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Determination of Iron, Zinc, Copper, Cadmium and Lead in Different Cigarette Brands in Yemen by Atomic Absorption Spectrometry

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Summary: The concentration levels of iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in different cigarette brands commonly produced and sold in Yemen were determined. Convenient sample treatment for cigarette tobacco of freshly opened packs was achieved by a sample preparation method based on dry digestion, and the concentrations of the analyzed metals were measured by flame atomic absorption spectrometry (FAAS). The mean values obtained for Fe, Zn, Cu, Cd and Pb in different Yemeni cigarette tobacco were 311, 52.2, 10.11, 1.71 and 4.06 µg/g dry weight, respectively. There is no more significance difference among cigarette brands tested. It was found that Fe was at the highest concentration followed by Zn, Cu, Pb and Cd. The average relative standard deviation (RSD) ranged from 2.92 to 11.90 %. The accuracy and precision of the results were checked by blank and recovery tests.
The results show that Yemeni cigarettes contain heavy metal concentration levels that are similar to those in foreign cigarette brands reported by other studies in the worldwide.

Introduction

Tobacco has been one of the most studied plants for well over 300 years[(1)]. The use of tobacco products constitutes the most significant cause of morbidity and mortality in the world. Tobacco-related disease originates from the smoking habit which represents the biological consequences of repeated inhalation exposure to numerous toxic constituents in tobacco smoke.

More than 4000 compounds have been identified in tobacco as well as in tobacco smoke[(2)]. Nicotine is recognized to be the major inducer of tobacco dependency[(3)]. Tobacco plant is well known for its capacity to concentrate toxic elements from its growing environment, which may cause a significantly serious damage on human health[(4,5)]. So smoking of cigarette tobacco is one way in which trace elements can enter the human body. The report of an international (WHO) program for assessment of human exposure to heavy metals, reported higher levels of

Cd and Pb in kidney cortex samples of smokers compared to non-smokers in Europe, United States and Japan[6].

In fact, some studies[7] clarified that the contents of certain toxic metals in fat and blood of tobacco smokers were much higher than those of nonsmokers. They also indicated that there is a definite correlation between trace element level in the body and that of certain diseases. Some metals are known to be carcinogenic and these are toxic and harmful to the human body even at the very low levels on intake. It has been found that some toxic elements inhaled through cigarette smoke are more easily taken up by the body than those in food or water. For instance, from 40 to 60% of the cadmium inhaled in smoke is absorbed into the bloodstream as opposed to the 5 to 10% absorbed through foods[8].

The potential health impact from smoking cigarettes that deliver high levels of toxic metals is not only limited to active smokers, the people around the smokers are also effected by side stream smoke[9]. The fact that children are more sensitive to the toxic effects of lead as compared to adults and passive smoking plays an important role in exposure of children to lead[10]. The monitoring of heavy metals in tobacco and food samples, therefore, is essential for protection of the environment and of our health.

Because of there are many factors may influence heavy metals in tobacco, it is expected that their levels are varied markedly in different cigarettes. Accordingly, the heavy metals content of cigarettes has been a subject of several studies in various areas in the world by using different digestion and analytical techniques among these are neutron activation analysis (NAA)[11], high performance liquid chromatography HPLC[12], atomic absorption spectrometry (AAS)[13-16], indicutively coupled plasma-atomic emission spectrometry (ICP-AEA)[17], inductively coupled plasma-mass spectrometry (ICP-MS)[18], anodic stripping voltammetry (ASV)[19], dispersive x-ray fluorescence (DXRF)[20]. Particularly, the need of elements analysis is expected to increase as the toxicity might be greatly enhanced by the synergistic effects of heavy metals.

Sufficient data are not available about the heavy metals concentrations in different branded cigarettes in Yemen, so the aim of this work is to determine the levels of iron (Fe), copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn) in most

cigarette brands produced and sold in Yemen, by using atomic absorption spectrometry.

Needless to say that atomic absorption spectrometry is one of the most widely used analytical techniques, and it has been applied for determination of numerous trace elements in cigarette materials[13-16].

Experimental

Reagents and Materials

All chemicals used were of analytical reagent grade (Merck). Double distilled water was used throughout for preparation of all solutions and at all other stages of analysis. Iron sulfate, zinc sulfate, copper nitrate cadmium nitrate and lead nitrate were obtained from Fisher Corporation, USA. Concentrated nitric acid HNO_3, 70% was LOBA Chemie product.

