CONCENTRATIONS OF SELECTED METALS (NA, K, CA, MG, FE, CU, ZN, AL, NI, PB, CD) IN COFFEE

KONCENTRACIJE IZBRANIH KOVIN (NA, K, CA, MG, FE, CU, ZN, AL, NI, PB, CD) V KAVI

Grażyna ADLER1*, Arkadiusz NĘDZAREK2, Agnieszka TÓRZ2

1Pomeranian Medical University, Department of Studies in Antropogenetics and Biogerontology, Żołnierska 48, 71-210 Szczecin, Poland
2West Pomeranian University of Technology in Szczecin, Department of Aquatic Sozology, Kazimierza Królewicza 4, 71-550 Szczecin, Poland

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ABSTRACT

Introduction: The health benefits and detrimental effects of coffee consumption may be linked to chemical compounds contained in coffee beans. The aim of our study was to evaluate the concentration of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), aluminum (Al), nickel (Ni), lead (Pb) and cadmium (Cd) in green and roasted samples of coffee beans purchased in Bosnia and Herzegovina, and to determine the potential health implications at current consumption level.

Methods: The concentrations were determined using a microwave high-pressure mineralization and atomic absorption spectrometer that measures total metal (ionic and non-ionic) content.

Results: The average metal concentrations (μg element/g coffee) in the green coffee beans were: Na: 18.6, K: 19898, Ca: 789, Mg: 1758, Fe: 60, Cu: 14, Zn: 3.6, Al: 4.2, Ni: 0.415, Pb: 0.076, and Cd: 0.015, while, in the roasted; Na: 23, K: 23817, Ca: 869, Mg: 1992, Fe: 41.1, Cu: 11.4, Zn: 5.41, Al: 4.19, Ni: 0.88, Pb: 0.0169, and Cd: 0.0140.

Conclusion: The level of investigated metals at the present level of consumption of coffee in Bosnia falls within the limits recommended as safe for health.

IZVLEČEK

Uvod: Zdravstvene prednosti in škodljivi učinki uživanja kave so lahko povezani z kemijskimi spojinami v kavnih zrnih. Cilj študije je oceniti koncentracije naslednjih spojin: natrij (Na), kalij (K), kalcij (Ca), magnezij (Mg), železo (Fe), baker (Cu), cink (Zn), aluminij (Al), nikelj (Ni), svinec (Pb) in kadmiij (Cd) v zelenih in praženih vzorcih kavnih zrn, kupljenih v Bosni in Hercegovini, ter določiti morebitne zdravstvene posledice glede na trenutno raven uživanja.

Metode: Koncentracije so bile določene z uporabo visokotlačne mineralizacije in atomic absorption spectrometerje, ki merijo skupno vsebino kovanec (ionska in neionska) content.

Rezultati: Povprečna koncentracija kovin (μg elementa/g kave) v zelenih kavnih zrnih je bila: Na: 18.6, K: 19898, Ca: 789, Mg: 1758, Fe: 60, Cu: 14, Zn: 3.6, Al: 4.2, Ni: 0.415, Pb: 0.076 in Cd: 0.015; v praženih zrnih pa: Na: 23, K: 23817, Ca: 869, Mg: 1992, Fe: 41.1, Cu: 11.4, Zn: 5.41, Al: 4.19, Ni: 0.88, Pb: 0.0169 in Cd: 0.0140.

Zaključek: Raven preverjenih kovin na trenutni stopnji uživanja kave v Bosni in Hercegovini se nahaja znotraj omejitev, ki so priporočene kot zdrave za zdravje.
1 INTRODUCTION

Coffee is, after water, amongst the most popular beverages consumed in the world (1). Numerous studies suggest that moderate coffee consumption has beneficial health impacts on humans. Coffee infusions show protective potential against oxidative stress through the presence of antioxidants and active components (2-4). They increase the antioxidant plasma capacity and might increase glutathione levels and thus reduce the levels of DNA damage (5, 6). Furthermore, some studies imply that habitual coffee consumption could protect against Parkinson’s and Alzheimer’s disease (7, 8). Coffee consumption may also be linked to improvements in fecal microbiota and colonic fermentation, may reduce the risk of developing liver, colorectal and prostate cancer as well as brain tumors (9-11). The meta-analysis by Lukic et al. confirmed the protective effect of coffee consumption on the risk of endometrial cancer. Additionally, authors suggest that increased coffee intake might be particularly beneficial for women with obesity (12). On the other hand, increased long-term coffee consumption may cause, among others: increasing plasma homocysteine and LDL (Low Density Lipoprotein) level, gastrointestinal disturbances (4, 13, 14). Moreover, repeated doses of coffee throughout the day may be linked to an elevation of cortisol secretion in the afternoon hours (15). What is even more, maternal consumption of coffee during pregnancy may increase the risk of the occurrence acute childhood leukemia (16, 17). It is also suggested that coffee consumption is a risk factor for osteoporosis in postmenopausal women (18).

