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# DETERMINATION OF MERCURY, CADMIUM AND LEAD CONTENTS IN DIFFERENT TEA AND TEAS INFUSIONS (*CAMELLIA SINENSIS*, L.)

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## ABSTRACT

The present paper deals with assessing the level of contamination of green ($n$ = 14) and black – fermented ($n$ = 10) teas of different origins (country of origin) (China, India, Japan, Nepal and Taiwan), which are normally available in Slovakia. The contents of the studied contaminants (mercury, cadmium and lead) were observed in samples of dried teas and their infusions. The contaminant contents were investigated by atomic absorption spectrometry with Zeeman background correction and a graphite furnace GF-AAS (Cd, Pb). The total mercury content was analyzed by CV-AAS method. Concentrations of the studied contaminants in the dried tea samples were as follows: green tea: Hg: 0.0027 ±0.0010 mg.kg$^{-1}$ (median ± standard deviation); Cd: 0.161 ±0.084 mg.kg$^{-1}$, Pb: 0.875 ±0.591 mg.kg$^{-1}$, black tea: Hg: 0.0022 ±0.0014 mg.kg$^{-1}$, Cd: 0.397 ±0.077 mg.kg$^{-1}$, Pb: 1.387 ±0.545 mg.kg$^{-1}$. The contents of the contaminants in the tea infusions were as follows: green tea: Hg: 0.03 ±0.04 µg.L$^{-1}$, Cd: 0.278 ±0.068 µg.L$^{-1}$, Pb: 1.975 ±0.503 µg.L$^{-1}$, black tea: Hg: 0.050 ±0.080 µg.L$^{-1}$, Cd: 0.291 ±0.054 µg.L$^{-1}$, Pb: 1.955 ±1.264 µg.L$^{-1}$. According to the currently valid maximum limits for a particular contaminant in Slovakia, it can be stated that the health standards were not exceeded in any of the tea samples. The limit value of the lead content (2.0 mg.kg$^{-1}$ DM) was exceeded (by 12.4%) only in one sample of the dried black tea from China (Yunnan – Golden Snow), however it is the limit value valid in China. The results of the analysis of 24 tea samples show that even regular consumption does not pose a health risk to consumers.

**Keywords**: mercury; cadmium; lead; teas; tea infusions; *Camellia sinensis*, L.

## INTRODUCTION

Tea (*Camellia sinensis*, L.) is one of the most popular non-alcoholic beverages consumed by over two-third of the world´s population due to its medicinal, refreshing and mild stimulant effects. Tea plays a major role in terms of the intake of a number of nutritional trace elements and biologicaly active compounds in humans **(Sharangi, 2009)**. The Tea plant is evergreen plant with three races (*C. sinensis, C. assamica* and *C.* c*ambodiensis*). Tea plant grows in more than 36 countries spread over all continents **(Jeszka-Skowron et al., 2015)**. Tea plant grows best in tropical and subtropical areas with adequate rainfall, good drainage and acid soils. Tea leaf comprises of two leaves and the terminal apical bud of a growing shoot of a tea bush. Made teas are classified into six main types such as white, green, oolong, black, compressed and flavored based on their respective manufacturing techniques **(The United Kingdom Tea Council Ltd., 2014)**.

Green teas (compared with other tea types) are characterized by the highest biological effect on consumers. They contain a broad spectrum of catechins, and/or flavan-3-ols and caffeine (theine) **(Naldi et al., 2014; Zimmermann and Gleichenhagen, 2011)**. Substances from the group of catechins and flavan-3-ols have high antioxidant ability **(Nováková et al., 2010)**.

Regular consumption of tea beverage has a positive effect on various aspects of human health. Tea drinking is associated with the reduction of blood serum cholesterol **(Dong et al., 2007)**, prevention of low density lipoprotein oxidation **(Zhang et al., 2009)**, decreased risk of cardiovascular disease and cancer **(Chung et al., 2003)**. Polyphenols are the most biologically active group of tea components, which have antioxidative, antimutagenic, and anticarcinogenic effects **(Yao et al., 2004)**. Tea contains also other compounds beneficial to human health like fluoride, caffeine and essential minerals **(Cabrera et al., 2003)**.

