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# Survey of content of cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, mercury, sodium and zinc in chamomile and green tea leaves by electrothermal or flame atomizer atomic absorption spectrometry

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**Abstract:** Due to the simplicity of tea preparation (pouring hot water onto different dried herbs) and its high popularity as a beverage, monitoring and developing a screening methodology for detecting the metal content is very important. The concentrations of Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Hg, Na and Zn in 8 samples of green tea (*Camellia sinesis*) and in 11 samples chamomile (*Matricaria chamomilla* L.) purchased both at local herbal pharmacies and supermarkets were determined using electrothermal atomizer atomic absorption spectrometry (ETAAS) and flame atomizer atomic absorption spectrometry (FAAS). The found concentrations in chamomile were: Cd (0.008 – 284 mg $kg^{-1}$), Ca (2.42 – 6.29%), Cr (0.91 – 6.92 mg $kg^{-1}$), Cu (6.27 – 11.39 mg $kg^{-1}$), Fe (133.5 – 534 mg $kg^{-1}$), Pb (0.561 – 1.277 mg $kg^{-1}$), Mg (2.27 – 3.73%), Mn (62.2 – 165.6 mg $kg^{-1}$), Hg (0.660 – 1.346 μg $kg^{-1}$), Na (0.91 – 1.28%) and Zn (63.37 – 108.5 mg $kg^{-1}$), in green tea Cd (36.29 – 202.1 mg $kg^{-1}$), Ca (2.77 – 6.40%), Cr (1.520 – 5.278 mg $kg^{-1}$), Cu (9.354 – 22.56 mg $kg^{-1}$), Fe (162.6 – 513.3 mg $kg^{-1}$), Pb (1.808 – 4.770 mg $kg^{-1}$), Mg (1.41 – 2.62 %), Mn (1.147 – 1.729 g $kg^{-1}$), Hg (1.045 – 2.802 μg $kg^{-1}$), Na (0.44 – 0.98%) and Zn (30.65 – 115.6 mg $kg^{-1}$), respectively. Principal Component Analysis (PCA) was applied to identify factors (soil, climate and country of origin) influencing the content of the measured elements in herbal samples. The proposed methodology developed in this work was successfully applied to the detection of metals in herbal samples. The analysis showed that the content of toxic metals in green tea samples was significantly higher and very close to the maximum dose recommended by the World Health Organization (WHO).

**Keywords:** trace elements; chemometrics; tea; ETAAS; FAAS; PCA.

## 1 Introduction

This work represents a continuation of our efforts for surveying of metal content in herbal products used for tea brewing [1,2] as well as other dietary products, e.g. olive oil [3] available at Croatian market. In this work, we expanded the list of analyzed metals for zinc, chromium and mercury and focused only on chamomile and green tea samples. Chamomile was selected as an example for a traditional herb used for brewing in Croatia. On the other hand, green tea was selected as a modern tea with an increasing popularity growth rate in share among consumers.

Teas have been part of the human civilization for at least the last five thousand years according to first written records report in ancient China. Nevertheless, it is hard to confirm, but it is reasonable to assume usage of herbs for medicine during man history from the Stone Age on.

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Recently, tea drinkers have become aware of contaminants, especially heavy metals, e.g. cadmium, lead and mercury, which have potential health hazards.

It is a well-known fact that plants are using leaves as storage places for all metal ions absorbed from soil and/or water, such as: cadmium, mercury, copper etc. [4] Due to mentioned factors, it is very important to observe the metal concentration in daily food and drink content and intake. This could be especially done for commonly accepted beverages like teas, since the metals extracted from the plant material during tea preparation can have either beneficial or harmful effect on human health [1]. It is very difficult to find literature or manuscripts dedicated to minerals and trace metals in chamomile [1,2]. The vast majority of the manuscripts [5-7] is focused on the most popular teas consumed in the world – black and green tea.

Minerals and trace elements have a number of key roles in the human body, e.g. Ca is important for bones and teeth growth and health [8], Fe is essential for oxygen transport [9]; Zn and Cu are built into the structure of enzymes etc. [10,11].

Cadmium (Cd) has been recognized as both, a human and multi-tissue animal carcinogen [1]. Cd could interfere with plant growth, as well as ion and water balances [1].

Iron (Fe) is an essential nutritional element for all forms of life [9]. Fe acts as a cofactor for many enzymes. It is crucial for the oxygen transport (as building part of hemoglobin) and the electron transfer [9]. Nevertheless, daily requirements of Fe may fluctuate between 8-18 mg for humans. Since Fe has pro-oxidant activity, it can be toxic in excess concentrations [12].

Calcium (Ca) is involved in a number of important physiological functions e.g. the rhythm maintenance of cardiac muscle and the excitability reduction both for nerves and muscles [8]. Elevated Ca concentration (especially above 2.6 mM) is called hypercalcemia, what could be related to the development of myeloma, hyperparathyroidism and vitamin D intoxication [13].