The contamination of food may be caused by various compounds found in the environment e.g. high doses of fertilizers or plant protection chemicals. These compounds, taken with food and then absorbed in the digestive tract, may have an influence on body cells and tissues. On the other hand, potential contamination may derive from package and storage, pollution, fungi and molds (19, 20). The amount of compounds taken with food and then absorbed in the digestive tract, may have an influence on body cells and tissues. On the other hand, potential contamination may derive from package and storage, pollution, fungi and molds (19, 20). The amount of compounds taken with coffee infusions depend on their contents in coffee beans, level of consumption and pattern of drinking coffee infusions, which is linked to the geographical region.

In Europe, the highest coffee consumption is observed in Finland, 12 kg per capita/year, and in Bosnia and Herzegovina (B&H) it is 6.2 kg per capita/year (21). Coffee infusions in Bosnia are prepared in a traditional way, which is widespread in the Balkans, the Middle East, Eastern Europe, North Africa, the Caucasus and Bali, known as Turkish coffee. During the first step roasted ground coffee is added to boiling water in the pot and heated until the foam level is rising. Afterwards, the coffee is flooded with a small amount of cold water. At this time the coffee is partially precipitating. Next, the coffee is heated until the foam level rises again. After a few minutes the coffee is ready to drink (19).

2 MATERIALS AND METHODS

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2.1 Coffee Samples

The coffee beans were roasted and purchased in small local stores in Sarajevo, B&H. Coffee roasting was carried out in specialized drum furnaces by store employees. The process of roasting can be described as follows: the gas flame under the drum heats and burns the coffee beans, the temperature being around 200 °C immediately after roasting. After this process, the beans are cooled down by air at ambient temperature.

We tested two samples of green coffee beans (G 1-2) and six (R 1-6) roasted. Two pairs of the same coffee beans, before and after roasting were examined: G 1a and R 1a and G 2b and R 2b. The measurements of metal concentration were performed in triplicate from each coffee sample.

2.2 Instrument

Grinded coffee beans weighing 1±0.01 g were digested with a mixture of concentrated HNO3 and HClO4 acids (5 and 1 mL, respectively) and then diluted to 25 mL with water. Coffee infusions were prepared by boiling 10±0.1 g of coffee in 200 mL of water. Coffee brewing time was 5 min. After cooling the infusions to 20 °C, 25 mL were taken for mineralization with a mixture of concentrated HNO3 and HClO4 acids (2.5 and 1 mL, respectively). After mineralization, the samples were diluted to 50 mL with water.

Mineralizations of coffee beans and coffee infusions were carried out using a high pressure microwave mineralizer, Speedwave Xpert (Bergof, Enningen, Germany). Metals were determined using the Hitachi Polarized Zeeman Atomic Absorption Spectrometer ZA3000 Series (Hitachi High-Technologies Corporation, Tokyo, Japan). Ca, K, Na, Mg were determined using the flame atomic absorption spectroscopy method (FAAS) in the air-acyetylene flame with Zeeman correction. Fe, Cu, Zn, Al, Pb Cd and Ni were measured by the flameless technique in a graphite furnace atomic absorption spectroscopy (GFAAS). Milli-Q water (18.2MΩ) was used to prepare coffee infusions and dilutions. Standardized calibration solutions...
dedicated for metal determination by atomic absorption with a concentration of 1000 mg/L were used for the calibration curves; for Mg, Ca, K, Na and Fe (Scharlau Chemie s.a., Barcelona, Spain), and for Zn, Cu, Pb, Cd Ni and Al (Merck, Darmstadt, Germany). Reliability of the analytical method was tested using a reference material - fish muscle ERM-BB422 (European Reference Materials, European Commission - Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium). The recovery of elements was in the range of 95-105%, and the precision for the reference material was 1.2-10.1%.

