ACADEMIA ROMÂNĂ

Revue Roumaine de Chimie

http://web.icf.ro/rrch/

Rev. Roum. Chim., **2018**, *63*(10), 903-909

HEAVY METALS IN COMMON TOBACCO BRANDS FROM LEGAL MARKET AND BLACK MARKET OF IAŞI – ROUMANIA

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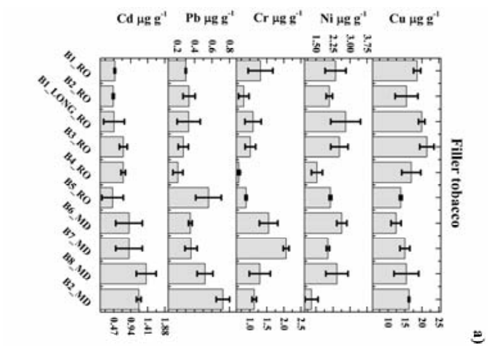
Received July 17, 2017

Tobacco consumption in human society represents a real threat for the health that leads to many diseases and in the end to death. This study was conducted for 2 years (2014-2015) in Iaşi City, Roumania. During this time eight different brands were purchased cigarettes, which are , common to every legal shop and black market. The aim of this study was to quantify the toxic metal levels in filler tobacco from common cigarettes brands purchased from local market and black (illegal) market of Iaşi City, Roumania, with the aid of HR-CS GF-AAS technique. We performed a general comparison between these brands and we calculated the dosage of toxic metal from each cigarette that was absorbed by people who smoked them.



INTRODUCTION

Tobacco consumption in human society represents a real threat for health that leads to many diseases and in the end to death. Many people start smoking cigarettes since their childhood without knowledge regarding the future health problems. Some of the carcinogenic compounds in tobacco smoke can be grouped in classes of: nitrosamines, polycyclic aromatic hydrocarbons and toxic metals.[1,2] The main toxic compounds resulted in tobacco smoke reported in scientific literature are: nicotine, carbon monoxide, heavy metals and metalloids (arsenic, cadmium, chromium, iron, lead, mercury, nickel and vanadium), hydrogen cyanide[3,4] and nitric oxide (NO).[5] Smoking has been associated with a series of infections: respiratory (pneumonia, tuberculosis, non-tuberculosis mycobacteria), exacerbation of COPD, cystic fibrosis, HIV infection (smoking increases the infections at persons with HIV that may lead to death), meningitis, otitis media, periodontics, bacteraemia, *H. pylori* infections, surgical site and nosocomial infections, inflammatory bowel disease, invasive fungal infections and bacterial vaginosis.[3]

Yu *et al.* [6] associated the cigarette smoking habit from China with men who have sex with men (MSM) among the Chinese population. They had a high prevalence of smoking that was possibly influenced by depression, intimate partner violence, alcohol abuse and drug use. In this case the cigarette smoking habit may be caused by changes in man behaviour.

Few years ago, an alternative of the classic tobacco cigarette considered healthier has been introduced on the market. This was the electronic cigarette without tar, carbon monoxide and other harmful compounds. A series of research

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publications proved the existence in appreciate amounts of numerous particles of tin, silver, nickel, aluminium, chromium and even trace of mercury in the resulted vapour.[7-10]

This study was conducted for 2 years (2014-2015) in City of Iaşi, Roumania. During this time we purchased cigarettes from eight different brands common to every legal shop and black market. The brands that were found on legal market of Iaşi City were produced in Roumania by the companies and distributed all around the country. The cigarettes from the illegal market of Iaşi (black market) were produced in the Republic of Moldova and Ukraine. We kept under the anonymous the name of the brands because we aimed to measure the level of contaminants from these products and not to give credit or discredit any studied brand.

The consumer of Iaşi may choose to buy the cheapest cigarettes in order to satisfy his needs without knowing the quality. Even if there are strong border controls between Roumania and Republic of Moldova somehow the smugglers are transporting the cigarettes into Iaşi City, which is located near the border and it is the largest from the east side of the country.

The aim of this study was to measure toxic metal levels of cadmium, lead, cooper, chromium and nickel in filler tobacco from common cigarettes brands purchased from the local market and black (illegal) market of Iaşi City, Roumania with HR-CS GF-AAS technique. We made a general comparison between the brands and we calculated the dosage of toxic metal from each cigarette that was absorbed by people who smoke.

