



# Estimation of toxic elements in the samples of different cigarettes and their impact on human health of Irish hypertensive consumers



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## ABSTRACT

**Background:** Cigarette smoking interferes with the metal homeostasis of the human body, which plays a crucial role for maintaining the health. A significant flux of heavy metals, among other toxins, reaches the lungs through smoking. In the present study, the relationship between toxic element (TE) exposure via cigarette smoking and hypertension incidence in population living in Dublin, Ireland is investigated.

**Methods:** The different brands of cigarette (filler tobacco, filter and ash) consumed by the studied population were analyzed for cadmium (Cd), nickel (Ni), and lead (Pb). The concentrations of TEs in biological samples and different components of cigarette were measured by inductively coupled plasma atomic emission spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked using certified reference materials.

**Results:** The filler tobacco of different branded cigarettes contains Cd, Ni and Pb concentrations in the ranges of 1.73–2.02, 0.715–1.52 and 0.378–1.16 µg/cigarette, respectively. The results of this study showed that the mean values of Cd, Ni and Pb were significantly higher in scalp hair and blood samples of hypertensive patients in relation to healthy controls, while the difference was significant in the case of smoker patients ( $p < 0.001$ ). The levels of all the three TEs were 2–3 folds higher in scalp hair and blood samples of non-hypertensive smoker subjects as compared to nonsmoker controls.

**Conclusion:** The high exposure of toxic metals as a result of cigarette smoking may be synergistic with risk factors associated with hypertension.

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## 1. Introduction

Hypertension (HT) is an increasingly important medical and public health issue. The prevalence of HT increases with advancing age (60–90 years) [1]. But today, the age criteria have been changed and even people below 30 years of age have HT problems because of the lack of exercise, fast foods, smoking, coffee and alcohol consumption [2]. Genetic effect may also be a factor [3]. Smoking, however, is an important source of exposure to toxic elements (TEs) such as cadmium (Cd), nickel (Ni) and lead (Pb), which have been proposed as causative agents of cigarette smoke-induced physiological disorders [4–6]. In fact, a study showed that serious symptoms (strong urges to smoke, feeling anxious or unsuccessful attempts at not smoking) appeared in youth within weeks or only days after the initial start of smoking [6].

Cigarette design has evolved considerably over the last few decades with the incorporation of new tobacco processes, papers, filters and several ingredients (flavor, humectants and casing materials), which either alone or in combination have the potential to modify the quantity and/or

the quality of the smoke yielded [7]. The tobacco plant absorbs TEs most probably from the soil, from fertilizers or from pesticides [8]. Other environmental factors that may influence the uptake of TEs by tobacco plants include the pH of soil, contaminated irrigated water and sewage sludge used as fertilizers. Tobacco smoking delivers 87 organic carcinogens to the lungs, in addition to TEs [9], which may partition into the smoke phase on combustion [10]. Some of these (Cd, Ni and Pb) readily pass into the bloodstream and may accumulate in specific organs, such as the kidney and liver [11]. There are a few studies that have reported on the large variations of heavy metal/TEs in the compositions of commercial tobacco products, which have tried to link smoking-related diseases with TEs derived from tobacco combustion [12].

The intake of trace and TEs may promote hypertensive and atherosclerosis disorders by increasing oxidative stress (for example, by catalyzing the production of reactive oxygen species or inhibiting their degradation) due to the deficiency of an antioxidant element and by increasing blood pressure levels [13]. The deficiency of essential nutrients, lack of homeostatic control or an excess intake of some TEs causes chronic physiological disorders, such as HT and cardiovascular disease [14].

Determinations of trace elements in human tissues and fluids were used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication, and to obtain information

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on environmental exposure [15]. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [16].

One of the most widely used analytical techniques for different element determinations in biological and environmental materials is inductively coupled plasma atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods: before all a possibility of simultaneous determination of many elements of interest, freedom from different chemical interferences and high detection power. ICP-AES also offers rapid, multi-element determinations. The sensitivity of ICP-AES is lower than that of either inductive coupled plasma mass spectrophotometer (ICP-MS) or atomic absorption graphite tube atomizer (AA-GTA), but ICP-AES can handle higher levels of total dissolved solids (TDS) than ICPMS and is much faster than AA-GTA [17,18]. Since ICP-AES is able to analyze samples with higher TDS, more concentrated solutions can be prepared allowing trace elements to be measured. The main advantage of microwave-assisted sample pretreatment is its requirement of small amount of mineral acids and a reduction in the production of nitrous vapors. Microwave systems keep blank levels low because only small volumes of reagents are required and allow more samples to be processed per hour than conventional digestion systems [19].

The aim and objective of our present study was to assess the concentrations of Cd, Ni and Pb in the scalp hair and blood samples of smoker and hypertensive patients. For a comparative study, 54 non-hypertensive individuals (smoker and nonsmokers) of the same age group (ranged 30–50 years), socioeconomic status, localities and dietary habits were selected as controls. The understudy elements were analyzed by inductive coupled plasma atomic emission spectrophotometer, after microwave-assisted acid digestion. Presently, we also evaluated and compared the status of toxic metals (TEs) (Cd, Ni and Pb), in different pre-smoking and post-smoking components (filler tobacco, filter and ash) of various imported branded cigarettes existing in Ireland.

