Assessment of heavy metal levels in roll-your-own cigarette and water pipe tobacco blends

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Abstract. The increased consumption of roll-your-own (RYO) cigarettes and water pipe (hookah), both globally and in Bulgaria, necessitated the current investigation aimed at the assessment of heavy metal levels in the tobacco blends used in those products. The concentrations of Mn, Zn, Cu, Cd, Pb, and Ni were determined by AAS in five brands of RYO tobacco (R1-R5) and seven brands of water pipe tobacco (W1-W7), all distributed on Bulgarian market. The average heavy metal contents (mg/kg) in the two types of tobacco blends, RYO and water pipe, respectively, were as follows: Mn - 214.20 and 37.89; Zn - 31.90 and 134.87; Cu - 12.06 and 10.07; Cd - 0.80 and 1.33; Pb - 1.60 and 3.57; Ni - 1.36 and 0.80. RYO cigarette tobaccos contained higher concentration of Mn, Cu and Ni, while water pipe tobaccos - Zn, Cd and Pb. Strong correlations were found between Cu and Zn (r=0.81), Cu and Cd (r=0.90) in RYO tobacco blends, and between Cu and Zn (r=0.86) - in water pipe blends, respectively. The results from the study give reason to recommend that this information be provided to the consumers, who consider the regarded tobacco products as a safer alternative to conventional cigarettes.

1 Introduction

In recent years, the popularity of certain tobaccoproducts traditionally used in the past has markedly revived, especially with regard to fine-cut tobacco blendsfor hand-rolling or hand-making of cigarettes (roll-your- own, RYO; make-your-own, MYO) and water pipe mixes (narghile, shisha, hookah). The distinctive growth in their consumption worldwide, in Europe and in our country, as well as the driving factors of those trends, have been revealed by a number of researchers [1 - 8]. The increased consumption of those products, especially among younger people, has been associated mostly with the attractive flavor and the lower price, compared with conventional cigarettes [9 - 12]. Besides, these products are still often considered as a “safer alternative” of commercial cigarettes [7, 13], although a series of studies revealing similar health risks in their consumption have been published already [1, 14, 15].

Tobacco consumption is responsible for about 700,000 deaths each year, and it is classified as the greatest health risk in the European Union [16]. The highmorbidity and mortality rates are associated with thebiological effects of the repeated inhalation of a series of toxic substances present in the smoke (including heavy metal ions), either synthesized through pyrolysis or transferred directly from the burning (smoldering) tobacco [17, 18]. In this regard, the determination of heavy metals (HMs) in the composition and in the emissions of various smoking products, as an important source of exposure to carcinogenic substances by active and passive smokers, has been the subject of serious research activities worldwide [19 - 22]. Metals mostcommonly associated with adverse health effects are arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni),and lead (Pb), due to their carcinogenicity and low daily exposure thresholds. Reasonably, most of the investigations on the concentrations of HMs in tobacco products and emissions have regarded different brands ofconventional cigarettes available on national or international markets, providing not just numerical data for HMs contents, but also evidence for substantial variations in those data due to the specifics of the tobaccos used in the composition of the respective tobacco blends. For example, the analysis of cigarettes sold in Brazil found the following average contents of HMs: As, 0.09 ± 0.024 μg/g; Cd, 0.65 ± 0.091 μg/g; Cr, 1.43 ± 0.630 μg/g; Ni, 1.26 ± 0.449 μg/g, and Pb, 0.27 ± 0.054 μg/g [23]. The same study reported the existence of strong correlations between some of the metals - Ni/Cr (r = 0.829), Ni/As (r = 0.799), Ni/Pb (r = 0.637),Cr/As (r = 0.621), and no correlation between Cd and therest of the metals. In another study, the average HM levels in different cigarette brands distributed in the USAwere as follows: As, 0.17 μg/g; Cd, 0.86 μg/g; Cr, 2.35 μg/g; Ni, 2.21 μg/g, and Pb, 0.44 μg/g [17]. In turn, the determination of 11 elements in the tobacco filler and in the smoke of twenty of the best-selling cigarette brands in Portugal [20] provided the following average

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concentrations: Al, 667.2 μg/g; Mn, 148.4 μg/g; Ba, 123.0 μg/g; Zn, 25.2 μg/g; Ni, 2.10 μg/g; Cr, 1.39 μg/g; Co, 0.84 μg/g; Cd, 0.79 μg/g; Pb, 0.54 μg/g; As, 0.14 μg/g, and Tl, 0.074 μg/g. A recent study tracing thecontent of Hg, Cd and Pb in cigarette brands sold in different countries (South Korea, Vietnam, Japan, Indonesia, Taiwan, Thailand, the United Kingdom,Belgium, Italy, Finland, and France) clearly differentiated between the national markets [19].

