Analysis of Tea for Metals by Flame and Graphite Furnace Atomic Absorption Spectrometry with Multivariate Analysis

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Abstract

The concentrations of iron, zinc, manganese, copper, chromium, nickel, lead, arsenic, and cadmium were determined using flame (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS) in nine herbal tea samples. Hawthorn, yarrow, elderflower, and bearberry are herbal teas for which the metal content has been rarely determined. The concentration of cadmium in St John's wort exceeded the maximum permissible limit of 0.3 mg/kg. The metals were also determined in aqueous extracts following 5, 10, and 20 min of boiling and using different preparation, including acidification with lemon juice. The influence of these factors on metal release was demonstrated using multivariate analyses by redundancy analysis (RDA) and principal component analysis (PCA). The metal release was not considerably affected by the boiling time, while the acidity of the medium was generally positively correlated. For the infusions, arsenic was detected only in acidified mint tea. The extraction efficiencies of metals were determined and classified as highly, moderately and poorly extractable, with chromium showing largest variations.

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INTRODUCTION

Herbal beverages (teas and herbal teas) are used worldwide due to their nutritional values and for health benefits, since they usually are not biologically aggressive (Pytlakowska et al. 2012). It is known that billions of cups are consumed worldwide (Polechońska et al. 2015). Herbal teas and teas globally are chemically complex: beside polyphenols, flavonoids, alkaloids, enzymes, minerals, proteins, amino acids, aroma-forming substance, vitamins, fibers, volatile oils, purines, carbohydrates and others, they contain a variety of minerals and trace elements (Szymczycha-Madeja, Welna, and Pohl 2012). While many are essential for human health and play a vital role in metabolic processes (Razic and Kuntic 2013), some may be toxic. For example, Cr, Cu, Fe, and Zn are considered essential nutrients, toxic when in high concentrations, while As, Pb, and Cd are toxic with no known beneficial properties (Korfali et al. 2013). The toxicity of trace metals and their impact on human health has drawn scientific attention for a long time. Toxic elements are prone to bioaccumulation, and thus can have a large number of negative effects on the nervous, immune, and reproductive systems (Razic and Kuntic 2013).

The World Health Organization established maximum permissible levels in raw plant materials only for As, Cd, and Pb (World Health Organization 2007). Determining the mineral level and trace elements present in herbs is a priority (Pytlakowska et al. 2012). The content of some elements in plant depends on the concentration of the element in soil, the ability of plant to accumulate the element (Lozak et al. 2002), and plant processing conditions. Additional sources include atmospheric dust, rainfall, plant protection agents, and fertilizer. However, when the tea is prepared, elements can be extracted into solution to different extents, depending on the plant

species, the solubility of the metal, medium, temperature, and pH (Gallaher et al. 2006; Street et al. 2006; Szymczycha-Madeja, Welna, and Pohl 2012).

The determination of heavy metals in food and tea has become a world-wide study, because metal monitoring provides information on the safety of food. *Camelia sinensis* is widely used in human consumption and is a frequently analyzed herb (Malik et al. 2008; Pękal, Biesaga, and Pyrzynska 2013; Song et al. 2013; Welna, Szymczycha-Madeja, and Pohl 2013; Mihaljev et al. 2014; Santos et al. 2015; Brzezicha-Cirocka, Grembecka, and Szefer 2016; Milani, Morgano, and Cadore 2016; Hocker et al. 2017). Aromatic herbal plants that have a pleasant aroma are also widely used, but the number of studies involving heavy metal determination is considerably lower. Mint, nettle, St. John's Wort, and thyme represent relatively well-studied herbs (mint: Gallaher et al. 2006; Gentscheva, Stafilov, and Ivanova 2010; Pytlakowska et al. 2012; Rubio et al. 2012; Tokalıoglu 2012; Prkić et al. 2013; Dghaim et al. 2015; Aziz et al. 2016; nettle: Łozak et al. 2002; Özcan et al. 2008; Kara 2009; Balabanova, Stafilov, and Bačeva 2015; Samolińska et al. 2017; St. John's Wort: Baranowska et al. 2002; Ražić et al. 2005; Pytlakowska et al. 2012; Muntean et al. 2013; Mihaljev 2014; thyme: Arpadjan et al. 2008; Özcan et al. 2008; Karadas and Kara 2012; Mihaljev 2014; Milani, Morgano, and Cadore 2016). For the rest of the examined tea samples, significantly lower number of studies have been performed (hawthorn: Özcan et al. 2008; Juranović Cindrić et al. 2015; Konieczynski, Arceusz, and Wesolowski 2015; yarrow: Ražić et al. 2005; Arpadjan et al. 2008; Mihaljev et al. 2014; elderflower: Gasser et al. 2009; Konieczynski, Arceusz, and Wesolowski 2015 and bearberry: Arpadjan et al. 2008; Kaličanin and Velimirović 2013). In general, the majority of studies deal with the determination of the total metals, while others also determined metals in the infusions. The determined metals differ among the studies: some authors focus on the determination of many metals, while others