Chromium (Cr) is one of the essential trace elements for both, human and animals [14]. Cr can be found in air, water and soil. The occurrence of Cr in soil ranges from 10 to 50 mg $kg^{-1}$ depending on the source. Cr usually occurs in two forms, Cr(III) and Cr(VI). Plants can assimilate Cr in both forms. Cr(III) has limited solubility in water and lesser toxicity, while Cr(VI) has greater solubility in water and is highly toxic to biota [15]. Cr can cause toxicity to the plants as exhibited by reduced root growth and phytomass, chlorosis, photosynthetic impairing, stunting and finally plant death [16].

Copper (Cu) is one of the micronutrients found in plants and animals, but it becomes toxic above a daily intake of 2 mg [17]. The usage of various cupreous compounds in agro-technical treatments can lead to copper increase on the cropped soils [17,18]. Due to the fact that Cu can be biogenic and toxic, Cu contents in human environment, e.g. water, food and beverages, have to be traced and controlled daily [17-21].

Lead (Pb) was very early identified and very well documented as one of the most common contaminants in the environment [22]. Humans are usually exposed to lead through occupational and environmental sources; for instance if contaminated water with Pb was used for herbal tea brewing, it could lead to Pb poisoning [23,24].

Manganese (Mn) is a micronutrient found naturally in plants and animals [25]. However, overexposure to Mn can have neurological effects which usually result from of the consumption of water with very high levels thereof [25].

Magnesium (Mg) is a vital mineral for humans. Mg is needed for normal functioning of the heart, muscles and nerves. It also participates in the activation of more than 300 enzymes that ensure the smooth operation of many metabolic processes. Ingestion of nutrients is only way for Mg entry to the human body [26,27].

The presence of small quantities of zinc (Zn) is crucial for the growth of both, plant and animal life. Zinc utilizes beneficial effects on cardiocirculatory function and prevention of black foot disease. On the other hand, zinc is widely used for the protection of steel against corrosion, dry batteries photoengraving and lithography [28].

Sodium is very important for maintaining a cation-anion balance. The loss of sodium can lead to a water loss. On the other hand, high sodium intake can be fatal for patients suffering hypertension [29].

Mercury is one of the metals that have been considered as a toxic element by European Commission since 2006 [30]. It can affect male reproductive functions including sperm counts, motility and morphology and spermatogenesis [29].

The investigated metals from the tea samples can be divided in three groups: biogenic (Ca, Mg, Na, Zn), biogenic/toxic (Cu, Cr, Fe, Mn) and toxic ones (Cd, Hg, Pb). In addition, the aim of this study was to show the differences in the metal content among samples that depend primarily on herb variety but also on applied treatments during the sample production from field to factory.

# 2 Materials and Methods

## 2.1 Apparatus and Reagents

The main conditions used for ETAAS and FAAS measuring were suggested by the manufacturer of atomic absorption spectrometer which were specified in our previous work [1].

The Cd, Cr and Pb measurements were carried out using a Model AAS vario 6 ETAAS atomic absorption spectrometer (Analytik Jena AG, Jena, Germany, 2001) equipped with a transversely heated graphite atomizer with an autosampler (Model MPE 50), a deuterium background correction system and a hollow cathode lamp, for cadmium operated at 3 mA (wavelength 228.8 nm), chromium at 3.5 mA (wavelength 357.9 nm) and lead operated at 3 mA (wavelength 283.3 nm), respectively. Pyrolytic coated graphite tubes with a PIN-platform (Analytik Jena, Part No. 407-A81.025) were used during the analytical determination (Analytik Jena AG, 2001). The injection volume was 20 μL and integrated absorbance (peak area) was used for signal evaluation. Ca, Cu, Fe, Na, Mg, Mn and Zn measurements were carried out using a Model AAS vario 6 FAAS atomic absorption spectrometer equipped with a deuterium background correction system and a hollow cathode lamp for calcium, copper, iron, sodium, magnesium and manganese (wavelength 422.7 nm for Ca; 324.8 for Cu nm; 248.3 nm for Fe, 589.6 nm for Na, 285.2 nm for Mg, 279.5 nm for Mn and 213.9 nm for Zn). The concentrations of Ca, Cu, Fe, Mg, Mn and Zn were determined by Flame AAS with $C_2H_2$/air burner, while Na was determined with $C_2H_2/N_2O$ burner (Analytik Jena AG, 2001). Argon (5N purity) was used as the purge gas at 300 mL $min^{-1}$, except in the atomization stage (gas stop). Acetylene, air and nitrogen(I) oxide were mixed on burner and used for atomization in flame. All of the gases used were 4N purity. Hg measurements were carried out using the mercury analyzer AMA 254 (Labeko, Czech Republic) equipped with low pressure mercury lamp. All samples were analyzed for mercury without microwave digestion directly from solid-state samples with oxygen burner (5N purity) built-in apparatus at 253.7 nm. The samples were weighed using a Mettler AX 205 (Mettler Toledo, Columbus, Ohio, United States of America) electronic balance. The analytical measurements were based on the absorbance peak areas like suggested by Mierzwa et al. [31]. The basic instrumental and experimental conditions for ETAAS determinations are shown in Table 1.