Regular consumption of tea (and/or herbal tea) also brings negative aspects on human health and it is therefore necessary to know the contents of some harmfull elements in teas and tea infusions **(Bobková et al., 2015)**. Some heavy metals, particularly Hg, Cd and Pb are characterized by high persistence in different environmental compartments or different agricultural (plant and/or animal) products, especially legumes **(Timoracká et al., 2011)**, potatoes **(Musilová and Bystrická, 2010)**, bee honey **(Roman and Popiela, 2011)** and high bioaccumulate ability in the consumer's body, causing various diseases such as cancer and/or Alzheimer disease **(De Sole et al., 2013)**. According to the findings of **Chen et al., (2011)**, contents of some hazardous metals (especially aluminum and mercury) are positively correlated with the levels of catechins or total phenolic contents.

Due to the fact that beverages prepared with hot or cold extraction of teas belong to the most popular non-alcoholic beverages, it is necessary to pay attention to the monitoring their phytochemical composition, especially in terms of the content of harmful ingredients, often naturally present in them. The paper focuses on determination of the content of monitored contaminants (Hg, Cd and Pb) in teas and their subsequent transfer to a tea beverage prepared by hot extraction.

## MATERIAL AND METHODOLOGY

For the purposes of this paper, 24 samples of tea (*Camellia sinensis* L.) that represent two basic groups of teas: green tea ($n$ = 10) and black – fermented tea ($n$ = 14) were chosen. The samples were obtained from Tea House of Good People, Nitra, Slovakia in 2014. The samples come from different countries of East Asia (China, India, Japan, Nepal and Taiwan). The main characteristics of the tea samples are shown in Table 1.

### *Pre-analytical and analytical procedure*

Before their mineralization, the samples of dried tea were homogenized in a ceramic mortar. The homogenized tea samples (1.0 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., Matthews, NC, USA) in a mixture of 5 mL $HNO_3$ (SupraPUR, Merck, Darmstadt, Germany) and 5 mL deionized water (0.054 $\mu S.cm^{-1}$) from Simplicity185 (Millipore SAS, Molsheim, France). Digestion conditions for the applied microwave system comprised of the heat which ran up to 160 °C for 15 minutes, keeping it constant for 10 minutes. A blank sample was carried out in the same way. The digest were subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell & Filtrak GmbH, Bärenstein, Germany) and filled up with deionized water to a volume of 50 mL **(Árvay et al., 2014)**.

To assess the degree of possible intoxication of a consumer resulting from the consumption of a tea beverage, 2 g of the sample was extracted with 200 mL of boiling water for 10 minutes and afterwards the extract was filtered through filter paper Filtrak 390 (Munktell & Filtrak GmbH, Bärenstein, Germany).

The determination of metals were performed in a Varian spectrAA240Z (Varian Inc., Mulgrave, VIC, Australia) atomic absorption spectrometer with Zeeman background correction. The graphite furnace technique was used for the determination of Cd and Pb (detection limits for GF-AAS: 10.0 and 10.0 $ng.kg^{-1}$ for Cd and Pb, respectively). The total mercury content was determined in the homogenized dried samples of teas (0.005 – 0.01 g) and tea infusions using a cold-vapour AAS analyzer AMA 254 (Altec, Praque, Czech Republic) with a detection limit of 1.5 $ng.kg^{-1}$ DM **(Svoboda et al., 2006)**. Calibration standard was used for the calibration of GF-AAS, CertiPUR® (Merck, Darmstadt, Germany).