Thehighest levels of Hg (with an average value of 19.95 ± 4.8 ng/g) were found in the cigarettes available in Vietnam and Thailand; respectively, the content of Cd (average, 1.34 ± 0.59 μg/g) was higher in Thailand, the UK andBelgium, and that of Pb (average, 2.30 ± 2.64 μg/g) – in Belgium, the UK and South Korea. Besides, the assessment of the distribution of Pb and Cd in 15 brands of cigarettes manufactured or sold in Selangor state, Malaysia [24] suggested very wide variations in mainstream smoke concentrations depending on brand price category ("cheap" vs. "expensive" cigarettes).

As already stated, data about the concentration ofHMs in the tobacco part and in the emissions of other tobacco products are relatively limited. A study comparing the content of HMs in nine imported cigarettebrands and nine brands of water pipe (shisha, hookah) tobacco in Hillah, Iraq [21] reported that Cd contentvaried from 0.02 to 0.06 mg/kg in cigarette tobaccos and from 0.05 to 0.08 mg/kg in water pipe tobaccos; respectively, Pb concentrations were in the range 0.11 - 0.19 mg/kg in cigarette tobaccos and 0.54 - 0.83 mg/kg in water pipe tobaccos.

An investigation including nine cigarette and four water pipe brands sold on the Iranian market, however, found the opposite distribution, i.e. higher average heavy metal concentrations in cigarettes compared with water pipe mixes [22].

The analysis of HMs in tobacco, based on a pool of 36 brandsrepresenting different tobacco products (cigarettes, cigars and water pipes) in Saudi Arabia also suggested that the number and the amount of the identified elements were strongly dependent on product type and brand [25].

The average metal concentrations in the tobacco filler in this study were as follows: Cr, 0.66mg/kg; Cd, 0.09 mg/kg; Cu, 2.61 mg/kg; Fe, 245.55mg/kg; Pb, 0.38 mg/kg; Mn, 3.99 mg/kg, and Zn, 1.64 mg/kg.

To the best of our knowledge, a very limited number of investigations on the levels of HMs in tobacco and tobacco-related products have been carried out in Bulgaria. Nevertheless, a previous study regarding microelement concentrations in five RYO tobacco brands available in Bulgaria reported the following ranges of content variation: Pb (0 - 1.3 mg/kg); Cd (0.5 - 1.3 mg/kg); Ni (0.23 - 1.13 mg/kg; Mn (182.5 - 215.2 mg/kg); Zn (25.6 - 36.9 mg/kg) [26].

Therefore, the objective of current study was to assess the heavy metal levels (Mn, Zn, Cu, Cd, Pb, and Ni) in different RYO/MYO and water pipe tobacco blends marketed in Bulgaria, thus providing new details to the data available for those types of smoking products.

2 Materials and methods

2.1 Tobacco materials

The investigation was carried out with five commercial brands of fine-cut tobacco for hand-rolled or hand-filled cigarettes (RYO/MYO tobacco blends) and seven brands of water pipe tobacco, all purchased from retailer shops in Plovdiv, Bulgaria. To facilitate data presentation, the RYO/MYO tobaccos in the study were labeled with codes from R1 to R5, and, respectively, the water pipe tobaccos – with codes from W1 to W7. All analyses were performed in the period 2021 - 2022.

2.2 Determination of the content of heavymetals (HMs)

The initial preparation of tobacco samples included the following steps: for RYO/MYO tobaccos – drying at22 ± 2°C temperature and milling (to particle size below 0.4 mm); for water pipe tobaccos – repeated rinsing with distilled water in order to achieve maximal separation of tobacco pieces from the rest of the ingredients of the respective mix (honey/molasses, glycerol, colorants, flavorings, etc.), then drying at 22 ± 2°C temperature and milling (to particle size below 0.4 mm).