## 2. Materials and methods

### 2.1. Apparatus

Agate ball mixer mill (MM-2000 Haan, Germany), was used for grinding the cigarette tobacco, filter and ash. Sieves made of nylon with mesh sizes of  $\varnothing < 50$  and  $65 \mu\text{m}$  were used to study the influence of particle size on extraction. A Varian Liberty 220 (Mulgrave, Victoria, Australia) inductively coupled plasma atomic emission spectrometer with the axially viewed plasma was used for the analysis. The Liberty Series II ICP features a 40 MHz free running RF generator, a 0.75 m Czerny-Turner monochromator with 1800 grooves/mm holographic grating used in up to 4 orders. The resolution of the spectrometer is typically 0.018 nm in 1st order, 0.009 nm in 2nd order, 0.007 nm in 3rd order and 0.006 nm in 4th order. The instrumental conditions are shown in Table 1. A Hinari Life style (Elstree) domestic microwave oven (maximum heating power of 800 W) was used for digestion of the scalp hair, blood and different cigarette component samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing the solutions.

### 2.2. Reagents and glass wares

Ultrapure water obtained from ELGA Lab Water system was used throughout the work. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were from Merck and checked for possible trace metal contamination. Working standard solutions of Cd, Ni and Pb were prepared immediately prior to their use, by stepwise dilution of certified standard solutions (1000 ppm) Fluka Kamica (Buchs), with 0.5 mol/l  $\text{HNO}_3$ . All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, the certified reference material (CRM), human hair NCSZN 81002b, Clincheck® control-lyophilized human whole blood and Virginia tobacco leaves (ICHTJ-cta-VTL-2) were used. All glassware

**Table 1**

Measurement conditions for inductive coupled plasma atomic emission spectroscopy Liberty 220 ICP-AES.

Parameters	Cd	Ni	Pb
Wavelength (nm)	226.5	231.6	220.5
Height (mm)	3	5	3
<i>Common parameters</i>			
Windows (nm) (above the coil)	0.027	Nebulizer type	V-groove
Scan (nm)	0.040	Nebulizer pressure	150 kPa
Integration (s)	3	Stabilization time	10 s
Replicates	3	Sample delay time	30 s
Sample uptake (s)	30	Rinse time	10 s
PMT (V)	650	Pump-tube	Orange–orange (inlet) Blue–blue (outlet)
Power (kW)	1.10	Snout purge	off
Plasma flow (l/min)	15.0	Fast pump	On
Auxiliary flow (l/min)	1.50	Background mode	Dynamic
Pump speed (rpm)	15	Max curve order	1
C.C. Limit	0.995		

and plastic materials used were previously soaked for 24 h in 5 mol/l nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

### 2.3. Sample collection and pretreatment

#### 2.3.1. Cigarette pretreatment

Five different commercially available branded cigarettes (BCs) were purchased from local market of Dublin (Ireland) during July and August 2010 (Table 2). The samples were in their original packaging, and placed in pre-washed dried plastic bags separately and stored at 4 °C until tested. The weight of each cigarette after dried at 80 °C was determined. A duplicate 4 composites samples of each branded cigarette ( $n = 10$ ) were taken randomly from 4 different batches (packed on different dates). For analysis of TES in cigarette tobacco, we separated all components of cigarette, tobacco, filter and wrapping paper of 5 cigarettes of each composite samples and dry it in a sterilized glass beaker for 48 h at 80 °C, the dried tobacco were ground with agate ball mixer mill and sieved through nylon sieves with mesh sizes of  $\varnothing 65 \mu\text{m}$ . The remaining 5 cigarettes of each corresponding composite batch of all branded cigarettes understudy were used for smoking by a volunteer to collect ash of cigarette in cleaned PTFE beaker separately at room temperature (30–35 °C). Cigarette smoking termination was carrying out when the burning line reached the butt length (different according to different brands). Care was taken to avoid any source of contamination, and this preparation was done in a clean room.

#### 2.3.2. Biological samples pretreatment

Before the start of this study, all controls and HT patients of both genders, age range 30–50 years, were informed through a consent form by the administration about the aim of study, and all agreed to participate and signed the form. A questionnaire was also administered to them to collect details regarding physical data, ethnic origin, health

**Table 2**

Information of branded cigarettes.

Sample code	Sample name	Description	Wt/cigarette (g)
BC1 <sup>a</sup>	Dunhill	International, filter deluxe UK	0.731 ± 0.008
BC2	Pine	Benhsn and hedges	0.548 ± 0.005
BC3	Marlboro	Filter class A cigarettes (USA)	0.869 ± 0.015
BC4	Silk cut	Japan tobacco	0.715 ± 0.009
BC5	John Player blue	Nottingham, England.	0.692 ± 0.013

<sup>a</sup> Branded cigarette.

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