RESULTS AND DISCUSSION

In Figure 1 the tobacco content per cigarette as average ± standard deviation from each brand was reported. There are significant differences of tobacco content between brands (***$p<0.001$ one way ANOVA). The quantity of tobacco varied between 0.46 and 0.91 grams per cigarette. The highest values were for B6_MD and the lowest for B5_RO. Because generally the standard mass of tobacco content was not the same for each brand, it was hard to control the consumer exposure to the toxins generated due to smoking. However, the more tobacco is in a cigarette the higher dose of heavy metals is for the consumers.

Filler tobacco

The total number of 240 samples analysed in this study provided (on the first step) important data regarding the concentrations of metals in the tobacco. All the concentrations were reported as mean ± standard deviation (SD). The results obtained for each brand were:

- B1_RO: **Cd** 0.49±0.02 μg g^{-1}, **Pb** 0.29±0.006 μg g^{-1}, **Cr** 1.31±0.36 μg g^{-1}, **Ni** 2.36±0.45 μg g^{-1} and **Cu** 18.6±1.04 μg g^{-1};
- B2_RO: **Cd** 0.45±0.02 μg g^{-1}, **Pb** 0.33±0.06 μg g^{-1}, **Cr** 0.82±0.15 μg g^{-1}, **Ni** 2.08±0.13 μg g^{-1} and **Cu** 15.46±3.36 μg g^{-1};
- B1_LONG_RO: **Cd** 0.47±0.28 μg g^{-1}, **Pb** 0.33±0.13 μg g^{-1}, **Cr** 1.09±0.24 μg g^{-1}, **Ni** 2.8±0.65 μg g^{-1} and **Cu** 19.93±0.92 μg g^{-1};
- B3_RO: **Cd** 0.73±0.1 μg g^{-1}, **Pb** 0.27±0.05 μg g^{-1}, **Cr** 1.01±0.15 μg g^{-1}, **Ni** 2.52±0.38 μg g^{-1} and **Cu** 21.47±2.09 μg g^{-1};
- B4_RO: **Cd** 0.73±0.06 μg g^{-1}, **Pb** 0.2±0.05 μg g^{-1}, **Cr** 0.68±0.03 μg g^{-1}, **Ni** 1.54±0.24 μg g^{-1} and **Cu** 16.86±2.81 μg g^{-1};
- B5_RO: **Cd** 0.43±0.29 μg g^{-1}, **Pb** 0.55±0.14 μg g^{-1}, **Cr** 0.89±0.03 μg g^{-1}, **Ni** 2.13±0.05 μg g^{-1} and **Cu** 13.78±0.38 μg g^{-1};
- B6_MD: **Cd** 0.89±0.36 μg g^{-1}, **Pb** 0.35±0.02 μg g^{-1}, **Cr** 1.55±0.26 μg g^{-1}, **Ni** 2.63±0.22 μg g^{-1} and **Cu** 12.38±1.68 μg g^{-1};
- B7_MD: **Cd** 0.89±0.36 μg g^{-1}, **Pb** 0.35±0.06 μg g^{-1}, **Cr** 2.06±0.08 μg g^{-1}, **Ni** 2.02±0.06 μg g^{-1} and **Cu** 14.98±1.45 μg g^{-1};
- B8_MD: **Cd** 1.36±0.27 μg g^{-1}, **Pb** 0.51±0.09 μg g^{-1}, **Cr** 1.29±0.3 μg g^{-1}, **Ni** 2.42±0.48 μg g^{-1} and **Cu** 15.41±3.64 μg g^{-1};
- B2_MD: **Cd** 1.16±0.06 μg g^{-1}, **Pb** 0.72±0.07 μg g^{-1}, **Cr** 1.13±0.06 μg g^{-1}, **Ni** 1.3±0.28 μg g^{-1} and **Cu** 16.2±0.2 μg g^{-1}.

A similar study was conducted in Roumania by Agoroaei *et al.* in 2014 [11] with a small sample size (15 samples). Some of their results were comparable with our results. The mean±SD of the metals concentration in tobacco were: Cd 1.06±0.28 μg g^{-1}, Pb 4.1±0.84 μg g^{-1}, Cr 4.37±1.71 μg g^{-1}, Ni 0.91±0.11 μg g^{-1} and Cu 9.46±2.09 μg g^{-1}. The values obtained were higher for Pb and Cr compared with our study.

Kazi *et al.* (2009) [12] analysed the heavy metals content in twelve brands from local market of Hyderabad, Pakistan, during 2005-2006. The authors did not mention the total number of samples used in their study (around 120 samples). They measured the following concentrations of heavy metals in filler tobacco (mean±SD): **Cd** 1.66±0.123 → 2.96±0.114 μg g^{-1}, **Pb** 0.399±0.021 → 1.39 ± 0.075 μg $g^{-1}$and **Ni** 0.725±0.065 → 1.34 ± 0.081 μg g^{-1}.