The four most popular Yemeni cigarette brands (coded A, B, C, and D) were chosen for this study, three packets of each brand were purchased at three different local markets.

Apparatus

The atomic absorption spectrometry analysis was made with a (NOVA 300, Germany), atomic absorption spectrometer equipped with hollow cathode lamps which used as radiation sources for: Pb, Cd, Cu, Fe and Zn, and they were operated at recommended currents. The optimum conditions for flame atomic absorption spectrometry with the fuel of acetylene-air are given in Table (1).

The weighing, heating, drying and ashing of the samples were carried out using a (EMID 310, USA) electronic balance, (SM 22, UK) hotplate, (FII 3410) oven and (Fur-Loo 1400) furnace, respectively. All instrumental conditions were used according to the manufacturer's recommendation. Various glassware and .polyethylene bottles were also used.

Physical Properties of Tested Cigarettes

A total of four local brands of king size filter cigarettes were tested. The weight of each cigarette and its components (tobacco, filter and paper) were measured. The

average weight of one cigarette for each brand was determined by weighing five sticks of each brand before and after removing the papers and filters.

The moisture content was determined by drying at 100 °C in an oven for a total time of 48 h.

Standard Solutions and Calibration Curves

The standard metal solutions of Cd(II), Pb(II), Zn(II), Fe(III) and Cu(II) were prepared by dissolving accurately weighed amounts of $Cd(NO_3)_2$, $Pb(NO_3)_2$, $ZnSO_4$, $FeSO_4$ and $Cu(NO_3)_2$ respectively, in appropriate amounts of bi-distilled water. Working standard solutions were daily prepared by diluting stock solutions with distilled water and nitric acid to the desired concentrations. All standards were made to a 10% (v/v) nitric acid solution, and they were stable for about one month.

Calibration curve for each element was constructed using the standard metal solutions previously prepared. Each measurement was performed at least four times to check the reproducibility, and readings were always performed in the linear range.

Preparation and Analysis of Tobacco Samples

From three packets of each brand, 15 cigarettes were taken at random, the filters and papers were removed and the pooled sample of tobacco was homogenized, using a spatula, and dried overnight in an oven at a temperature of 80°C.

Four 1.00 g portions of the dried tobacco sample of each individual cigarette brand were accurately weighed and ashed at 450°C in a muffle furnace. The ash was treated with concentrated HNO_3 and heated to near dryness on a hot plate at approximately 70°C.

After cooling, to room temperature, it was taken up in HNO_3 and filtered through No. 4 Whatman filter paper into a 25 ml volumetric flask where it was made to volume with distilled water and nitric acid. Colorless solutions were transferred into polyethylene bottles, which were tightly capped and kept as stock sample solutions until analyzed. The experimental consisted of four replicates for each brand.

Sample dilutions required for individual metal were took place at the time of analysis, in which the stock sample solutions were further diluted according to

different concentration of heavy metals, so that their absorbance fall within the desired calibration range with a good signal-to-noise ratio and very little matrix effect.

Aliquots of the sample solutions were analyzed for iron, zinc, copper, lead and cadmium by atomic absorption spectrometry using acetylene–air flame at recommended conditions of instrument. Device setting was controlled every five readings with internal quality control and retests of metal standards. The quantitative analysis was achieved by interpolating the relevant calibration curves prepared from metal standard solutions in the same acid concentration.

Glassware and polyethylene containers were cleaned and dried in such manner to ensure that no any contamination occurs. Blank samples were treated in the same way, in which, the procedure was carried out without a tobacco sample. Duplicate samples of some brands were taken randomly from two different batches and analyzed within the same experimental conditions.

All blanks, standards and samples were made to a 10% (v/v) nitric acid solution.

Because no appropriate reference materials were available and to determine whether there was any loss of analyte as a result of the analysis process, the recovery of spiked samples was used. To do this, sample solution aliquots whose Fe, Zn, Cu, Pb and Cd concentrations had been already determined were spiked with known amounts of each metal and the concentrations were measured.