### *Statistical analysis and risks assessment*

All statistical analyses were carried out using the statistical software Statistica 10.0 *(Statsoft,* USA). Descriptive data analysis included minimum value, maximum value, median, mean and standard deviation. The limit of statistical significance was set up at $p$ <0.05 for all descriptive statistical analysis. In order to assess the level of contamination of the dried green and black teas, the results of the studied contaminant contents were compared with maximum allowable levels (MAL) of the contaminants that are listed in relevant legislative regulations valid in China and Slovakia.

## RESULTS AND DISCUSSION

### *Heavy metals in the tea samples (dried)*

The contents of the studied elements in the dried tea samples are shown in Table 1. The contents varied in different intervals, depending mainly on the type of tea. Significantly higher concentrations of mercury were recorded in green teas compared with black – fermented teas, which is caused by technological processes (higher temperature during the tea fermentation). Hg concentration was 0.0027 ±0.0010 $mg.kg^{-1}$ (median ±standard deviation) in the green teas and 0.0022 ±0.0014 $mg.kg^{-1}$ in the black teas. Paradoxically, the highest concentration of Hg was recorded in one sample of black tea (Darjeeling Singbulli: 0.00511 $mg.kg^{-1}$). Almost identical results of mercury content in Pu-Erh teas (fermented teas) recorded **Cao et al., (2010)**, who found in 17 samples concentration interval of 0.0002 – 0.0390 $mg.kg^{-1}$ with an average of 0.0030 $mg.kg^{-1}$. The maximum allowable levels of the monitored contaminants defined by the legislative standards of China, and/or Codex Alimentarius of Slovakia were not exceeded in the results of the Hg content in the tea samples.

In the case of cadmium and lead, the situation was different. Higher concentration of Cd and Pb was recorded in black teas compared with green teas. The cadmium content ranged from 0.161 ±0.084 $mg.kg^{-1}$ in the green teas and 0.397 ±0.077 $mg.kg^{-1}$ in the black teas. Similar conclusions were published by many authors who analyzed samples of green and black teas from different countries. For example **Salahinejad and Aflaki, (2010)** analyzed samples of black tea from India and recorded a cadmium content ranging from ND-0.770 $mg.kg^{-1}$. **Moreda-Pinero et al., (2003)** found Cd content ranging from 2.22 to 2.39 $mg.kg^{-1}$ in different kinds of teas from India, Japan, Kenya, Malaysia, etc. Lower Cd content was found in samples of green tea from China and Japan, ranging from 0.051 to 0.114 $mg.kg^{-1}$ **(Marcos et al., 1998)**. Approximately 3 – 4 fold higher concentrations were found in lead concentration compared with cadmium. The Pb content was 0.875 ±0.591 $mg.kg^{-1}$ in the green teas and 1.387 ±0.545 $mg.kg^{-1}$ in the black teas. The relatively high value of the standard deviation indicates high variability of the set of values (Table 1). Similar results reported **Lv et al., (2013)**, who recorded Pb concentration ranging from 0.66 – 4.66 $mg.kg^{-1}$ with an average of 2.32 $mg.kg^{-1}$ in fermented Pu-Erh teas. It confirms the findings of **Cao et al., (2010)**.

Relatively large concentration interval of lead in individual tea samples can be caused by harvest period and age of the tea leaves. **Han et al., (2006a)** reported 2 – 2.5 times higher Pb content (as well as other elements) in older leaves compared with younger ones. Other major factors affecting the different concentrations of lead in teas are local environmental conditions **(Qin and Chen, 2007)**, use of fertilizers **(Frankl et al., 2005)** and technological processing. **Han et al., (2006b)** state that technological processes are major source of the product contamination