Verma *et al.* (2010) [13] conducted a study on Indian market of tobacco. They measured the following concentrations of heavy metals in similar samples (mean±SD): **Cd** 0.3±0.05 → 0.87±0.07 μg g^{-1}, **Pb** 0.79±0.25 → 5.79±1 μg g^{-1}, **Cr** 2.79±0.57 → 5±0.5 μg g^{-1}, **Ni** 7.21±1.19 → 10.24±1.07 μg g^{-1} and **Cu** 9.01±1.45 → 15.01±1.11 μg g^{-1}.They analysed 30 samples.

Armendáriz *et al.* (2015) [14] studied 33 brands of cigarettes marketed in Spain for the concentration of heavy metals in tobacco. They obtained the following concentrations (mean±SD): **Cd** 0.4±0.15 → 1.47±0.77 μg g^{-1}, **Pb** 0.33±0.06 → 3.6 ± 1.65 μg g^{-1}, **Cr** 0.73±0.23 → 3±1.52 μg g^{-1} and **Ni** 1.33±0.21 → 4.87±2 μg g^{-1}. They concluded that the metal content in tobacco depends on factors such as land where the plant was cultivated and manufacturing process used, which requires a series of harmful chemicals.

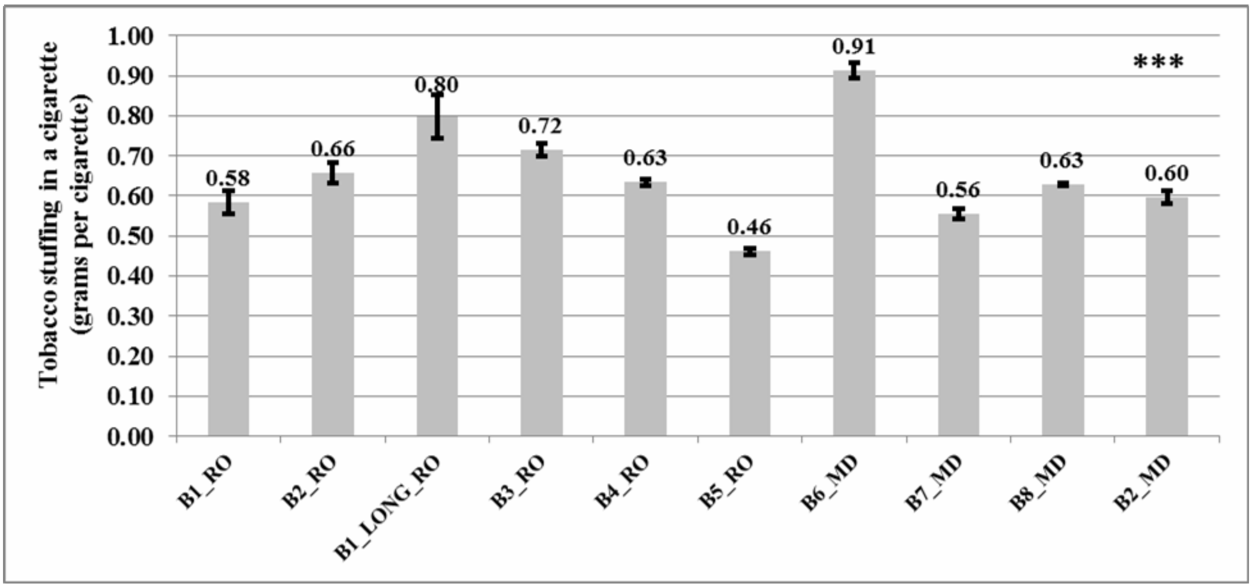

Fig. 1 – Tobacco stuffing in each cigarette from the studied brands reported as mean±SD (***p<0.001 one way ANOVA).

