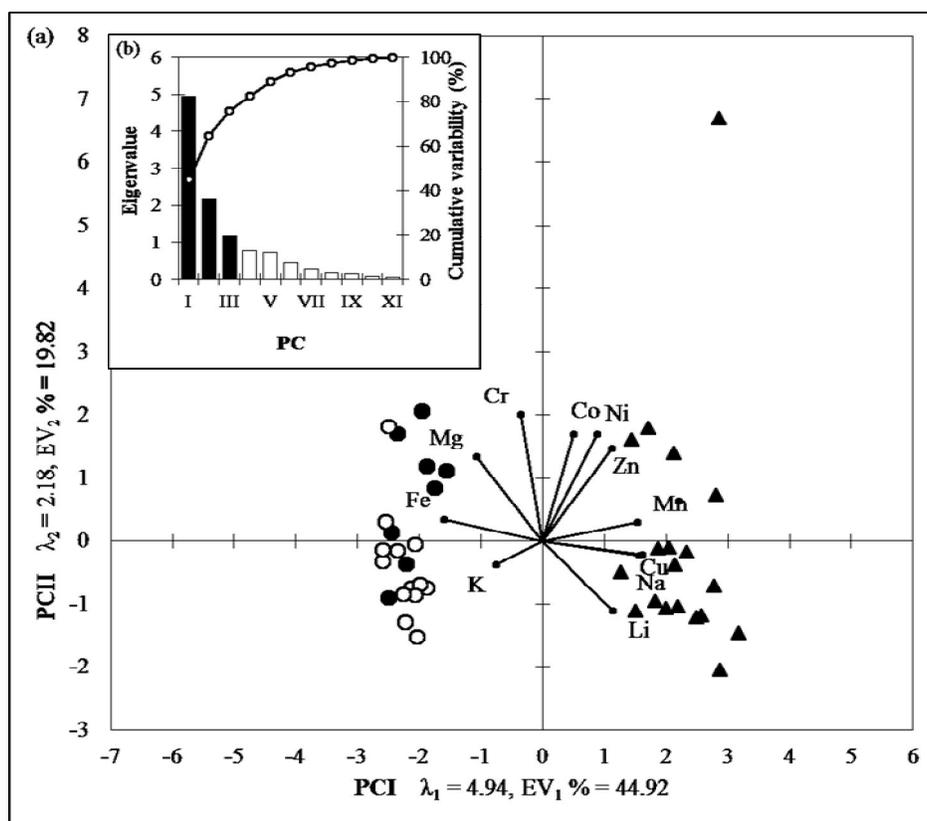
Figure 1. **Principal component analysis (a) Scree plot (b) Biplot showing score and loading plots simultaneously (λ_1, and λ_2 are eigenvalues and EV$_1$, and EV$_2$ explained variance %) Samples: ●Local, ○Local branded, ▲Imported.**

Table 3. **Component matrix for metals (significant components are shown in bold).**

Variables	PCI	PCII	PCIII
Fe	-0.405	0.089	-0.046
Zn	0.284	0.373	-0.075
Mg	-0.275	0.342	0.165
Mn	0.393	0.076	0.188
Cu	0.413	-0.057	-0.025
Co	0.127	0.431	0.090
Cr	-0.093	0.512	-0.472
Ni	0.226	0.433	-0.039
Na	0.398	-0.061	-0.259
K	-0.190	-0.097	-0.722
Li	0.290	-0.282	-0.330

The remaining imported samples have a position in the score plot that corresponds to the direction of Co, Ni, Zn, and Mn in the loading plot. These relations were due to high values of these variables in imported brands. Conversely, the local branded samples appeared in the region rich of K and Fe. The content of Cr, Mg, and Fe were found to be higher in local brands compare to the other two brands.

Another important interpretation of the loading plot is variable relationships. Variables plotted in the same direction from the center are positively correlated, while those on opposite sides of the origin are negatively correlated. The cluster variable plot (Fig. 2a) strengthens the findings of a loading plot in similarity level (Fig. 1b). A strong correlation of Mn with Cu and Na was observed in the loading plot and the cluster of the same variables have shown at the similarity level of 91.19 and 87.16 % respectively. Li was correlated at the similarity level of 86.17 %. Zn, Ni, and Co appeared in the same quadrate and clustered at the similarity level of 81.64, 79.92, and 67.39 %. A positive correlation between Fe and Mg was observed in the loading plot and they formed a

separate cluster at 76.89 % similarity level. K was the only element appeared in a separate quadrate and shown the minimum similarity level (66.88 %) in the cluster variable plot.