All sample preparations, as well as ETAAS and FAAS measurements, were carried out according to the standard method HRN EN 14084:2005, corresponding to the British Industrial Standard EN 14084:2003. All results shown in this manuscript were calculated using the arithmetic mean of measured values of absorptions in triplicate. The obtained recovery results were found to be in the range from 95 to 102%.

Tea leaf samples were digested in a closed microwave system, CEM Model Mars 5 (CEM Corporation, United States of America) which is a microwave oven equipped with internal pressure and temperature control systems. For the microwave digestion were used 4 mL of nitric acid (suprapure) and 2 mL of hydrogen peroxide (suprapure), purchased from Merck, Germany. This oven has a variable power range (up to 630 W) adjustable in 1 % increments and a programmable timer. The lined Teflon® vessels with a volume of 100 mL and a pressure-relief valve were used. Temperature and pressure in the vessel during wet digestion were between 210°C and 240°C and 0.70 MPa and 1.0 MPa, respectively. The first cycle of microwave digestion lasted for 25 minutes and the second one 15 minutes. After the cooling down period, all the samples were transferred into 50 mL volumetric flasks and diluted with supra pure water. Table 1 shows the physical properties of ETAAS employed in this work:

Table 2 illustrates a linear range, LOD (limit of detection), LOQ (limit of quantification) and RSD (relative standard deviation) for surveyed metals.

LOD and LOQ were calculated by next equations:

$$\mathrm{LOD} = \frac{3\sigma}{S} \qquad \mathrm{LOQ} = \frac{10\sigma}{S}$$

Where is:

$\sigma$ – standard deviation of the slope of calibration curve for each element

$S$ – slope of calibration curve for each element

The concentrations at the linear range represent the lowest and the highest standard concentrations, respectively. Every calibration curve was calculated from measured absorptions for three standard solutions according HRN EN 14084:2005 for examined metal.

The results of the calibration curves for Cd, Ca, Cu, Fe, Pb and Mg were taken from analysis described in our previous work [1] because both analysis were done simultaneously.

## 2.2 Chemicals

All solutions were prepared by dissolving the chemicals in supra pure water (with a conductivity 0.04 μS $cm^{-1}$) using Millipore Simplicity (Millipore, United States of America). Hydrogen peroxide, s.p. (supra pure) and nitric

acid, s.p., were obtained from Merck, Germany. All metal standard solutions (analytical concentration metal in the form of metal nitrate in nitric acid solution was 1000 ± 2 mg $L^{-1}$) were obtained from Merck. All the matrix modifiers employed such as: palladium nitrate, p.a. (pro analysis) and magnesium nitrate, p.a. were also obtained from Merck. Pyrolysis and atomization curves were established in the presence of a chemical modifier – 0.1% $Pd(NO_3)_2$ + 0.05% $Mg(NO_3)_2 \times 6H_2O$. The modifier was prepared from Merck stock solution: Art.1.07289 palladium matrix for graphite furnace AAS and Art.1.05855 magnesium nitrate hexahydrate. The final volume added of modifier was 5 µL.

**Table 1:** Physical properties of ETAAS for the determination of Cd and Pb in tea samples.

| | Cd | Cr | Pb |
|---|---|---|---|
| Wavelength, nm | 228.8 | 357.9 | 283.3 |
| Slit/nm | 0.5R | 0.8R | 0.5R |
| Purge gas | Ar | Ar | Ar |
| Drying temperature, °C | 120 | 120 | 120 |
| Ramp, hold, s | 72/50 | 65/40 | 72/50 |
| Ashing temperature, °C | 900 | 1100 | 1200 |
| Ramp, hold, s | 13/10 | 5/5 | 4/4 |
| Atomization, °C | 1300 | 2100 | 2050 |
| Ramp, hold, s | 3.3/3 | 4.3/4 | 5/4 |
| Clean-up, °C | 2300 | 2400 | 2300 |
| Hold, s | 4 | 4 | 4 |
| L'vov platform | Yes | Yes | Yes |
| Integration time, s | 4 | 4 | 4 |
| Injected sample volume, µL | 20 | 20 | 20 |
| Modifier volume, µL | 5 | 0 | 3 |

## 2.3 The analyzed samples

The samples of commercially available teas were purchased at local supermarkets (samples 1 to 19, Table 3). A mixture of plant leaves in tea bags formed the analyzed samples. There were three tea bags from tea packages used for the preparation of the samples by mixing in mortar. The weighted sample mass was about (0.4000±0.0010) g.