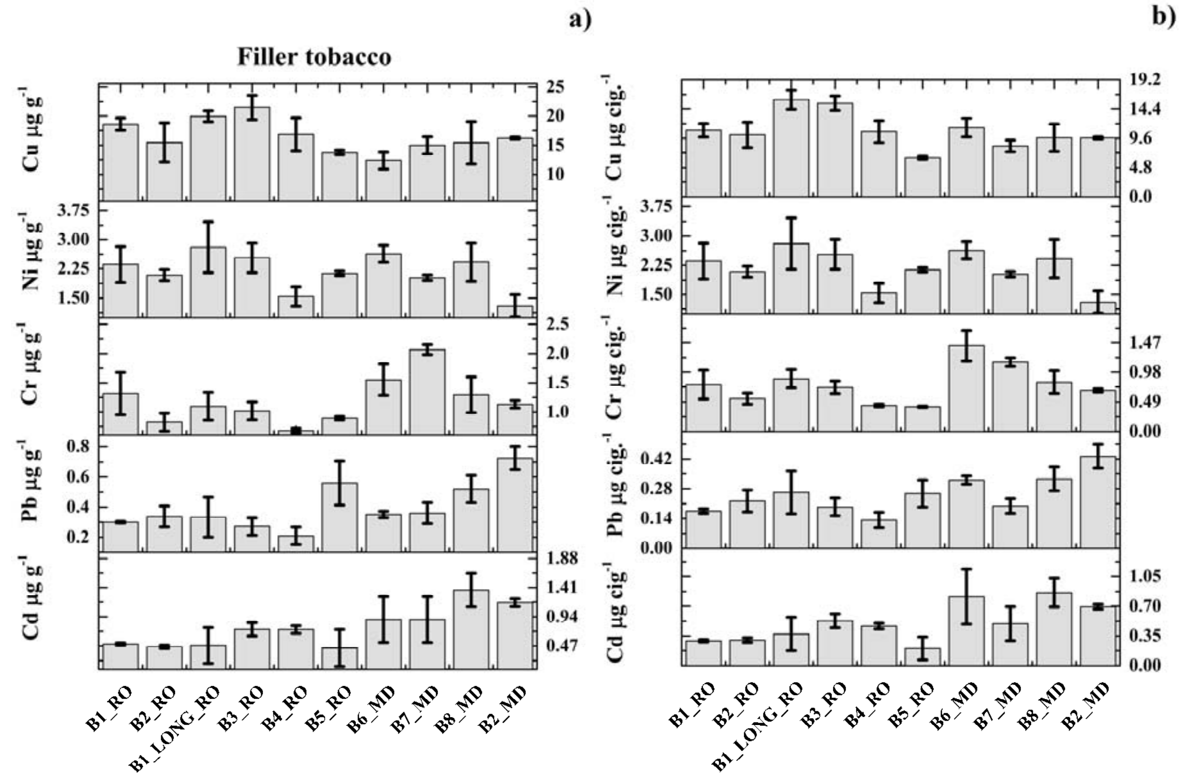

Fig. 2 – Heavy metals concentration in tobacco (a) and a cigarette (b) reported as mean±SD.

Filler tobacco — **Cigarette**

←Cadmium (Cd)→

Cd µg per gram	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	*p<0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B4_RO	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	**p<0.01	*p<0.05
B6_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05
B7_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		**p<0.01	*p<0.05
B8_MD	**p<0.01	**p<0.01	**p<0.01	p>0.05	p>0.05	**p<0.01	p>0.05	**p<0.01		p>0.05
B2_MD	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	*p<0.05	p>0.05	

Cd µg per cig.	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	**p<0.01	p>0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	**p<0.01	p>0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B4_RO	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		**p<0.01	p>0.05	**p<0.01	*p<0.05
B6_MD	*p<0.05	*p<0.05	p>0.05	p>0.05	p>0.05	**p<0.01		**p<0.01	p>0.05	p>0.05
B7_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01		**p<0.01	p>0.05
B8_MD	**p<0.01	**p<0.01	*p<0.05	p>0.05	p>0.05	**p<0.01	p>0.05	**p<0.01		p>0.05
B2_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	

←Lead (Pb)→

Pb µg per gram	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	*p<0.05	p>0.05	p>0.05	*p<0.05	**p<0.01
B4_RO	p>0.05	p>0.05	p>0.05	p>0.05		**p<0.01	p>0.05	p>0.05	**p<0.01	**p<0.01
B5_RO	*p<0.05	p>0.05	p>0.05	*p<0.05	**p<0.01		p>0.05	p>0.05	p>0.05	p>0.05
B6_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	**p<0.01
B7_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	**p<0.01
B8_MD	p>0.05	p>0.05	p>0.05	*p<0.05	**p<0.01	p>0.05	p>0.05	p>0.05		p>0.05
B2_MD	**p<0.01	**p<0.01	**p<0.01	**p<0.01	**p<0.01	p>0.05	**p<0.01	**p<0.01	p>0.05	

Pb µg per cig.	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B4_RO	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	**p<0.01	p>0.05	**p<0.01	**p<0.01
B5_RO	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	*p<0.05
B6_MD	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05		p>0.05	p>0.05	p>0.05
B7_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	**p<0.01
B8_MD	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05		p>0.05
B2_MD	**p<0.01	**p<0.01	*p<0.05	**p<0.01	**p<0.01	*p<0.05	p>0.05	**p<0.01	p>0.05	