focus on the determination of only toxic elements. However, several studies dealt with the effect of boiling time on metal release from herbs, and others on the influence of the acidity of the medium (i.e. Mehra and Baker 2007; Özcan et al. 2008; Altıntıg, Altundag, and Tuzen 2014; Sembratowicz and Rusinek-Prystupa 2014; Garba et al. 2015; Jeszka-Skowron, Krawczyk, and Zgoła-Grześkowiak 2015; Pantelić et al. 2015). This study is designed to update knowledge and determine the levels of Fe, Zn, Mn, Cu, Cr, Ni, Pb, As, and Cd in green and eight herbal teas that include hawthorn, St John's wort, nettle, elderflower, bearberry, thyme, yarrow, and mint. The total metal concentrations were determined, as well as the content of metals in various infusions using flame atomic absorption spectrometry (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS). The effect of boiling time, the infusion preparation, and the acidity of the medium were simultaneously examined and the results evaluated using multivariate analyses, principal component analysis (PCA), and redundancy analysis (RDA) as novelties of this work. The extraction efficiencies of the metals were also evaluated.

MATERIAL AND METHODS

Sampling and Pretreatment

Eight herbal tea samples and one green tea sample were obtained from local markets as bags for analyses: Hawtorn (*Crataegus monogyna* Jacq.), St John's wort (*Hypericum perforatum* L.), nettle (*Urtica dioica* L.), elderflower (*Sambucus nigra* L.), green tea (*Camellia sinensis* (L.) Kuntze), bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.), thyme (*Thymus serpyllum* L.), yarrow (*Achillea millefolium* L.), and mint tea (*Mentha piperita* L.). Nettle, green tea, bearberry and mint consist of leaves, while the other samples are combinations of plant parts such as flowers

and leaves. For each sample, the bags were opened separately, mixed and homogenized, and dried at 105°C to constant weight before analysis.

Reagents and Instrumentation

All chemicals and reagents were of analytical grade. Ultrapure water (pH between 6.0 and 7.0, and conductivity of 0.0 µS/cm) was used to prepare all solutions. Working standards for creating the calibration curve were made from a stock solution (AccuStandard). The filtration was conducted using Macherey-Nagel MN 615 paper. The weight of the samples was measured on a Mettler Toledo AB204-S/PH and a Elektron ELP-06 furnace was used for the heating of the crucibles. The pH was measured on a WTW inoLab pH 730 meter and conductivity using a WTW LF 191 meter. An atomic absorption spectrometer (AA Analyst PinAAcle 900T, PerkinElmer) was used for the determination of the metals by flame (FAAS) and graphite furnace with Zeeman background correction (GFAAS). The absorption wavelengths for each metal and operating conditions of the spectrometer are summarized in **Table 1**.

Analysis of Dried Tea

The total metals were determined for the dried tea and also from the residues that remained on the filters after 5, 10, and 20 min of boiling. The residues were dried at 105°C prior to the determination of total metals. One gram of the each te sample was accurately weighed and decomposed in a silica crucibles at 480°C for 4 h (Gallaher et al. 2006). The mineral content was determined based on the mass after ashing. After cooling and measuring to the constant weight, 3 mL of aqua regia were added to the ash. The samples were heated for 10 min at 100°C and diluted to 25 mL using ultrapure water (Street et al. 2006; Malik et al. 2008).

