

## Original Research Article

# Evaluation of some toxic metals in blood samples of smokers in Saudi Arabia by inductive coupled plasma mass spectrometry

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### Abstract

**Purpose:** To determine some toxic elements in the blood of cigarette and tobacco pipe smokers in Riyadh, Saudi Arabia.

**Methods:** The study setting was Riyadh, the capital city of Saudi Arabia, Riyadh City. Male volunteers, aged 20 - 58 year, whose blood samples were collected, were classified into three groups of cigarette smokers (N = 30), tobacco pipe smokers (N = 30) and non-smokers (24). Inductive coupled plasma mass spectrometry (ICP-MS) measurement was conducted to determine the concentration of toxic metals in the blood samples after microwave acid digestion.

**Results:** The mean concentrations of cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg) and nickel (Ni) were  $0.23 \pm 0.30$ ,  $26.42 \pm 20.08$ ,  $19.43 \pm 10.00$ ,  $8.77 \pm 6.98$  and  $79.57 \pm 70.51$ , respectively, for cigarette smokers;  $0.31 \pm 0.19$ ,  $8.11 \pm 8.60$ ,  $4.80 \pm 3.01$ ,  $13.05 \pm 3.01$  and  $9.96 \pm 5.00$ , respectively, for tobacco pipe smokers; and  $0.07 \pm 0.20$ ,  $3.89 \pm 5.82$ ,  $10.09 \pm 2.75$ ,  $10.44 \pm 5.37$  and  $1.60 \pm 2.77$ , respectively, non-smokers.

**Conclusion:** The results showed that the blood concentrations of heavy metals in smokers were higher than those in non-smokers.

**Keywords:** Blood, Heavy metals, Smokers, Tobacco pipe smokers, Inductive coupled plasma mass spectrometry

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## INTRODUCTION

Tobacco use is one of the main risk factors for a number of chronic diseases, including cancer, lung diseases, and cardiovascular diseases [1,2]. Numerous studies have been concerned with smoking in Saudi Arabia population [3,4]. Tobacco and their products contain many organic carcinogens in addition to toxic elements [5]. Some of these elements pass into the blood

stream and may accumulate in specific organ [5]. Smoking is an established source of toxic elements such as Cd, Hg, As, Pb and Ni, which are known to cause physiological disorders [7-9].

Studies have been carried out in various cities to determine the levels of toxic elements in smokers using either inductive coupled plasma (ICP)-optical emission spectrometry [10] or ICP - mass spectrometry [11-15]. Most of the reported data

[10-15] show that the concentrations of heavy metals in smokers are higher than in non-smokers. The present investigation was aimed to assess the concentration of Cd, Pb, As, Hg and Ni in the blood of cigarette smokers and tobacco pipe smokers resident in Riyadh, Saudi Arabia, using inductive coupled plasma mass spectrometry, after microwave acid digestion. An attempt was also made to draw a correlation between smoking and the concentration these toxic elements.

## EXPERIMENTAL

### Chemical and reagents

High-purity hydrogen peroxide 35 % (w/v) and analytical reagent grade nitric acid 69- 71 % (w/v), hydrochloric acid of purity (36 – 38 %) Riedel-de Haën, Seelze, Germany. High purity water used for diluting samples, blanks and standard was obtained using Water Purification System Type, PURELABE OPTION-Q15 of ELGA Lab Water, British. Standard solution of Pb, Cd, As, Hg, Ni, and rhodium (Rh) of concentration of 1 mg mL<sup>-1</sup> of Perkin Elmer was used.

### Apparatus

Inductive coupled plasma mass spectrometry (ICP/MS) NexION 300D, the system manufactured by Perkin Elmer, New York, USA. The instrumental conditions are shown in Table 1. Deionized water was obtained using Water Purification System Type, PURELABE OPTION-Q15 from Elga Lab Water, British. A microwave digestion system manufactured by Milestone (Ethos One) Sorisole, Italy. The instrumental conditions are shown in Table 1.