←Chromium (Cr)→

Cr µg per gram	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	**p<0.01	p>0.05	p>0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	*p<0.05	p>0.05	p>0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05
B4_RO	*p<0.05	p>0.05	p>0.05	p>0.05		p>0.05	*p<0.05	*p<0.05	*p<0.05	p>0.05
B5_RO	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		*p<0.05	**p<0.01	p>0.05	p>0.05
B6_MD	p>0.05	*p<0.05	p>0.05	p>0.05	*p<0.05	*p<0.05		p>0.05	p>0.05	p>0.05
B7_MD	**p<0.01	*p<0.05	*p<0.05	*p<0.05	*p<0.05	**p<0.01	p>0.05		**p<0.01	**p<0.01
B8_MD	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	**p<0.01		p>0.05
B2_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05	

Cr µg per cig.	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	*p<0.05	*p<0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	**p<0.01	*p<0.05	p>0.05	p>0.05
B4_RO	p>0.05	p>0.05	*p<0.05	p>0.05		p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05		*p<0.05	**p<0.01	p>0.05	p>0.05
B6_MD	**p<0.01	**p<0.01	**p<0.01	**p<0.01	**p<0.01	*p<0.05		p>0.05	*p<0.05	**p<0.01
B7_MD	p>0.05	**p<0.01	p>0.05	*p<0.05	**p<0.01	**p<0.01	p>0.05		p>0.05	*p<0.05
B8_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05		p>0.05
B2_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01	*p<0.05	p>0.05	

←Nickel (Ni)→

Ni µg per gram	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05	p>0.05	**p<0.01
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05
B4_RO	p>0.05	p>0.05	**p<0.01	p>0.05		p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05
B6_MD	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05		p>0.05	p>0.05	**p<0.01
B7_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05
B8_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05		*p<0.05
B2_MD	*p<0.05	p>0.05	**p<0.01	*p<0.05	p>0.05	p>0.05	**p<0.01	p>0.05	*p<0.05	

Ni µg per cig.	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B2_RO	p>0.05		**p<0.01	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B1_LONG_RO	**p<0.01	**p<0.01		p>0.05	**p<0.01	**p<0.01	p>0.05	**p<0.01	*p<0.05	**p<0.01
B3_RO	p>0.05	p>0.05	p>0.05		**p<0.01	**p<0.01	p>0.05	*p<0.05	p>0.05	**p<0.01
B4_RO	p>0.05	p>0.05	**p<0.01	**p<0.01		p>0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05		**p<0.01	p>0.05	p>0.05	p>0.05
B6_MD	**p<0.01	**p<0.01	p>0.05	p>0.05	**p<0.01	**p<0.01		**p<0.01	**p<0.01	**p<0.01
B7_MD	p>0.05	p>0.05	**p<0.01	*p<0.05	p>0.05	p>0.05	**p<0.01		p>0.05	p>0.05
B8_MD	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	**p<0.01	p>0.05		*p<0.05
B2_MD	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05	**p<0.01	p>0.05	*p<0.05	

←Copper (Cu)→

Cu µg per gram	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05
B2_RO	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B1_LONG_RO	p>0.05	p>0.05		p>0.05	p>0.05	*p<0.05	**p<0.01	p>0.05	p>0.05	p>0.05
B3_RO	p>0.05	p>0.05	p>0.05		p>0.05	**p<0.01	**p<0.01	*p<0.05	*p<0.05	p>0.05
B4_RO	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B5_RO	p>0.05	p>0.05	*p<0.05	**p<0.01	p>0.05		p>0.05	p>0.05	p>0.05	p>0.05
B6_MD	*p<0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05		p>0.05	p>0.05	p>0.05
B7_MD	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05
B8_MD	p>0.05	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05
B2_MD	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	

Cu µg per cig.	B1_RO	B2_RO	B1_LONG_RO	B3_RO	B4_RO	B5_RO	B6_MD	B7_MD	B8_MD	B2_MD
B1_RO		p>0.05	*p<0.05	*p<0.05	p>0.05	*p<0.05	p>0.05	p>0.05	p>0.05	p>0.05
B2_RO	p>0.05		**p<0.01	**p<0.01	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
B1_LONG_RO	*p<0.05	**p<0.01		p>0.05	**p<0.01	**p<0.01	*p<0.05	**p<0.01	**p<0.01	**p<0.01
B3_RO	*p<0.05	**p<0.01	p>0.05		*p<0.05	**p<0.01	p>0.05	**p<0.01	**p<0.01	**p<0.01
B4_RO	p>0.05	p>0.05	**p<0.01	*p<0.05		*p<0.05	p>0.05	p>0.05	p>0.05	p>0.05
B5_RO	*p<0.05	p>0.05	**p<0.01	**p<0.01	*p<0.05		*p<0.05	p>0.05	p>0.05	p>0.05
B6_MD	p>0.05	p>0.05	*p<0.05	p>0.05	p>0.05	*p<0.05		p>0.05	p>0.05	p>0.05
B7_MD	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05	p>0.05		p>0.05	p>0.05
B8_MD	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05	p>0.05	p>0.05		p>0.05
B2_MD	p>0.05	p>0.05	**p<0.01	**p<0.01	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	

Fig. 3 – The results of Tukey HSD test for multiple comparisons between the heavy metals concentration in tobacco and cigarette in the studied brands (***p<0.01, *p<0.05*).