2.3 Statistical Analysis
The measurements of metal concentration were performed in triplicate from each coffee sample. For quantitative variables in each group, the minimum and maximum values, the arithmetic mean, SD and IQR were calculated. The differences of metals’ concentration were performed using the ANOVA test.

### Table 1a. Concentration of metals in green coffee (μg/g=ppm).

| No of green coffee | Na  | K   | Ca   | Mg   | Fe  | Cu  | Zn  | Al  | Ni  | Pb  | Cd  |
|--------------------|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|
| G1a                | 10.6| 19583| 695  | 1733 | 75.1| 18.5| 4.04| 5.65| 0.44| 0.1436| 0.1415|
| G2b                | 26.6| 20213| 882  | 1783 | 45.2| 9.4  | 3.09| 2.73| 0.39| 0.0084| 0.0152|
| Mean               | 18.6| 19898| 789  | 1758 | 60  | 14   | 3.6 | 4.2  | 0.415| 0.076| 0.015 |
| SD                 | ±11.31| ±445.48| ±132.23| ±35.36| ±21.14| ±6.43| ±0.67| ±2.06| ±0.04| ±0.0956| ±0.0005 |
| IQR                | (8)  | (315)  | (93.5) | (25) | (14.95) | (4.55) | (0.48) | (1.46) | (0.03) | (0.068) | (0.0004) |

### Table 1b. Concentration of metals in roasted coffee (μg/g=ppm).

| No of roasted coffee | Na  | K   | Ca   | Mg   | Fe  | Cu  | Zn  | Al  | Ni  | Pb  | Cd  |
|----------------------|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|
| R1a                  | 24.2| 20300| 868  | 2127 | 41.8| 16.5| 5.07| 3.53| 0.72| 0.0202| 0.0124|
| R2b                  | 36.3| 22133| 888  | 2120 | 49.1| 10.3| 6.18| 6.01| 0.69| 0.0131| 0.0126|
| R3                   | 21.6| 32933| 864  | 2097 | 37.4| 13.3| 5.16| 4.25| 1.14| 0.0115| 0.0111|
| R4                   | 14.3| 25167| 904  | 2007 | 36.2| 6.9  | 4.96| 4.44| 0.71| 0.0196| 0.0222|
| R5                   | 13.0| 20867| 750  | 1803 | 46.7| 14.6| 5.89| 5.17| 0.84| 0.0215| 0.0120|
| R6                   | 28.6| 21500| 939  | 1800 | 35.2| 6.5  | 5.22| 1.74| 1.16| 0.0155| 0.0135|
| Mean                 | 23  | 23817| 869  | 1992 | 41.1| 11.4| 5.41| 4.19| 0.88| 0.0169| 0.0140|
| SD                   | ±8.80| ±747.12| ±64.30| ±153.91| ±5.80| ±4.13| ±0.50| ±1.47| ±0.22| ±0.0041| ±0.0041 |
| IQR                  | (11.4) | (3383)  | (35) | (260.3) | (9)  | (6.5) | (0.63) | (1.28) | (0.35) | (0.006) | (0.001) |

Samples G1 and G2- green; R1 to R6- roasted; G1a and R1a and G2b and R2b- pairs of the same coffee beans, before and after roasting; SD- standard deviation; IQR- interquartile range.

3 RESULTS
The ranges of values of metals’ concentration (μg element/g coffee) in the samples of green coffee beans are presented in Table 1a. In the samples of roasted coffee beans, there were: Na: 13.0-36.3, K: 20300-32933, Ca: 750-939, Mg: 1800-2127, Cu: 5.6-16.5, Zn: 4.96-6.18, Al: 1.74-6.01, Ni: 0.69-1.16, Pb: 0.0115-0.0215 and Cd: 0.0111-0.0222 μg/g of coffee. The average contents of Na, K, Ca, Mg, Zn, Ni and Cd in roasted coffee samples compared to green coffee were higher, while average contents for Al have not changed, and for three metals: Fe, Cu and Pb were lower (see Table 1a and 1b). The ranges of values of metals’ concentration (μg/100mL) in the coffee infusions were as follows: Na: 25.5-63.9, K: 81467-132333, Ca: 1037-1827, Mg: 4600-8463, Fe: 8.93-24.50, Cu: 1.20-6.86, Zn: 5.53-13.17, Al: 2.03-8.43, Ni: 0.78-1.82, Pb: 0.133-0.558, and Cd: 0.036-0.061 (see Table 2).
Table 2. Concentration of metals in coffee infusions, (μg/100mL).