**Table 1:** Operating conditions for inductive coupled plasma mass spectrometry

RF power	1250W
Nebulizer gas flow	0.92 L/min
Lens voltage	9.25V
Analog stage voltage	-1762.5V
Pulse stage voltage	1050V
Replicates per sample	3
Reading/replicates	20
Scan mode	Peak hopping
Dwell time	40 ms
Integration	1200ms
Internal standard	Rhodium 500 ppb

### Sample collection and pretreatment

Blood samples were obtained from 30 cigarette-smoking volunteers (aged between 20 and 50 years, with the period of smoking ranging from 4

to 40 years). Blood samples for 30 tobacco pipe smoking volunteers (aged between 20 and 54 years, with a period of smoking ranging from 2 - 25 years); and for 24 non-smoking volunteers (aged 20 to 58 years) were also collected. All the volunteers were men resident in Riyadh City. The method was developed and validated for a clinical trial for determination of heavy metals and conducted in accordance with the Declaration of Helsinki [16] and approved by the Research Ethics Committee of Ministry of Health Riyadh, Saudi Arabia (approval ref no. 4810). Samples were collected and classified on the basis of the period of smoking; 5 ml blood was withdrawn and placed in tubes containing lithium heparin (8 ml), and placed in a refrigerator at a temperature of -20 °C until analyzed.

### Preparation of standard solutions samples

Two working solutions, each of 100 and 10 µg/mL, of Cd, Pb, As, Hg, Ni and Rh were prepared by suitable dilution of standard solution with de-ionized water.

### Sample digestion

The blood sample (0.75 ml) was transferred into a Teflon pots; HNO<sub>3</sub> (2 ml), 2 ml of H<sub>2</sub>O<sub>2</sub>, and 8 ml of deionized water were added into each pot. The pots were placed in a microwave, closed, and heated to digest it according to the conditions shown in Table 2 [17]. After digestion, the samples were left to cool at room temperature (25 °C); the resulting solution filtered with Whatman No. 42 filter paper, transferred into a 50 ml volumetric flask and filled to the mark with deionized water. Blank and standard solutions were prepared in a similar manner. The final sample solution was analyzed by ICP-MS.

### Validation of the proposed method

Validation of the proposed method was performed using ICP-MS for analysis of heavy metals in cigarette, tobacco pipe and non-smoker blood samples. The validation parameters including linearity, sensitivity, selectivity, precision, and accuracy were examined. The proposed method was checked by adding known concentration of the tested elements in deionized water. Also the proposed method was tested by applying of the proposed method to analysis of the study elements in certified reference material (KATS no. 2008242) [18].

### Statistical analysis

The results are presented as mean ± standard deviation (SD) and were analyzed by one-way

analysis of variance (ANOVA). Statistical significance was set at  $p < 0.05$ , and analysis was carried out using Microsoft Excel for Windows 2010 and Statistical Package for the Social Sciences (SPSS), version 16.0.

## RESULTS

After digestion in microwave, ICP-MS allows for the simultaneous determination of Cd, Pb, As, Hg and Ni at low concentrations in the blood. The optimum conditions for ICP - MS were obtained, viz, good linearity, correlation coefficient, accuracy and precision. Calibration curves were obtained in the range of 10 - 100 µg/L for cadmium, lead, arsenic and nickel, nickel [19]. Mercury was in the range of 100 - 300 µg/L. All calibrations were carried out using rhodium as internal standard [19]. The regression equation of the calibration curves were  $y = 6218.4x - 1424.1$ ,  $y = 4017.7x - 202.53$ ,  $y = 5745.6x + 8050.6$ ,  $y = 1174x + 2400$  and  $y = 10002x + 335.44$  for Cd, Pb, As, Hg and Ni, respectively, where the correlation coefficient ( $r^2$ ) were 0.9999, 0.99919, 0.9992, 0.9989 and 0.99992 respectively. Mean recovery was 97.37, 98.9, 101.95, 95.0 and 103.13% for Cd, Pb, As, Hg and Ni respectively. The relative standard deviation was 4.6, 1.5, 1.2, 6.2 and 1.7 % for Cd, Pb, As, Hg and Ni, respectively (Table 3). On the other hand, analysis of the certified sample by the proposed method showed that recovery was 100.77, 102.67, 104.01, 95.0 and 102.92 % for Cd, Pb, As, Hg and Ni, respectively, while relative standard deviation was 4.7, 3.3, 5.2, 6.2 and 3.1 % for Cd, Pb, As, Hg and Ni, respectively (Table 4).