**Table 1** Basic characteristics of tea samples and heavy metals content in tea and tea infusion samples.

| Name | Sub-name | Country of origin | Heavy metals in teas (mg.kg$^{-1}$ DM) | | | Heavy metals in tea infusions (µg.L$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Hg | Cd | Pb | Hg | Cd | Pb |
| **Black teas** | | | | | | | | |
| Darjeeling | Jungpana 2013 SF | India | 0.00193 | 0.269 | 1.036 | 0.00030 | 0.440 | 4.530 |
| Darjeeling | Muscatel | India | 0.00082 | 0.338 | 1.392 | 0.00004 | 0.275 | 0.340 |
| Darjeeling | Singbulli | India | 0.00511 | 0.382 | 1.382 | 0.00009 | 0.261 | 2.580 |
| Nepal | Guranse 2013 FF | Nepal | 0.00177 | 0.491 | 1.456 | 0.00004 | 0.282 | 2.940 |
| Aiko | -- | Japan | 0.00247 | 0.324 | 0.370 | 0.00005 | 0.277 | 1.770 |
| Assam | Bherjan Bio | India | 0.00101 | 0.425 | 1.624 | 0.00001 | 0.336 | 1.900 |
| Ruby | -- | Japan | 0.00132 | 0.447 | 1.901 | ND | 0.257 | 1.360 |
| Yunnan | Golden Snow | China | 0.00365 | 0.411 | **2.248**[b] | 0.00009 | 0.314 | 2.850 |
| Yunnan | Black Needles | China | 0.00369 | 0.431 | 0.767 | 0.00005 | 0.318 | 0.315 |
| Gaba | Black | Taiwan | 0.00279 | 0.260 | 1.078 | 0.00006 | 0.299 | 2.010 |
| **Descriptive statistics** | | Minimum | 0.00082 | 0.260 | 0.370 | ND | 0.257 | 0.315 |
| | | Maximum | 0.00511 | 0.491 | 2.248 | 0.00030 | 0.440 | 4.530 |
| | | Median | 0.00220 | 0.396 | 1.387 | 0.00005 | 0.291 | 1.955 |
| | | St. dev. | 0.00137 | 0.077 | 0.545 | 0.00008 | 0.054 | 1.264 |
| **Green teas** | | | | | | | | |
| Lung Ching | Superior | China | 0.00416 | 0.222 | 1.832 | 0.08600 | 0.276 | 1.830 |
| Pi Lo Chum | Extra | China | 0.00226 | 0.230 | 1.475 | 0.03000 | 0.338 | 2.090 |
| Mao Feng | Fu Tien | China | 0.00365 | 0.090 | 0.448 | ND | 0.304 | 2.360 |
| Tai Mu | Long Zhu | Chinan | 0.00278 | 0.117 | 0.880 | 0.05300 | 0.238 | 3.190 |
| Gaba | Imperial | Taiwan | 0.00237 | 0.189 | 0.996 | 0.02300 | 0.222 | 2.090 |
| Kate | Benituki | Japan | 0.00339 | 0.272 | 1.315 | 0.03900 | 0.319 | 1.790 |
| Gaba | Katsura | Japan | 0.00202 | 0.134 | 0.157 | 0.01800 | 0.382 | 1.770 |
| Gyokuro | Asahina | Japan | 0.00435 | 0.049 | 0.196 | 0.07100 | 0.255 | 1.240 |
| Genmaicha | Extra | Japan | 0.00268 | 0.050 | 0.299 | 0.04500 | 0.132 | 1.890 |
| Sakura | Kukicha | Japan | 0.00119 | 0.245 | 1.048 | ND | 0.360 | 2.460 |
| Sencha | Shizouka | Japan | 0.00227 | 0.245 | 0.870 | 0.13300 | 0.276 | 2.270 |
| Matcha | Iri Genmaicha | Japan | 0.00351 | 0.094 | 0.315 | 0.11300 | 0.279 | 1.750 |
| Sencha | Ashikubo | Japan | 0.00445 | 0.072 | 0.717 | 0.01900 | 0.210 | 1.300 |
| Shambala | Nepal Exclusive | Nepal | 0.00226 | 0.262 | 1.968 | ND | 0.358 | 2.440 |
| **Descriptive statistics** | | Minimum | 0.00119 | 0.049 | 0.157 | ND | 0.132 | 1.240 |
| | | Maximum | 0.00445 | 0.272 | 1.968 | 0,13300 | 0.382 | 3.190 |
| | | Median | 0.00273 | 0.161 | 0.875 | 0,03450 | 0.278 | 1.975 |
| | | St. dev. | 0.00098 | 0.084 | 0.591 | 0,04208 | 0.068 | 0.503 |
| **Maximum Allowable Levels** | | | **0.300**[a] **0.500**[c] | **1.000**[a, c] | **2.000**[b] **10.0**[c] | -- | -- | -- |