| No of coffee | Na   | K    | Ca    | Mg    | Fe    | Cu    | Zn    | Al    | Ni    | Pb    | Cd    |
|--------------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| R 1a         | 57.0 | 8693 | 1277  | 6593  | 11.30 | 2.82  | 6.10  | 4.93  | 0.78  | 0.558 | 0.042 |
| R 2b         | 59.8 | 9567 | 1633  | 8463  | 24.50 | 2.62  | 13.17 | 4.63  | 1.63  | 0.357 | 0.059 |
| R 3          | 42.8 | 1323 | 1037  | 6893  | 15.87 | 6.86  | 6.13  | 8.33  | 1.62  | 0.163 | 0.041 |
| R 4          | 25.5 | 1035 | 1233  | 4600  | 8.93  | 2.15  | 5.53  | 5.40  | 0.82  | 0.133 | 0.036 |
| R 5          | 19.6 | 8146 | 1263  | 7380  | 16.13 | 2.70  | 6.67  | 8.43  | 1.04  | 0.192 | 0.061 |
| R 6          | 63.9 | 9526 | 1827  | 8073  | 15.27 | 1.20  | 8.13  | 2.03  | 1.82  | 0.140 | 0.046 |
| Mean         | 44.8 | 9920 | 1378  | 7000  | 15.33 | 3.04  | 7.62  | 5.63  | 1.29  | 0.257 | 0.048 |
| SD           | ±18.71 | ±1794 | ±292.36 | ±1369.18 | ±5.33 | ±2.86 | ±2.43 | ±0.46 | ±0.168 | ±0.010 |
| IQR          | (29.3) | (12550) | (303.5) | (1231.8) | (3.8) | (0.5) | (1.66) | (0.75) | (0.17) | (0.015) |

SD- standard deviation; IQR- interquartile range

All analyzed metals passed from ground coffee beans to coffee infusions. The highest average diffusion showed K and Mg, more than 83 and 70%, respectively. The average level of diffusion from 38 to 27% showed: Na, Pb, Ca, Ni and Zn and Al. While, the lowest: Fe, Cu and Cd, each less than 11% (see Table 3).

Table 3. Penetration of metals to coffee infusions, (%).

| No of coffee | Na   | K    | Ca    | Mg    | Fe    | Cu    | Zn    | Al    | Ni    | Pb    | Cd    |
|--------------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| R 1a         | 47.1 | 85.6 | 29.4  | 62.0  | 5.4   | 3.4   | 24.0  | 28.0  | 55.3  | 6.8   | 21.5  |
| R 2b         | 32.9 | 86.4 | 36.8  | 79.8  | 10.0  | 5.1   | 42.6  | 15.4  | 54.4  | 9.4   | 47.6  |
| R 3          | 39.6 | 80.4 | 24.0  | 65.8  | 8.5   | 10.3  | 23.8  | 39.2  | 28.2  | 7.3   | 28.1  |
| R 4          | 35.7 | 82.3 | 27.3  | 45.8  | 4.9   | 6.2   | 22.3  | 24.3  | 13.6  | 3.2   | 23.2  |
| R 5          | 30.2 | 78.1 | 33.7  | 81.8  | 6.9   | 3.7   | 22.6  | 32.6  | 17.9  | 10.3  | 24.9  |
| R 6          | 44.7 | 88.6 | 38.9  | 89.7  | 8.7   | 3.7   | 31.1  | 23.3  | 18.0  | 6.8   | 31.4  |

The analysis in pairs of the same coffee beans, before and after roasting showed that, in roasted coffee beans (R 1a), concentrations of Na, Ca, Zn and Ni were significantly higher than in green coffee beans (G 1a), while in roasted beans R 2b, concentrations of Na, K, Mg, Zn, Al, Ni and Pb were significantly higher than in green beans G 2b. It should be noted that, in green coffee beans (G 1a), concentrations of Al and Pb were significantly higher than in roasted coffee beans (R 1a) (see Table 4).