The resultant dry samples were subjected to mineralization in a muffle furnace at 500°C for 5 h, conditioned in a desiccator and then the ash was dissolved in 3M HCl. The content of the respective mineral elements (mg/kg) was determined on an atomic absorption spectrometer SpectrAA 220 (Varian, Australia) at the following wavelengths: Mn - 279.5 nm, Zn - 213.9 nm, Cu - 324.8 nm, Cd - 228.8 nm, Pb - 217.0 nm, and Ni - 232.0 nm.

Mean values from two parallel measurements of each sample have been presented further in the study.

3 Results and discussion

3.1 Heavy metal content in RYO/MYO tobaccos

The results from the determination of the content of HMs in the analyzed tobacco blends for RYO/MYO cigarettes are presented in Table 1.

Table 1. Concentration of heavy metals in five RYO/MYO tobacco blends

Sample	Concentration, mg/kg					
	Mn	**Zn**	**Cu**	**Cd**	**Pb**	**Ni**
R1	176.60	30.80	11.90	0.50	2.00	2.30
R2	240.10	33.90	11.70	0.40	3.00	0.60
R3	223.00	28.10	10.90	0.70	1.00	2.10
R4	223.10	35.70	14.80	1.80	2.00	1.40
R5	208.20	31.00	11.00	0.60	0.00	0.40
AVG	**214.20**	**31.90**	**12.06**	**0.80**	**1.60**	**1.36**

As seen from the data, Mn content in the regarded RYO/MYO tobaccos varied in a relatively wider range, from 176.60 mg/kg (R1) to 240.10 mg/kg (R2). The average Mn concentration (214.20 mg/kg) was higherthan the average values reported for cigarette tobaccos

originating from other markets – Portugal (148.4 µg/g) [20] and Saudi Arabia (3.985 mg/kg) [25]. Those differences in current and previous studies suggested that metal content might vary on a national and regional basis, due to the different tobaccos used in the respectiveblends and the applied technologies for their processing. The range of Zn variation between the brands was from 28.10 mg/kg (R3) to 35.70 mg/kg (R4). The values were fully compliant with the data for different cigarette brands in Iran (from 18.1 to 42.2 µg/g; average 27.02 µg/g) [27] and for RYO tobaccos in Bulgaria (in the range 25.6 - 36.9 mg/kg) [26], but higher than the averageZn concentrations in other studies [20, 25]. The average Cu content in the analyzed RYO/MYO tobaccos (12.06 mg/kg) was higher than the respective data (2.61 mg/kg) in a previous study [25], but the results were very close to those for Iranian cigarettes (5.18-17.6 µg/g) [27]. Four of the samples (R1, R2, R3 and R5) showed nearly identical Cu contents (between 10.90 and 11.90 mg/kg), while one of them (R4) differed significantly with a higher Cu concentration (14.80 mg/kg). The content of Cd in the samples was within therange from 0.40 mg/kg (R2) to 1.80 mg/kg (R4). The parallel with published data showed that the average Cd level in the analyzed tobaccos (0.80 mg/kg) was completely in line with the data reported for cigarettes sold in the USA, Malaysia and Portugal, respectively, 0.86 µg/g [17], 0.80 µg/g [24] and 0.79 µg/g [20]. Our results, however, differed to some extent from the data inother studies, being higher than the average Cd concentrations in cigarette tobaccos from Brazil [23],Iraq [21] and Saudi Arabia [25], but lower than those in other countries; 2.71 µg/g [27] and 1.34 µg/g [19].

The average Pb concentration (1.60 mg/kg) was lower than previously reported data; 2.07 µg/g [27], 3.05µg/g [24] and 2.30 µg/g [19]. The element was not detected in one of the brands (R5), while the rest of the samples fell in the range from 1.00 mg/kg (R3) to 3.00 mg/kg (R2). Correspondingly, Ni content in the RYO/MYOtobaccos in the study varied between 0.40 mg/kg (R5) and 2.30 mg/kg (R1), with an average level of 1.36 mg/kg. Our results were very close to the data for Ni content in cigarettes traded in Brazil (1.26 µg/g) [23] andconsiderably lower than those in other studies; 17.93µg/g [27], 2.21 µg/g [17] and 2.10 µg/g [20], respectively. Comparing the average concentrations of HMs in the analyzed RYO/MYO tobaccos marketed in Bulgaria the following descending order of element abundance could be derived: Mn > Zn > Cu > Pb > Ni > Cd. In that regard, our observations were fully compliant with similar data found in previous research works [20, 25].