In our study there were significant differences between the investigated brands. In Figure 2(a) the concentrations of investigated metals in filler tobacco are presented. The variability of each metal was significantly different from one brand to another. The one-way ANOVA test of single variability applied for each metal had the result of significance ***$p<0.001$. The highest concentrations of Cd, Pb and Cr were obtained for the brands purchased from the black market. In case of copper the brands from legal market had a significant higher concentration compared with those from black market. In case of nickel two brands had the lowest concentration B4_RO and B2_MD. In Figure 3 the results of multiple comparisons between brands of the metals concentration provided by Tukey HSD test are presented. The level of significance was marked with **$p<0.01$ and *$p<0.05$.

We calculated the quantity of each studied metal in a cigarette based on the measurements presented in Figure 1. The mass of each cigarette, which is different from one cigarette to another in the studied brands may influence the exposure of the consumer to the studied metals. This can be observed in Figure 3 where the results of multiple comparisons between the metal content per cigarette from each brand provided by Tukey HSD test are presented. The level of significance was marked with **$p<0.01$ and *$p<0.05$. The highest concentrations of Cd and Pb per cigarette were measured in some of the brands from the black market and Cr in all of studied brands from the black market of Iasi.

Afridi *et al.* (2013) [15] investigated the toxic elements in different cigarettes in Dublin, Ireland and they associated them with high risk of hypertension. The results reported as mean ± SD of metal per cigarette were: **Cd** 1.73±0.082 → 2.02±0.054 μg cig.$^{-1}$, **Pb** 0.378±0.034 → 1.16±0.062 μg cig.$^{-1}$ and **Ni** 0.715±0.059 → 1.52 ± 0.065 μg cig.$^{-1}$.

In scientific literature, the quantity of metal converted from the cigarette mass to smoke was calculated, which passes through the filter in the mouth of the smoker. Afridi *et al.* (2013) estimated the following concentration of heavy metals that were converted from a cigarette tobacco mass to smoke: **Cd** 54 - 62%, **Pb** 48.4 – 56.6% and **Ni** 50 – 58%. In general, smokers can absorb 10-30% of the total amount of cadmium from a standard cigarette.[16,17] Based on these results we estimated for each studied brand the heavy metal dose resulted from a cigarette reported as nanogram per kilogram body weight of smoker (81 kg male and 66 kg female). We use the maximum percentage of 30% for each studied metal that can be absorbed from a cigarette by a Roumanian male with a 81 kg body weight and a female with 66 kg body weight.

In Table 1 the results of heavy metals absorbed by smokers for each studied brand and metal are presented. In general, the highest exposure was estimated for a female smoker because her body weight is lower than that of a male smoker. The highest dosage of cadmium, lead and chromium were found for B6_MD, B7_MD, B8_MD and B2_MD brands from the black market. For instance, B2_RO and B2_MD brands represent the same brand, but the content of some toxic metals was almost double in the cigarette purchased from the black market: **Cd** (1.11 ng kg^{-1} b.w. M for B2_RO compared with 2.57 ng kg^{-1} b.w. M for B2_MD), **Pb** (0.82 ng kg^{-1} b.w. M for B2_RO compared with 1.6 ng kg^{-1} b.w. M for B2_MD), **Cr** (2 ng kg^{-1} b.w. M for B2_RO compared with 2.5 ng kg^{-1} b.w. M for B2_MD), **Ni** (5.08 ng kg^{-1} b.w. M for B2_RO compared with 2.88 ng kg^{-1} b.w. M for B2_MD) and **Cu** (37.6 ng kg^{-1} b.w. M for B2_RO compared with 35.78 ng kg^{-1} b.w. M for B2_MD).

Table 1

Metal absorbed from a cigarette in the body by an average Roumanian male and female smoker, expressed as nanogram per kilogram body weight (ng kg $^{-1}$ b.w.)