Each sample was put in Teflon® vessels with 4 mL of nitric acid and 2 mL of hydrogen peroxide for microwave digestion.

The data for the origin of green tea samples in Table 3 are related to country of production. It was very hard to establish precisely where the green teas were from. Practically no producers mentioned a country where the raw tea was purchased. Virtually completely all of Croatian industrial growth of chamomile plants is based in Slavonia. Slavonia is a Croatian region famous for its plains used for agriculture production. Due the usage of artificial fertilizers, soil in Slavonia is laden with different heavy metals e.g. Cd and Cu.

The statistical data analysis was made using the R program ver. 2.9.2 [32]. Principal component analysis (PCA) was applied to analyze the significance of each element in the data structure.

The teas purchased at drugstores were dried and produced in factories on different ways depending on the plant used.

Ethical approval: The conducted research is not related to either human or animals use.

**Table 2:** Linear range, LOD, LOQ and RSD for all surveyed metals.

| Parameters | Cd | Ca | Cu | Fe | Na |
|---|---|---|---|---|---|
| Linear range/µg $L^{-1}$ | 0.20-1.00 | 500-4000 | 100-500 | 100-1000 | 2000-10000 |
| LOD/µg $L^{-1}$ | 0.08 | 26 | 28 | 26 | 92 |
| LOQ/µg $L^{-1}$ | 0.26 | 86 | 94 | 88 | 307 |
| RSD/% | 2.00 | 1.60 | 1.89 | 0.56 | 0.25 |

| Parameters | Cr | Pb | Mg | Mn | Zn | Hg |
|---|---|---|---|---|---|---|
| Linear range/µg $L^{-1}$ | 10-20 | 10-50 | 100-1000 | 100-1000 | 500-1000 | 0.20-20 |
| LOD/µg $L^{-1}$ | 0.03 | 0.07 | 18 | 20 | 61 | 0.01 |
| LOQ/µg $L^{-1}$ | 0.10 | 0.24 | 62 | 66 | 205 | 0.04 |
| RSD/% | 3.15 | 2.30 | 1.53 | 2.25 | 0.77 | 0.22 |

LOQ – limit of quantification
RSD – relative standard deviation

**Table 3:** Information of investigated herbal tea samples.

| Sample | Herb latin name | Origin | Trademark |
|---|---|---|---|
| 1 | *Matricaria chamomilla* L. | Macedonia/industrial growth | Good Nature |
| 2 | *Matricaria chamomilla* L. | Poland/industrial growth | Rumianek |
| 3 | *Matricaria chamomilla* L. | Poland/industrial growth | Grande |
| 4 | *Matricaria chamomilla* L. | Germany/industrial growth | Alnatura |
| 5 | *Matricaria chamomilla* L. | Croatia/industrial growth | Franck |
| 6 | *Matricaria chamomilla* L. | Croatia/industrial growth | Biofarm |
| 7 | *Matricaria chamomilla* L. | Croatia/industrial growth | Agristar |
| 8 | *Matricaria chamomilla* L. | Croatia/industrial growth | Ultra plus |
| 9 | *Matricaria chamomilla* L. | Croatia/industrial growth | Cedevita |
| 10 | *Matricaria chamomilla* L. | Croatia/industrial growth | Naturavita |
| 11 | *Matricaria chamomilla* L. | Croatia/industrial growth | Travar "MB" |
| 12 | *Camellia sinesis* | Croatia | Podravka |
| 13 | *Camellia sinesis* | Ukraine | Twinings |
| 14 | *Camellia sinesis* | Croatia | Cedevita |
| 15 | *Camellia sinesis* | Croatia | Biofarm |
| 16 | *Camellia sinesis* | Croatia | Holiplant |
| 17 | *Camellia sinesis* | Austria | Teekanne |
| 18 | *Camellia sinesis* | Croatia | Travar "MB" |
| 19 | *Camellia sinesis* | Russia | Russian green tea |

## 3 Results and Discussion

Amongst all tested metals, WHO classifies Ca, Na and Mg as minerals, Cr, Cu, Fe, Mn and Zn as trace metals. Minerals or trace metals, even the same ones, could have high importance and unwanted side effects at the same time for human health. The WHO recommends a recommended dietary allowance (RDA). RDA for Ca is 1.3 g, Na 1.5 g, Mg 0.42 g, Cr 35 μg, Cu 0.9 mg, Fe 18 mg, Mn 2.3 mg and Zn 11 mg. It could be interesting to see if herbal beverage consumption helps for reaching RDA for minerals and trace elements. We tried to give an answer to this question. The results from the next tables and in the Discussion section give an answer.

Tables 4 and 5 show the calculated concentrations of each tested metal in every sample, as well as the mean values, the standard deviations, the minima, the maxima and median values.