**Table 5:** ANOVA data

Element	F-test	P-value
Cd	6.810	0.002
Pb	22.409	0.000
As	4.807	0.011
Hg	161.288	0.000
Ni	29.197	0.000

## DISCUSSION

In this study, we determine the concentration of toxic metals (Pb, Cd, As, Hg and Ni) in blood samples of cigarette, tobacco pipe, and non-smokers using ICP-MS method to determine any correlation between toxic metals concentration and smoking habitats. The developed ICP-MS was validated by analysis of standard sample and spiking known concentration of toxic metals. The validated method showed good linearity, accuracy, precision and lower limit of detection.

Smoking is one of the major sources of intake of the toxic element [5]. The tobacco absorb the element mainly from soil or from the environment. Toxic elements pass into the blood stream and accumulate in kidneys, which damage their ability to regulate the water balance in the body. This function (water unbalance), lead to water retention and salt retention. Therefore many diseases like blood pressure [6] and cardiovascular disease [1] were occurred. On the other hand, smoking causes the inhalation of cadmium, arsenic, nickel and mercury.

Nickel vapor reacts with carbon monoxide in tobacco to form carcinogenic carbonyl compounds [5].

**Table 3:** Determination of heavy metal concentrations in spiked de-ionized water using ICP-MS

Element	Added (µg/L)	Found (µg/L)	Recovery (%)*	SD	RSD (%)
Cd	6.47	6.30	97.37	0.29	4.6
Pb	18.15	17.95	98.89	0.26	1.5
As	56.02	57.11	101.94	0.69	1.2
Hg	40.0	38	95.00	0.00%	6.2
Ni	58.1	59.92	103.1	1.02	1.7

\*Mean of 5 measurements

**Table 4:** Heavy metal levels in certified sample, determined by ICP-MS

Element	Recommended value (mg/kg)	Measured value* (mg/kg)	Recovery (%)	RSD (%)
Cd	1.3	1.31	100.77±0.06	4.7
Pb	60	61.6	102.77±2.03	3.3
As	13.4	0.73	104.01±0.73	5.2
Hg	0.04	0.038	95.00± 0.002	6.2
Ni	26	26.76	102.92± 0.83	3.1

\*Mean of five determinations

Exposure to mercury causes damage to brain [21], while arsenic causes damage to nerve cells in patients suffering from diabetes and heart disease, and to blood vessels [22]. Smoking, in some circumstances, may lead to cancer diseases, especially prostate cancer, based on varying mechanisms, for example, increase in total and free testosterone, genetic variation, carcinogenic effect [23].

The results obtained in the present study show that mean blood cadmium, lead and nickel concentration were higher in smokers than in non-smokers, and are higher than those previously reported by other workers [24,25]. On the other hand, the mercury and arsenic reported for both smokers and non-smokers in the present study are in accord with an earlier report [24].

To find a correlation between the toxic metals and smoking habitat and non-smokers, variance test has been carried out using statistical analysis. The significance of toxic elements (Cd, Pb, As, Hg, and Ni) were smaller than the significance off null hypothesis. Therefore there are significance difference between the concentration of toxic metals in smokers and non-smokers. These findings are in agreement with previous studies [10-15].

Thus, the findings obtained indicate that toxic metal blood levels are much higher in smokers than in non-smokers. Health care professionals need to offer appropriate counsel and therapies to patients suffering from ailments such as blood pressure [10,11], cardiovascular diseases [1], Alzheimer's diseases [13] and prostate cancer [15].

## CONCLUSION

Inductive coupled plasma-mass spectrometry has been developed for the determination of Cd, Pb, As, Hg, and Ni in cigarette, tobacco pipe and non-smokers smokers in Saudi Arabia (population). The study shows that the concentration of heavy metals in smokers is much higher than non-smokers. The findings of this study lend some support to the link between heavy metals in human body and smoking habitat.

## DECLARATIONS

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### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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