3.2 Heavy metal content in water pipe (hookah) tobaccos

Table 2 presents the results from the determination of the same HMs in the water pipe tobacco mixes included in the study (W1 – W7).

Similar to the results from the assessment of RYO/MYO tobaccos above, there were also variations in the concentration of metals between the samples. The content of Mn varied in a relatively wide range, from 27.80 mg/kg (W2) to 50.00 mg/kg (W7). Those values were considerably higher than the average Mn levels reported in different tobacco products from Saudi Arabia [25], but they were significantly below the concentrations registered in other studies [20, 26].

Table 2. Concentration of heavy metals in seven water pipe mixes

Sample	Concentration, mg/kg					
	Mn	**Zn**	**Cu**	**Cd**	**Pb**	**Ni**
W1	29.80	199.70	12.20	1.10	3.00	0.40
W2	27.80	280.90	16.80	1.00	4.00	0.30
W3	36.50	60.40	8.50	1.60	4.00	0.80
W4	40.30	56.80	6.50	0.90	2.00	1.10
W5	33.70	149.80	7.00	1.80	3.00	0.60
W6	47.10	114.80	9.80	1.00	5.00	1.70
W7	50.00	81.70	9.70	1.90	4.00	0.70
AVG	**37.89**	**134.87**	**10.07**	**1.33**	**3.57**	**0.80**

The average Zn concentration in the water pipe tobacco mixes was 134.87 mg/kg, but the variations were substantial, from 56.80 mg/kg to 280.90 mg/kg. The levels registered in the study were significantly higher than the data reported earlier [20, 25 - 27], but it should be noted that those studies regarded different types of tobacco products (cigarettes, RYO tobacco, and others). Thus, possible reasons for such variations could be the specific characteristics of the tobaccos included in the respective blends, the agroecological conditions of their cultivation, and the applied processing technologies.

Similarly, Cu concentrations also varied significantly, between 6.50 mg/kg (W4) and 16.80 mg/kg (W2). The average Cu concentration (10.07 mg/kg), however, was very close to that found for Iranian cigarettes [27], although considerably higher than the average value for the tobacco products sold in Saudi Arabia (2.61 mg/kg) [25].

Cd concentrations in the studied water pipe tobaccos varied from 0.90 mg/kg to 1.90 mg/kg, with an average value of 1.33 mg/kg. The results were in full compliance with the data in previous studies, regarding both RYO tobaccos in Bulgaria [26] and cigarette tobaccos on the international market [19]. Some numerical variations also existed, and the registered Cd levels in the study were higher than the respective data for different typesof tobacco products found on other markets (Brazil, USA, Iraq, Portugal, Malaysia, Saudi Arabia) [17, 20, 21, 23 - 25]. Most probably, the factors behind those deviations were related, as already stated, to the selectionof tobaccos in the blends and their composition, the abundance of HMs and the pH of the soils, the coefficient of Cd absorbance, and others [23, 25].

The average Pb concentration in the water pipe tobacco samples was 3.57 mg/kg, varying in the range from 2.00 mg/kg to 5.00 mg/kg. Although the average was very close to the data for cigarette tobaccos in the Selangor state, Malaysia (3.05 mg/kg) [24], our results showed higher Pb levels in the studied water pipe mixes compared to previous studies, both in hookah tobacco (0.54 - 0.83 mg/kg) [21] and in other tobacco products [17, 20, 23 - 25]. The content of Ni in the analyzed samples varied between 0.30 mg/kg and 1.70 mg/kg, being fully compliant with the respective range of variation observed for RYO tobaccos in Bulgaria [26]. The average content

(0.80 mg/kg) was lower than the average metal concentration in previous studies [17, 20, 23, 27]. Summarizing the data in Table 2, the following order of HMs accumulation in the studied water pipe tobacco mixes could be derived: Zn > Mn > Cu > Pb > Cd > Ni.

Our results did not reveal a clear trend of HMs distribution, i.e. higher or lower concentrations in one of the studied types of tobacco products, RYO/MYO and water pipe blends (Table 1 and Table 2), respectively. For example, Mn was found at about 5-time higher concentration in the RYO/MYO tobacco, while Zn was present in a considerably (4-time) higher concentration in the water pipe tobaccos. Partially, the results supported a previous observation for higher Pb and Cd levels in hookah tobaccos, compared with cigarette tobaccos [21], but no other data for further parallel were available.