One way normal analysis of means (ANOM) was applied on the data to identify the variables contributing to discrimination (Fig. 2b). It was found that Fe contributes predominately in discrimination, while a significant contribution to Co, Cr, Cu, K, Li, Mn, and Ni were also observed in the same. No significant difference ($p < 0.05$) was observed in the mean values of Mg, and Zn in the tobacco of studied brands.

Significant differences ($\alpha = 0.05$) were observed in the values of physicochemical properties for local, local branded and branded samples (Fig. 2c). It indicates the difference in processing methods adopted to maintain the peculiar taste of the tobacco by different manufacturers.
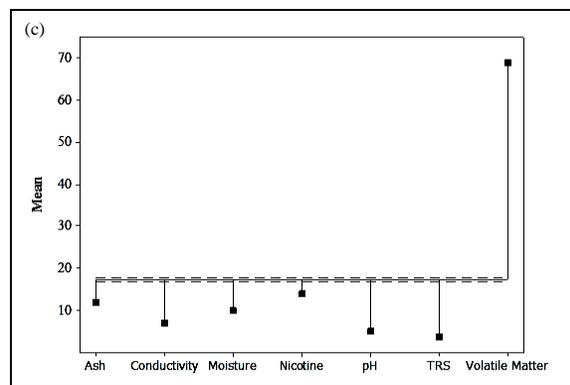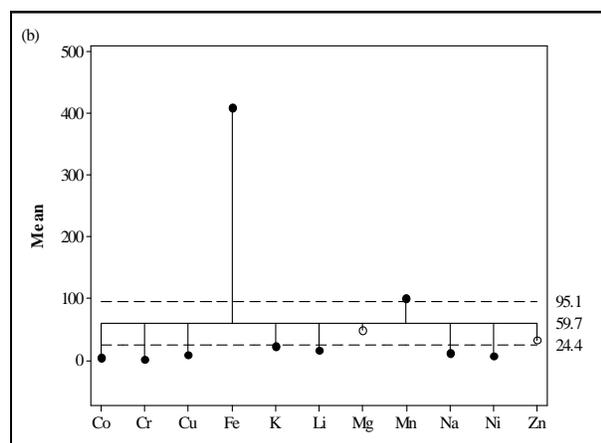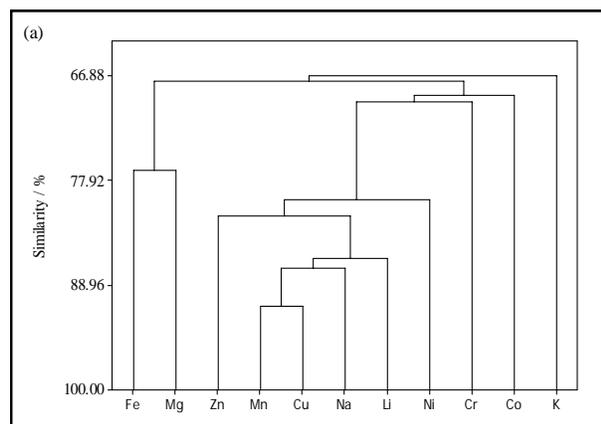

Figure 2. **(a): Cluster analysis showing dendrogram with single linkage and Euclidean distance (b): ANOM for metal content (c): ANOM for physicochemical properties.**

Conclusion

Cigarette smoking is one of the major causes of throat, mouth and lung cancer among Pakistan-people along with other coronary diseases. This concern leads us to study and compare the composition of tobacco used in different brands. The amount of nicotine in the cigarettes marketed in Pakistan was found to be many times higher than the maximum permissible limit. It is also higher than the imported and local brands used in Jordan. It shows that the cigarettes available in Pakistan are more injurious to health compare to Jordan cigarettes as far as nicotine content concerned. A suitable amount of moisture in branded samples reflects the conservation of the appropriate conditions for the cultivation of a tobacco crop. High ash in local samples is attributed to the mineral rich soil. The metal content of the branded samples were found to be significantly different to the other two classes. PCA denoted significant differences between different brands of tobacco in their metallic content. Fe and volatile matter were identified as the main discriminating parameters, contributing to the difference in tobacco composition of branded samples from the rest ones. The study suggested the continuous and regular monitoring of tobacco and strict regulations on import and export of various brands available in the local market.

Acknowledgment

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