In analyzed samples the highest contents, by mean value, were recorded for Ca and Mg for both chamomile and green tea. The third metal by content in chamomile is sodium which is reasonable (importance in water balances), but in green tea samples manganese was the third one. It is a very interesting thing that manganese content in analyzed green teas were about 10 times higher than in chamomile. Unfortunately, green tea had higher contents of mercury, lead and cadmium too. Those contents were higher about 2 times than in chamomile.

After qualitative analysis, we performed a t-test on independent groups to analyze the difference in each metal content for two tea plants, chamomile and green tea. Principal component analysis was used to group samples in two plant types, chamomile and green tea. The variables were metal content and the cases were samples. The results are given in Table 6.

Normality condition for t-test was performed by the assessment of data diverging form the Normal plot. In case of chamomile data the normality was very good for all metals except for mercury for which the normality was still satisfactory (Figure 1.).

For the metals in green tea the normality was very good for all metals but lower, still satisfactory for chromium and sodium (Figure 2 and 3).

Metals whose were analyzed in this study were from group of biogenic elements for humans (Fe, Cu, Mn, Zn, Mg and Ca), beneficial elements (Na and Cr) and toxic elements (Cd, Pb and Hg).

Higher contents of Cu, Mn, Cd, Pb and Hg were found in green tea while Mg and Na were higher in chamomile. The content of Fe, Zn, Ca and Cr were similar in both

**Table 4:** Elemental content in analyzed tea chamomile samples expressed as a dry mass of the sample (mean of three measurements)

| Sample | Ca g kg$^{-1}$ | Mg g kg$^{-1}$ | Na g kg$^{-1}$ | Zn mg kg$^{-1}$ | Cr mg kg$^{-1}$ | Cu mg kg$^{-1}$ | Fe mg kg$^{-1}$ | Mn mg kg$^{-1}$ | Cd mg kg$^{-1}$ | Hg μg kg$^{-1}$ | Pb mg kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5.88 | 3.29 | 1.10 | 99.5 | 4.31 | 8.29 | 360 | 152 | 0.678 | 0.660 | 1.07 |
| 2 | 3.65 | 2.51 | 0.990 | 102 | 2.89 | 7.02 | 210 | 120 | 0.508 | 0.694 | 1.15 |
| 3 | 6.29 | 2.27 | 1.21 | 83.2 | 6.92 | 4.09 | 423 | 184 | 0.719 | 1.35 | 0.970 |
| 4 | 4.93 | 3.04 | 1.27 | 67.9 | 4.22 | 11.4 | 534 | 62.2 | 0.082 | 0.819 | 1.11 |
| 5 | 5.44 | 3.11 | 1.02 | 92.3 | 3.60 | 8.99 | 435 | 166 | 0.456 | 0.953 | 1.28 |
| 6 | 5.76 | 2.88 | 1.28 | 109 | 3.31 | 6.27 | 292 | 54.7 | 0.279 | 0.954 | 0.777 |
| 7 | 5.30 | 3.34 | 0.915 | 87.3 | 2.08 | 9.42 | 198 | 141.7 | 0.805 | 0.957 | 0.836 |
| 8 | 2.42 | 3.73 | 1.16 | 99.0 | 3.61 | 9.06 | 210 | 111 | 0.696 | 1.26 | 0.775 |
| 9 | 6.11 | 3.50 | 1.07 | 63.4 | 3.70 | 7.99 | 480 | 144 | 0.545 | 0.968 | 1.06 |
| 10 | 4.68 | 3.53 | 0.988 | 66.9 | 1.74 | 7.25 | 134 | 132 | 0.483 | 0.637 | 0.598 |
| 11 | 5.19 | 3.46 | 1.18 | 89.5 | 0.909 | 8.49 | 69.0 | 113 | 285* | 0.895 | 0.561 |
| Mean | 5.06 | 3.15 | 1.11 | 87.2 | 3.39 | 8.02 | 304 | 125 | 0.525 | 0.922 | 0.925 |
| SD** | 1.15 | 0.45 | 0.12 | 15.3 | 1.58 | 1.89 | 152 | 39.7 | 0.219 | 0.226 | 0.231 |
| Minimum | 2.42 | 2.27 | 0.91 | 63.4 | 0.909 | 4.09 | 69.0 | 184 | 0.082 | 0.637 | 0.561 |
| Maximum | 6.29 | 3.73 | 0.91 | 109 | 6.92 | 11.4 | 534 | 54.7 | 0.805 | 1.35 | 1.28 |
| Median | 5.30 | 3.29 | 1.10 | 89.5 | 3.60 | 8.29 | 292 | 132 | 0.527 | 0.953 | 0.970 |