ND – not detected.
DW – dry weight.
[a] Maximum allowable levels of Cr, Cd, Hg, As and F in tea (NY659-2003) **(MOAC, 2002)**.
[b] Maximum allowable levels of contaminants in green foods – tea (NY/T 288-2002) **(MOAC, 2003)**.
[c] Maximum allowable levels of contaminants – Codex allimentarius of Slovakia **(PKSR, 2006)**.

by lead, especially spreading out. MAL of the cadmium and lead content defined by relevant legislative standards was not exceeded almost in all samples. An exception was one sample of Chinese black tea (Yunnan – Golden Snow), where MAL was exceeded by 12.4% (Table 1), which was reflected also in the Pb content of the tea infusion. However, this concentration does not posses any health risk arising from a long-term and regular consumption of the tea beverage. According to MAL valid in the Slovak Republic the Pb content was not exceeded.

***Heavy metals in the tea infusion samples***

The contents of the monitored elements in the tea infusions are listed in Table 1. The concentration of mercury in the infusions of green tea were 0.03 ±0.04 $\mu g.L^{-1}$ and 0.05 ±0.08 $\mu g.L^{-1}$ in the black tea infusions. The highest concentration of Hg was recorded in the black tea Darjeeling Jungpana 2013 SF infusion (0.30 $\mu g.L^{-1}$). The cadmium content ranged from 0.278 ±0.068 $\mu g.L^{-1}$ in the green tea infusions and 0.291 ±0.054 $\mu g.L^{-1}$ in the black tea infusions. Similarly to mercury, the highest concentration of cadmium was recorded in the sample of Darjeeling Jungpana 2013 SF (0.44 $\mu g.L^{-1}$). Similar results obtained Nookabkaew et al., (2006) and Sofuoglu and Kavcar, (2008), who recorded, cadmium contents at intervals from 0.04 to 0.24 $\mu g.L^{-1}$ and 0.02 – 0.79 $\mu g.L^{-1}$, respectively in samples of green and black tea from China, India, Japan and Turkey.

The lead content was in the range of 1.975 ±0.503 $\mu g.L^{-1}$ in the green tea infusions and 1.955 ±1.264 $\mu g.L^{-1}$ in the black tea infusions. The relatively high value of the standard deviation in the black teas is caused by high levels of lead in the sample of Darjeeling Jungpana 2013 SF (4.53 $\mu g.L^{-1}$). This value is by almost 132% higher in comparison with other values (median). Our results are considerably lower than results of green and black tea from Iran and China recorded by **Nookabkaew et al., (2006)** and **Karimi et al., (2008)**.

## CONCLUSION

The present paper aims at determinating of the studied xenobiotics – heavy metals (Hg, Cd and Pb) in samples of green (*n* = 14) and black – fermented (*n* = 10) teas originating from different countries and beverages prepared from them. Our results show that MAL values defined by appropriate legislative norms were not exceeded in the majority of the dried tea samples. An exception was only one sample of Chinese fermented tea (Yunnan – Golden Snow), in which the allowable amount of lead was exceeded by 12.4%. The contents of the monitored contaminants in the tea infusions were not compared with the limit values. It can be concluded that even regular consumption of the tea beverages prepared from the tea samples does not pose a health risk to consumers.

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