Atomic absorption measurements were performed under the manufacturer's recommended wavelength, slits and lamp currents (Table 1). Value reported by the instrument software was expressed as μg/mL in solution. After deducting the blank, this value multiplied by the dilution of the sample and divided by the original sample weight being digested. All concentrations have been expressed as μg/g and μg/cigarette on dry weight basis.

Table (1) Operating conditions for flame atomic absorption spectrometer which were used in analyzed metals.

Variable	Fe	Zn	Cu	Cd	Pb
Wavelength (nm)	248.3	213.9	324.8	228.8	283.3
HCL current (mA)	12.0	7.0	3.0	4.0	5.0
Slit (nm)	0.2	0.5	0.5	0.5	0.5

Fuel flow rate, (L/min)	0.7	0.7	0.7	0.7	0.8

Results and Discussion

Various systems are affected by a deficiency or excess of trace elements such as zinc, copper and iron, or by the negative effects of elements that contribute to metal pollution poisoning such as lead and cadmium. As a consequence, any of the specific diseases caused by any of the elements may occur, or may contribute as important factors in the occurrence of various diseases[16].

Tobacco plant is naturally amenable to absorb, accumulate and concentrate relatively high levels of heavy metal species into its leaves[2]. The possible sources of heavy metals for tobacco presumably include natural uptake from soil and surface contamination by industrial activities[5,15]. The widespread use of chemical fertilizers and pesticides, and the irrigation with residual water are among the causes of contamination of tobacco. Moreover, the packaging and other processes (including the additives) used to bring raw tobacco items to the consumer can significantly increase the total concentration of trace metals in the finished products of cigarettes[4,21].

Thus, different cigarette brands could yield markedly different levels of heavy metals depending on where the tobacco was grown, and where the cigarette was produced.

In this study, the concentrations of some heavy metals in Yemeni cigarettes were measured.

The whole information about branded cigarettes understudy are described in Table (2)

Table (2) Information about the Yemeni cigarette brands.

		Wt/cigarette (g)			
Sample code	Sample name	Cigarette stick	Tobacco	Filter	Warping paper
A	Kamaran	0.935	0.751	0.143	0.041
B	Mareb	0.903	0.720,	0.140	0.040

C	Ghamdan	0.916	0.732	0.142	0.042
D	Kreater	0.912	0.731	0.140	0.041
Mean		0.916	0,734	0.141	0.041

The weights of the cigarettes range from 0.90 to 0.94 g. The weight of cigarettes removing paper and filter varies from 0.72 to 0.75 g per cigarette. The average of moisture content in tobacco was between 12.3–13.5%.

As given in experimental section the cigarette tobacco samples were decomposed before analysis by dry-ashing procedure in which care has been taken to avoid sample contamination, and analyte losses that may occur in such method.

The sample size was restricted to that amount which could be accurately weighted, completely digested, and could produce easily measurable atomic absorption signals within the linear range of each metal. In fact they always were in excess in order to obtain solutions having around 1.00 g of tobacco in a total volume of 25 ml. These amounts were found to be adequate for analysis.

The analytical results and standard deviations (SD) of the concentration levels of zinc, iron, copper, cadmium and lead in the four cigarette brands (labeled A, B, C and D), are shown in Table (3). These results confirm that tobacco is a notable source of many heavy metals pollutants.

Table (3) Determination results of iron, zinc, copper, cadmium and lead in the cigarettes tobacco understudy.

Sample code	Fe	Zn	Cu	Cd	Pb	Fe	Zn	Cu	Cd	Pb
	(μg/g)					(μg/cigarette)				
A	350	75.0	12.70	2.85	6.01	263	56.3	9.54	2.14	4.51
B	345	46.3	9.64	1.20	4.49	248	33.34	6.94	0.86	3.23
C	286	58.1	8.87	1.13	3.25	209	42.5	6.49	0.83	2.38
D	262	29.5	9.23	1.65	2.50	192	21.6	6.75	1.21	1.83
Mean	311	52.2	10.11	1.71	4.06	248	38.3	7.42	1.26	2.98
SD	37	4.7	0.82	0.05	0.27	27	3.4	0.60	0.04	0.20

The concentration of iron in tobacco of different cigarette brands was found to be between 262 and 350 μg/g. The concentration of lead was found to have relatively divergent concentration values in the different brands. Here maximum concentration was found to be 6.01 μg/g in A brand, whereas a minimum concentration of 2.50 μg/g was obtained from D brand.