* Did not included in statistical calculations
** Standard deviation

**Table 5:** Elemental content in analyzed green tea samples expressed as dry mass of the samples (mean of three measurements)

| Sample | Ca g kg$^{-1}$ | Na mg kg$^{-1}$ | Mg g kg$^{-1}$ | Zn mg kg$^{-1}$ | Cr mg kg$^{-1}$ | Cu mg kg$^{-1}$ | Fe mg kg$^{-1}$ | Mn g kg$^{-1}$ | Cd mg kg$^{-1}$ | Pb mg kg$^{-1}$ | Hg μg kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 5.06 | 566 | 2.6. | 48.8 | 3.06 | 18.0 | 513 | 1.15 | 36.3 | 1.81 | 1.65 |
| 13 | 3.68 | 983 | 2.23 | 60.3 | 1.59 | 16.2 | 271 | 1.29 | 106 | 2.58 | 1.55 |
| 14 | 6.40 | 585 | 2.05 | 98.5 | 2.58 | 20.8 | 234 | 1.73 | 202 | 4.77 | 2.80 |
| 15 | 5.30 | 437 | 2.42 | 77.6 | 3.23 | 12.6 | 234 | 1.35 | 154 | 2.51 | 1.05 |
| 16 | 2.77 | 473 | 1.41 | 30.7 | 1.52 | 9.35 | 163 | 1.64 | 84.1 | 4.33 | 1.47 |
| 17 | 4.85 | 542 | 2.02 | 50.5 | 2.70 | 22.6 | 343 | 1.36 | 104 | 2.89 | 2.33 |
| 18 | 3.63 | 664 | 1.72 | 116 | 1.76 | 13.9 | 269 | 1.76 | 86.3 | 3.85 | 2.31 |
| 19 | 5.88 | 552 | 2.04 | 88.3 | 5.28 | 17.3 | 197 | 1.16 | 60.8 | 1.50 | 1.05 |
| Mean | 4.70 | 600 | 2.06 | 71.3 | 2.71 | 16.3 | 278 | 1.43 | 104 | 3.03 | 1.77 |
| SD* | 1.24 | 169 | 0.378 | 28.7 | 1.23 | 4.33 | 109 | 0.248 | 52.5 | 1.18 | 0.641 |
| Minimum | 2.77 | 437 | 1.41 | 30.7 | 1.52 | 9.35 | 163 | 1.15 | 36.3 | 1.50 | 1.05 |
| Maximum | 6.40 | 983 | 2.62 | 116 | 5.28 | 22.6 | 513 | 1.76 | 202 | 4.77 | 2.80 |
| Median | 4.96 | 559 | 2.04 | 68.9 | 2.64 | 16.8 | 252 | 1.35 | 95.0 | 2.73 | 1.60 |

* Standard deviation

tea plants. Noteworthy findings are significantly higher content of toxic metals in green tea leaves. The next thing done for some better insight was PCA. Projections of variables in PCA were given in Figure 4.

The first and second component explained 64.84% of the total variance. A contribution of Na and Mg in first component was highly positive and a contribution of Cu, Hg, Mn, Pb and Cd was high and negative (Figure 5). The contribution of the second principal component of Fe, Cr and Ca was high and positive and contribution of other metals was low.

**Table 6:** Descriptive statistics of metal content in chamomile and green teas leaves.

| | Chamomile | | | | | Green tea | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Minimum | Maximum | *N* | Mean | SD | Minimum | Maximum | *N* |
| | mg kg$^{-1}$ | | | | | mg kg$^{-1}$ | | | | |
| Ca | 5060 A | 1147 | 2420 | 6293 | 11 | 4695 A | 1236 | 2772 | 6400 | 8 |
| Mg | 3149 B | 449 | 2273 | 3728 | 11 | 2062 A | 378 | 1414 | 2616 | 8 |
| Na | 1108 B | 123 | 915 | 1277 | 11 | 600 A | 169 | 437 | 983 | 8 |
| Zn | 87.2 A | 15.3 | 63.4 | 109 | 11 | 71.3 A | 28.7 | 30.7 | 116 | 8 |
| Cu | 8.02 A | 1.89 | 4.09 | 11.39 | 11 | 16.34 B | 4.33 | 9.35 | 22.6 | 8 |
| Cr | 3.390 A | 1.58 | 0.909 | 6.92 | 11 | 2.714 A | 1.23 | 1.52 | 5.28 | 8 |
| Fe | 304 A [a] | 152 | 69.0 | 534 | 11 | 278 A | 109 | 163 | 513 | 8 |
| Mn | 125 A | 39.7 | 54.7 | 184 | 11 | 1427 B | 248 | 1147 | 1763 | 8 |
| Cd | 26.4 a | 85.7 | 0.0824 | 285 | 11 | 104 b | 52.5 | 36.3 | 202 | 8 |
| Hg | 0.0009 A | 0.0002 | 0.0006 | 0.0013 | 11 | 0.002 B | 0.0006 | 0.001 | 0.003 | 8 |
| Pb | 0.925 A | 0.232 | 0.561 | 1.28 | 11 | 3.03 B | 1.18 | 1.50 | 4.77 | 8 |

Different letters correspond to statistically different content of metals. Uppercase and lowercase letters correspond to significance level at 0.01 and 0.05, respectively.