	Cd		**Pb**		**Cr**		**Ni**		**Cu**	
	M (81kg)	F (66kg)	M (81kg)	F (66kg)	M (81kg)	F (66kg)	M (81kg)	F (66kg)	M (81kg)	F (66kg)
B1_RO	1.08	1.33	0.65	0.80	2.87	3.52	5.14	6.31	40.36	49.53
B2_RO	1.11	1.37	0.82	1.01	2.00	2.46	5.08	6.23	37.60	46.15
B1_LONG_RO	1.38	1.69	0.97	1.19	3.22	3.95	8.21	10.08	58.99	72.40
B3_RO	1.96	2.40	0.72	0.88	2.69	3.31	6.70	8.23	56.81	69.72
B4_RO	1.72	2.12	0.49	0.60	1.60	1.96	3.61	4.43	39.60	48.60

Table 1 (continued)

B5_RO	0.75	0.92	0.95	1.17	1.52	1.87	3.65	4.48	23.57	28.93
B6_MD	3.01	3.69	1.19	1.46	5.26	6.46	8.92	10.95	41.99	51.53
B7_MD	1.83	2.25	0.74	0.91	4.25	5.22	4.16	5.11	30.85	37.86
B8_MD	3.18	3.91	1.21	1.48	3.01	3.69	5.63	6.91	35.86	44.01
B2_MD	2.57	3.15	1.60	1.97	2.50	3.07	2.88	3.54	35.78	43.91

EXPERIMENTAL

Each brand received the following label that shows also the country's origin (RO for Roumania and MD for Republic of Moldova): B1_RO, B2_RO, B1_LONG_RO, B3_RO, B4_RO, B5_RO, B6_MD, B7_MD, B8_MD and B2_MD. In case of B1_RO and B1_LONG_RO they belong to the same brand but one of them has longer cigarettes than standard ones. The B2_RO and B2_MD was the same brand with different points of production (Roumania and Republic of Moldova). Each 20 cigarette package from legal market was under Roumanian Minister control with a specific label, which proved the payment of a tobacco tax. The cigarettes from black market were labelled by the Republic of Moldova minister control for tobacco tax. In this study we purchased randomly 4 packs for each studied brand per year, in January, March, June and September. There were analyzed cigarettes from a total number of 80 packs. A total number of 240 cigarettes were analyzed.

Sample preparation

A number of 3 cigarettes from each studied pack was randomly extracted. The tobacco filling from each one was carefully separated and weighted with minimum limit for measurements of 0.0001 g. After this step, samples of 0.3 g of dry tobacco were taken for metal digestion. Special TFM vessels for metal digestion were inserted. The sample was mixed with 4 mL of 65% nitric acid (Merck, Darmstadt) and 1 mL of 30% hydrogen peroxide (EMSURE stabilized for higher temperature from Merck, Darmstadt). The mixture was kept fot 25 minutes to react before microwave digestion. Thereafter, the TFM pressure vessels were inserted in the MWS-2 Berghof microwave digester, programed for three step plant digestion protocol described by Strungaru *et al.*(2015).[18] After mineralization, the liquid samples were transferred into 50 mL dilution BRAND flasks that were filled up to the sign with ultrapure water produced by LaboStar system (Siemens) from double distilled water.

Apparatus optimization for metal analysis and method validation

The studied metals were measured with a HR-CS GF-AAS with platform, contrAA600 (Analytik, Jena, Germany). The calibration was made using stock solutions for Cu, Cd, Pb, Cr and Ni, diluted from standard solutions with certificate (Merck, Darmstadt), with concentrations of 1000 mg L^{-1} for each element. Since the tobacco from cigarettes represents in fact parts from tobacco plants, we validated our optimized method with Certified Reference Material BCR-679 white cabbage dry powder. This was certified by European Commission Joint Research Centre Institute for Reference Materials and Measurements. We prepared a number of 6 samples from this material in the same way as normal sample. These samples were randomly analysed during the metal measurements. The results obtained for each element were compared with the certified values of the concentration from the analysis table. The results obtained for each element were expressed as average ± confidence 95%: Cd (1.66±0.07 µg g^{-1} certified value with 1.64±0.05 µg g^{-1} obtained value), Cu (2.89±0.12 µg g^{-1} certified value with 2.75±0.2 µg g^{-1} obtained value), Cr (0.6±0.1 µg g^{-1} certified value with 0.7±0.075 µg g^{-1} obtained value) and Ni (27±0.8 µg g^{-1} certified value with 26.32±0.6 µg g^{-1} obtained value). Lead was not mentioned on the certificate and it was not analyzed from these samples. The optimization of AAS was done according to the protocol described by Strungaru *et al.* (2015).[18]

Statistical analysis

Shapiro-Wilk normality test was performed for the data sets. One-way ANOVA followed by the Tukey HSD test was conducted to demonstrate the significant variance of toxic metal between the cigarette brands.[18] All the statistical analyses were carried out with OriginPro v.9.3 (2016) software created by OriginLab Corporation, USA.