Table 4. Empirical levels of significance for differences of metals’ concentrations by ANOVA test.

| Metal | G 1a vs. R 1a | G 2b vs. R 2b |
|-------|---------------|---------------|
| Na    | <0.001*       | 0.013*        |
| K     | 0.509         | 0.008*        |
| Mg    | <0.001*       | 0.802         |
| Fe    | 0.004         | 0.427         |
| Cu    | 0.335         | 0.235         |
| Zn    | 0.017*        | 0.007*        |
| Al    | 0.004*        | 0.0066*       |
| Ni    | 0.020*        | 0.012*        |
| Pb    | <0.001*       | 0.030*        |
| Cd    | 0.173         | 0.135         |

*statistically significant differences
4 DISCUSSION

Coffee beans from miscellaneous geographical areas are characterized by different organoleptic features and chemical composition (20, 22, 23). The final content of elements in the coffee beans is also influenced by technological processes: drying, burning, roasting and storage (19, 20). Mineral components and toxic elements pass to the beverage during the brewing process. Their final content in infusion is influenced by methods of brewing and occurrence other mineral components that could be present e.g. in water used to prepare beverage (24).

On the one hand metals play an important role in the human body e.g. as components of enzymes. On the other hand, their increased supply may cause adverse health effects, like damage to internal organs, endocrine disruption and poor reproductive capacity and genotoxicity linked to cancers. They may also be involved in the pathogenesis of neurodegenerative diseases (25-28). Moreover, it should be noted that lead accumulates in the human body, causing distant effects (29).

The tolerable upper intake level (mg), which exceeds a recommended intake for the examined elements per year (for adults with average body weight 70 kg), is as follows: Na: 730000, K: 2775000-127750000 (probably oral lethal dose for human varies), Ca: 912500, Cu: 1825, Zn: 9125, Al: 3650, Ni: 365, Pb: 91, Cd: 9.125. Very large doses of magnesium, higher than 1825000 mg, are associated with its toxicity, and oral dose of 1533000 mg Fe can be lethal (30-34).

The exposure on metals from coffee is usually a long-term low-concentration exposure. Coffee is a rich source of elements and compounds. The data on the differences in concentrations of metals in coffee beans is important due to the popularity of this beverage and the occurrence of a safe consumption limit for elements that come from the diet, dietary supplements and the environment and could be present e.g. in water used to prepare beverage during the brewing process. Their final content in infusion is influenced by methods of brewing and occurrence other mineral components that could be present e.g. in water used to prepare beverage (24).

In 2015, Oliveira et al. have analyzed mineral contents in infusions prepared from roasted coffee beans from: Brazil, Ethiopia, Colombia, Mexico, Sudan, Guatemala, Papua New Guinea, Kenya, Cuba, Timor, Mussulo and China. Authors have noted similar values for Na, as in our studies of coffee infusions, but lower values for: Ca, Mg, K and Fe (39). The authors observed that the mineral profiles of the beverages were linked to both, inter- and intracontinental differences. It is interesting that Mn and Ca were found to be the best chemical descriptors for origin of coffee beans. South American origin coffees were on average richer in the analyzed elements, except for Ca, while coffee beans from Central America had lower mineral contents (excluding Mn). Unfortunately, among the above mentioned studies authors rarely investigated these elements, so such comparison is impossible.
Ashu and Chandravanshi studied content of selected metals in coffee infusions in Etiopian coffee beans and noted similar Ca, Fe and Cu concentrations compared to our results, but higher values for Na and Zn, and lower values for K and Mg (20). Pb and Cd concentrations were below their method detection limits, just like in research by Grembecka et al. (23).

Average intakes of Na, K, Ca, Mg, Fe, Cu, Zn, Al, Ni, Pb and Cd (mg/per capita/year), based on the average metal content of roasted coffee purchased in Bosnia (see table 1b) and coffee consumption in B&H were: 142.6, 147665.4, 5387.8, 12350.4, 254.82, 70.68, 33.54, 25.98, 5.46, 0.105, 0.071 respectively. Note that the intake values are the entire population averages, which means that coffee drinkers will tend to ingest more coffee and metals than values shown.

Therefore further investigations are needed, especially on metals that have harmful effects on health, and also those that accumulate in the body, like Pb. Research should focus on countries with high coffee consumption. Again, the fact that even low levels of some metals, e.g. zinc, may interfere with HDL (High Density Lipoprotein) concentrations, Pb is accumulated and the effects of its accumulation can be distant in time should be taken into account (29, 40).

5 CONCLUSION
All in all, the level of studied components in coffee purchased in Bosnia is within the recommended limits.

CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

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ETHICAL APPROVAL
Not applicable.

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ETHICAL APPROVAL
Not applicable.
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