3.3 Correlations between the studied HMs in RYO/MYO and water pipe tobaccos

The next step in the investigation was to make an attempt to find functional dependencies between the individual HMs in the studied RYO/MYO and waterpipe tobacco blends, as suggested in some previous studies [19, 23].

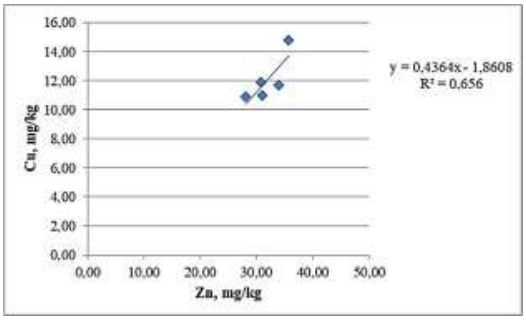

Fig. 1. Linear correlation between Zn and Cu concentrations in the studied RYO/MYO tobacco blends

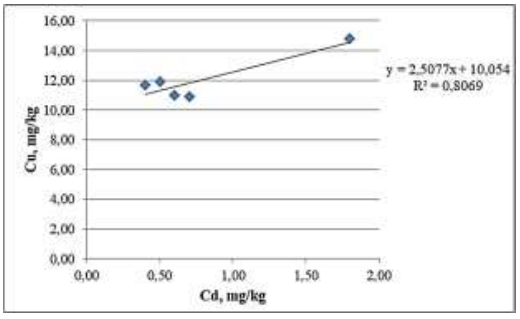

Fig. 2. Linear correlation between Cd and Cu concentrations in the studied RYO/MYO tobacco blends

The fitting of the experimental data to linear regression models found such correlations between Zn and Cu (Fig. 1), and Cd and Cu (Fig. 2) contents in the compared RYO/MYO tobaccos, while similar dependency existed only between Zn and Cu in the water pipe tobacco mixes (Fig. 3). Strong positive correlations were proven between the elements,respectively, Zn/Cu (r = 0.810) and Cd/Cu (r=0.898) in RYO/MYO tobaccos, and Zn/Cu (r = 0.859) in water pipetobaccos. The correlations between the rest of the metals in the comparison series were weak, either positive or negative.

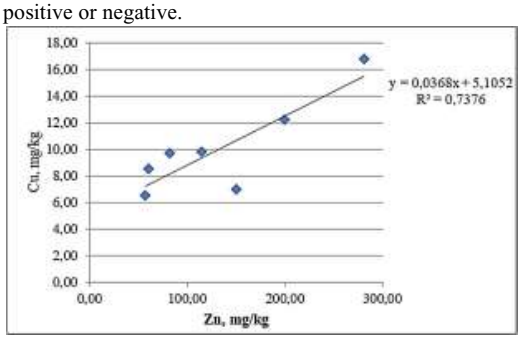

Fig. 3. Linear correlation between Zn and Cu concentrations in the studied water pipe tobacco mixes

4 Conclusions

The investigation on the levels of heavy metals (Mn, Zn, Cu, Cd, Pb, Ni) in RYO/MYO and water pipe tobaccos marketed in Bulgaria allowed to make the following conclusions:

There was considerable variation in the content of HMs in the studied tobacco products. The average HM concentrations (mg/kg) in the regarded RYO/MYO and water pipe tobaccos, respectively,were as follows: Mn - 214.20 and 37.89; Zn - 31.90 and 134.87; Cu - 12.06 and 10.07; Cd - 0.80 and 1.33; Pb - 1.60 and 3.57; Ni - 1.36 and 0.80.

The average concentration of HMs in the RYO/MYO tobaccos blends was in the order Mn > Zn > Cu > Pb > Ni > Cd; respectively, in the water pipe tobacco mixes the order was Zn > Mn > Cu > Pb > Cd > Ni.

There was no tendency for higher HMs concentrations in RYO/MYO tobaccos blendscompared with the water pipe tobacco mixes in the study.

Strong positive correlations were observed between some individual elements; Zn/Cu (r = 0.810) andCd/Cu (r = 0.898) in the analyzed RYO/MYO tobaccos, and Zn/Cu (r = 0.859) in the water pipe tobaccos.

The study provides new information for the content and distribution of heavy metals in RYO/MYO and water pipe tobaccos, which might be useful for expanding the available data on harmful substances in different types of tobacco products distributed on theBulgarian market. The study also provides grounds for future research in that direction, aiming at the provision of relevant information to tobacco consumers.

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