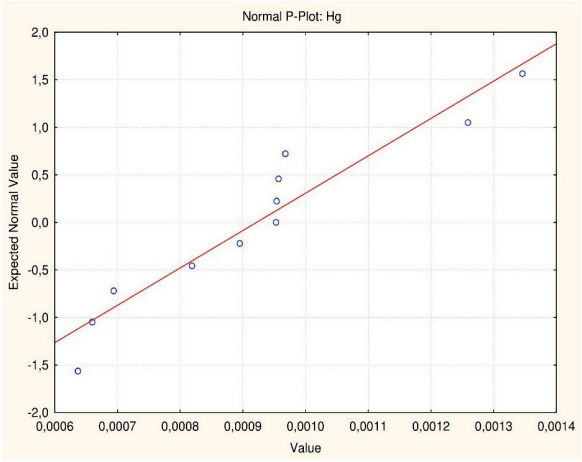


**Figure 1:** Normality data test for mercury in chamomile samples.

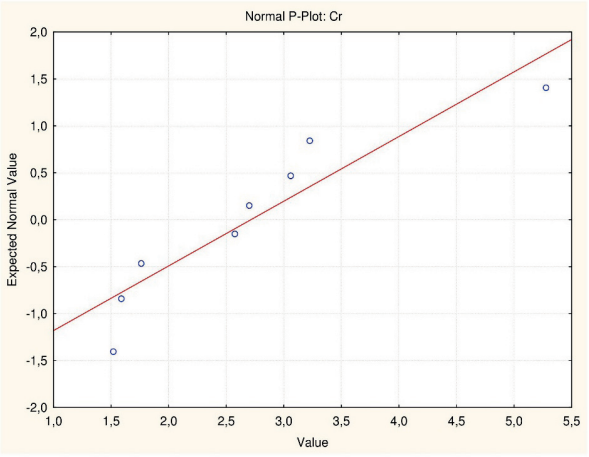


**Figure 2:** Normality data test for chromium in green tea samples.

The principal component analysis was performed in order to distinguish tea plants based on 11 metal contents, as presented in Figure 5. Samples from chamomile were grouped on the right plane of the coordinate system while samples of green tea were grouped on the left plane. This proves that the total metal content in plants used for tea making could be used for classification of tea plants. Although all analyzed tea samples, chamomile and green tea, were produced by European companies, their country of origin lies at different continents, green tea (e.g. China, Sri Lanka) and chamomile (European countries – Poland, Germany, Croatia etc.), with very distinctive soil and climate leading to observed variations..

The results reported in this manuscript were compared with similar investigations reported in Croatia [1,2] and Poland [4] as illustrated in Table 7. In addition, there were also differences in the soil composition, the Polish ones were chernozem and diluvial soils whereas the Croatian were “terra rosa” and chernozem.

In Table 7 metal contents for three plants, mint, chamomile and green tea are given. In this study, higher Ca and Mg values were recorded in the same plant, chamomile. Our both investigations were practically in the same time period, November 2016-February 2017, except the second one was cover period January-May 2017. This is very interesting because it indices different metal content

**Table 7:** Comparison of the previously published results for tea samples purchased at Croatian and Polish market expressed on dry basis (mean±SD)/mg kg$^{-1}$.

| Reference | Ca(%) | Cd | Cu | Fe | Mg(%) | Mn | Zn | Cr | Pb |
|---|---|---|---|---|---|---|---|---|---|
| [4] chamomile | 0.018±0.002 | N/A | 1.55±0.03 | 16.8±0.3 | 0.028±0.002 | 13.8±0.1 | 88.8±0.5 | N/A | N/A |
| [2] (marketplace) | N/A | 0.170±0.174 | 4.69±0.84 | 134±80 | 0.54±0.516 | 158±231 | 11.9±6.26 | 19.7±16.4 | 1.46±0.237 |
| [2] (supermarket) | N/A | 0.252±0.050 | 3.08±1.10 | 451±437 | 0.077±0.062 | 66±26 | 4.03±9.22 | 50.7±34.1 | 0.522±0.590 |
| [1] | 1.00±0.41 | 0.173±0.152 | 26.2±3.70 | 208±124 | 0.356±0.051 | 121±6.28 | N/A | N/A | 1.04±0.57 |
| This investigation (chamomile) | 5.06±1.15 | 26.4±85.7 | 8.02±1.89 | 304±152 | 3.15±0.45 | 125±39.7 | 87.2±15.3 | 3.39±1.58 | 0.925±0.232 |
| This investigation (green tea) | 4.70±1.24 | 104±52.5 | 16.3±4.33 | 278±109 | 2.06±0.38 | 1.43±0.25* | 71.3±28.7 | 2.71±1.23 | 3.03±1.18 |