CONCLUSIONS

The heavy metals investigated were present in all 240 samples in significant amounts. The concentrations of cadmium and lead were significantly higher in brands purchased from the black market. This suggested that tobacco of a lower quality was purchased from the black market regarding heavy metal contaminants. Their source could be the chemical treatments made in the industrial process of tobacco, which implicate such metals. On the other hand, copper was significantly higher in the brands from legal market. This may have the origin in the agricultural processes of tobacco plant growing or industrial process. In case of nickel, it was not possible to make a separation between the studied markets. Based on dosage estimation, a female smoker is under a higher risk than a man smoker taken into consideration their body weight and the metal resulted from a cigarette. We suggest that it is necessary a legislation able to determine the cigarette producer to have a standard dose of tobacco in a slim, normal and long cigarette. This may help to calculate in the future the risk exposure with higher accuracy and to associate different diseases caused by tobacco smoking. There are many answers to be found about the origin of cadmium in tobacco and of other metals.

Acknowledgments. This study was financially supported by the project Resources pilot center for cross-border preservation of the aquatic biodiversity of Prut River MIS-ETC 1150 Roumania – Ukraine – Republic of Moldova.

REFERENCES

1. S. H. Hecht, *J. Natl. Cancer Inst.*, **1999**, *9* , 1194–1210.
2. R. S. Pappas, G. M. Polzin, C. H. Watson and D. L. Ashley, *Food Chem. Toxicol.*, **2007**, *45*, 202–209.
3. A.R. Surleva and G. Drochioiu, *J. Chem. Educ*., **2013**, *90*, 1654–1657.
4. G. Drochioiu, I. Mangalagiu and V. Tataru, *Analyst*, **2000**, *125*, 939-941.
5. C. Feldman and R. Anderson , *J. Infection*, **2013**, *67*, 169-184.
6. F. Yu, E. J. Nehl, T. Zheng, N. He, C. J. Berg, A. F. Lemieux, L. Lin, A. Tran, P. S. Sullivan and F. Y. Wong, *Drug and Alcohol Depen.*, **2013**, *132*, 265-270.
7. M. Williams, A. Villarreal, K. Bozhilov, S. Lin and P. Talbot, *PLoS One*, **2013**, *8*, e57987.
8. M. L. Goniewicz, J. Knysak, M. Gawron, L. Kosmider, A. Sobczak, J. Kurek, A. Prokopowicz, M. Jablonska-Czapla, C. Rosik-Dulewska, C. Havel, P. Jacob and N. Benowitz, *Tob. Control.*, **2014**, *23*, 133–139.
9. M. Laugesen, S. Thornley, H. McRobbie and C. Bullen, *SRNT Conference Portland Oregon*, **2008** (Conference abstract).
10. W. Schober, K. Szendrei, W. Matzen, H. Osiander-Fuchs, D. Heitmann, T. Schettgen, R. A. Jörres and H. Fromme, *Int. J. Hyg. Environ. Health.*, **2014**, *217*, 628–637.
11. L. Agoroaei, N. Bibire, M. Apostu, M. Strugaru, I. Grigoriu and E. Butnaru, *Rev. Chim.* (Bucharest), **2014**, *65*, 2148-2150.
12. T. G. Kazi, N. Jalbani, M. B. Arain, M. K. Jamali, H. I. Afridi, R. A. Sarfraz and A. Q. Shah, *J. Hazard. Mater.*, **2009**, *163*, 302-307.
13. S. Verma, S. Yadav and I. Singh, *Food Chem. Toxicol.*, **2010**, *48*, 2291-2297.
14. C. R. Armendáriz, T. Garcia, A. Soler, Á. J. G. Fernández, D. Glez-Weller, G. L. González, A. H. Torre and C. R. Gironés, *Environ. Res.*, **2015**, *143*, 162-169.
15. H. I. Afridi, T. G. Kazi, F. N. Talpur, D. Brabazon and S. Naher , *Clin. Chim. Acta* , **2013**, *426*, 51-57.
16. P. Andujar, L. Bensefa-Colas and A. Descatha, *Rev. Med. Interne*, **2010**, *31*, 107-115.
17. S. A. Strungaru, G. Plavan and M. Nicoara, *J. Surv. Fish. Scien.*, **2016**, *2*,1-15.
18. S. A. Strungaru, M. Nicoara, O. Jitar and G. Plavan, J. *Environ. Health Sci. Eng.*, **2015**, *13*, 1-11.