Alvarado et al.[13] reported iron concentrations in cigarette tobacco in different Venezuelan brands between 340 and 466 μg/g, and lead values were between 6.5 and 12.4 μg/g.

There is no significant different in average concentration of Cd in all cigarette brands tested ranging from 1.13–2.85 μg/g. The minimum amount of Cd was observed in C, while highest amount was observed in A (Table 3). The maximum concentration of copper was found to be 12.70 μg/g in A, and the minimum concentration was found to be 8.87 μg/g in C brand.

Compared with the minimum and maximum reported results for cadmium and copper in cigarettes of some countries, cadmium was 0.90 in UK[17] and 2.96 in Pakistan[15], while copper was 7.73 in Korea[17] and 25.4 in Turkey[16]. The zinc concentration was between 29.3 and 75.0 μg/g (Table 3).

Chengjie et al.[12] found zinc concentrations in cigarette tobacco between 65.7 and 89.8 μg/g. Metal amounts have been found relatively high in cigarette which are produced under the brand labeled of A. Except in few cases of Fe which were comparatively low levels, the results were compared with those reported in many other studies[11-20].

Linearity of Calibration Graphs

Under the experimental condition, AAS measurements for standard solutions of zinc, iron, copper, cadmium and lead ions were measured. Straight line calibration curves between atomic absorbance and each ion concentrations (over the desired concentration ranges) were obtained. The values of regression equations and correlation coefficients, listed in Table (4), indicating acceptable linearity of each

calibration curve. The slopes and the intercepts of the calibration curves were also included in the regression equations.

The detection limit (LD) was defined as 3s/m, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. Detection limit of each analysed metal was calculated and listed in Table (4).

Precision and Accuracy

As described in experimental section, in addition to the repeats of each sample at least four times, the reproducibility of the procedure was also checked on separate two composite samples of some cigarette brands understudy, within the same experimental conditions. The results of blank samples indicated that there are no contamination from solvents or glassware used in the analysis. Recovery studies were undertaken to assess the accuracy of these measurements. The values obtained for recovery from spiked tobacco samples indicated no appreciable loss during sample preparation.

Table (4) Linear regression of calibration curves, detection limits (DL), relative standard deviations (RSD) and recovery results obtained for the analyzed metals.

Components	Regression Equation	Linear Range (μg/ml)	Coefficiet	[a] Detection Limit (μg/ml)	[b] RSD % min-max (mean)	[c] Recovery% (n = 4)
Fe	Y=0.053x+0.0421	5.0 - 15.0	0.9915	0.07	6.86-16.03 (11.9)	97.4
Zn	Y=0.025x+0.0467	0.5 - 4.0	0.9868	0.06	2.84-19.31 (9.00)	101.5
Cu	Y=0.043x- 0.0011	0.1 - 0.7	0.9946	0.10	3.36- 19.34 (8.11)	98.8
Cd	Y=0.597x- 0.0028	0.02 - 0.15	0.9986	0.01	1.77 – 7.50 (2.92)	99.0
Pb	Y=0.040x+0.0051	0.07 - 0.40	0.9999	0.04	4.40 – 10.32 (6.65)	102.6

[a] The DL was calculated as 3 times the standard deviation of blank signal and dividing this by the slope of the calibration graph.
[b] RSD was obtained from the determination of each sample for four times.
[c] The results of a recovery test by adding certain amount of each metal into measured sample solutions.

Conclusion

In present study, the dry digestion of heavy metals followed by flame atomic absorption spectrometry had been successfully applied for the determination of Fe, Zn, Cu, Cd and Pb in the tobacco of Yemeni cigarette brands. The precision of the procedure together with its efficiency, rapidity, low cost and environmental acceptability makes it a good alternative for the determination of heavy metals in plant material. This study confirms that tobacco is a notable source of many heavy metals. There is no significant difference in concentration of heavy metals in four cigarette brands understudy. The results indicate that concentrations of Fe, Zn, Cu, Cd and Pb in Yemeni cigarette tobacco are compared with those reported by many other studies in the worldwide. Moreover, the results obtained gives very important information for the smokers to know that heavy metals, with addition of other organic toxicants in tobacco effect adversely their health.

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