N/A - not available

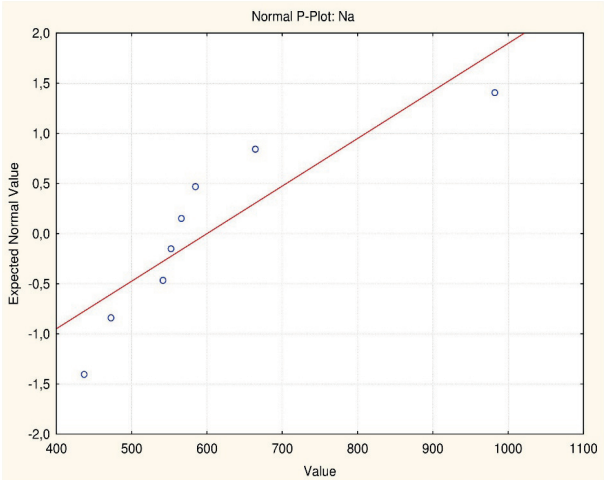


**Figure 3:** Normality data test for sodium in green tea samples.

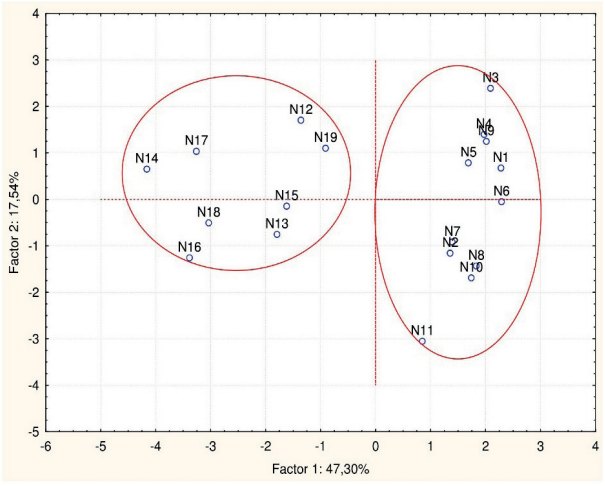


**Figure 5:** PCA diagram of interrelations of 19 teas.

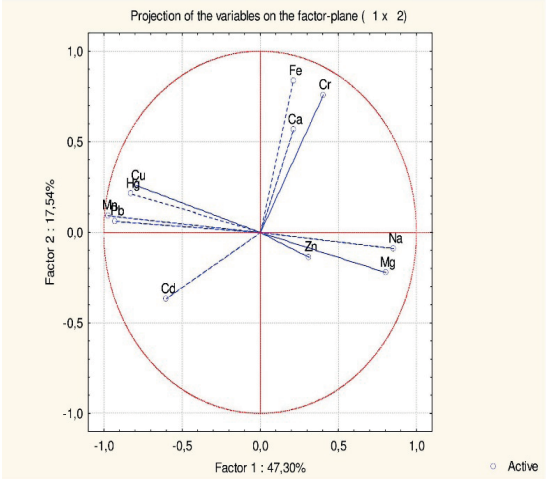


**Figure 4:** Projections of variables in PCA.

during season. It could be possible that different weather conditions can influence to metal content in plants. On the other hand, we did not found harvesting year at packages. This statement right now is hard to confirm, but in our next investigations, we shall take care about that. Since about 5% of the metal content is usually extracted by tea brewing such high Mg content found both in chamomile and green tea is excellent source for it.

During this investigation, we found a chamomile sample (sample 11) with very high Cd content (285 mg kg$^{-1}$). A small company with poor quality control produced and put on this market sample 11. Fe and Cu contents were similar to the previous investigations [1,4]. Both chamomile and green tea have lower affinity to Cr than mint [2], of a dozen times. This investigation showed the spossible beneficial of green tea consumption. The obtained Mn

content was very high, about 1.43%. Since the RDA for Mn is 2.3 mg, it could be an excellent source for Mn.

By analyzing the content of Hg and Pb, it can be seen that the content of Pb is practically the same as in the literature [1,2,4]. On the one hand, Hg content, Table 4 and 5, it is twice as high as in green tea compared to chamomile. Since the recommended lethal dose of mercury ranges from 20 to 60 mg per kg of body mass, the consumption of green and chamomile tea is (therefore) quite safe.

## 4 Conclusion

Since the same metals can be both beneficial and harmful to human organism, it is reasonable to conduct the metal survey in very popular beverages such as teas. In this work, we show the results of our analysis of 11 chamomile and eight green tea samples for 11 different metals.
Since this investigation is the extension of our previous one, we established seasonal changing of Ca and Mg content for chamomile. Chamomile beverages are very popular in Croatia. On the other hand, this fact is very interested for local population because chamomile is planted in Croatia. The next very important finding was establishing very high Mn content in green teas what supports tea drinking both as cultural phenomenon and as possible trace metal source. Levels of Hg and Pb were not dangerous to human health.

In the end, we suggest that there is the need for more rigorous and continuous controls of herbal products available at markets.

**Conflict of interest:** Authors state no conflict of interest.

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