Analysis of Tea Extracts

Five sets of aqueous extracts were prepared by weighing approximately 2 g of each tea sample with treatment using 100 mL of boiling ultrapure water. One set was boiled for 5 min, the second for 10 min, and the third for 20 min. For the fourth set, boiling water was added to the tea that was covered and left at room temperature for 5 min. In the fifth set of samples, 5 mL of lemon juice was added to the tea that was covered and left for 5 min at room temperature. The liquors were passed through filter paper and diluted to 100 mL using ultrapure water. For the determination of Cr, Cd, Ni, As, Pb, Cu, Fe, Mn, and Zn, the samples were evaporated to 25 mL. In each tea infusion, the pH and conductivity were measured.

Quality Control

All polyethylene bottles and glassware were washed with detergent and tap water, left in 10% HNO_3 overnight, and thoroughly rinsed with ultrapure water. The crucibles were heated at 480°C to constant weight. Blanks were prepared by following the procedure for dry tea and tea infusions. All samples and standards were analyzed in triplicate.

The limits of detection and quantification for all metals were calculated by analyzing 10 replicates of a standard low concentration and multiplying the standard deviation by 3 and 10, respectively, as presented in **Table 2**.

The accuracy of the total element concentrations was characterized using the Apple Leaves 1515 standard reference material from the National Institute of Standards and Technology. The recoveries are shown in **Table 2**.

Statistical Analysis

Statistical analyses were done using CANOCO for Windows, Version 5.0 (Ter Braak and Šmilauer 2012). The first principal component analysis (PCA) was used to evaluate the relationship between the total metal content determined from the residues in the filters after boiling at 5, 10, and 20 min. Supplementary variables included the tea samples, time of boiling, and portion of the plant included in the making of the tea. The second PCA was used to demonstrate the relationship between the same supplementary variables and metals released in water after boiling for 5, 10, and 20 min. The goal of PCA was to determine if the presence of significant differences in metal concentrations after boiling for 5, 10, and 20 min.

For a better understanding of metal extraction, the relationship of pH to the extraction of metals from the samples was examined using redundancy analysis (RDA). The metal concentrations were compared for samples boiled for 5 min, samples covered and left at room temperature for 5 min, and sample treated with lemon juice covered and left at room temperature expressed as µg/g. The pH was used as an explanatory variable, while conductivity, the type of sample, and the sample preparation were supplementary. RDA was employed using the center and standardize option because the gradient was 0.7 SD units long. The relationship between pH and metal release was characterized.

RESULTS AND DISCUSSION

Many methods of digestion have been applied (Karak and Bhagat 2010; Pohl et al. 2016). For our study, a dry digestion method was employed. The total concentrations of Cr, Cd, Ni, As, Pb, Cu, Fe, Mn, and Zn from all tea samples are shown in **Table 3**.

Bearberry accounted for relatively high concentrations of Cr, Ni, As, Fe, and Zn. The content of Cu and Mn was highest in green tea, and Pb in hawthorn and mint tea. The highest concentrations of Cd were in St John's wort and yarrow.

Fe and Mn are essential nutrients, and Zn and Cu essential micronutrients. Cr is an essential microelement which at higher concentrations may be toxic (Korfali et al. 2013). The higher levels of Fe in bearberry were probably due to the soil particles adhering to the samples. High levels of Cr are due to accumulation by adsorption of Cr to iron oxides and hydroxides in soil, as the correlation between these metals is usually high (Korfali et al. 2013). The most abundant trace elements were Cu, Fe, and Zn (Szymczycha-Madeja, Welna, and Pohl 2012). These elements are commonly determined in tea, although rarely in herbal tea and medicinal plants. Ražić et al. (2005), for example, examined mint tea, yarrow, nettle and St John's worth and found similar values for Cu and Zn, while Fe and Mn were higher than in our samples, except for Fe in St John worts. The same authors also examined bearberry and their results showed lower values of these metals compared to this study.

As, Cd, and Pb are toxic and the maximum permissible levels in raw plant materials are established to be 1.0, 0.3, and 10 mg kg^{-1}, respectively (World Health Organization 2007) The concentrations in plant are usually low, but can vary among species (Szymczycha-Madeja, Welna, and Pohl 2012). Sometimes those elements are not detected or are below the detection limit. The contents of As and Pb were below the maximum permissible levels in all samples. As was not detected at all in St John's wort, nettle, hawthorn, and elderflower. Juranović Cindrić et al. (2015) also reported that in hawthorn, As was not detected. However, higher As levels were found in bearberry, while the concentration of Pb was highest in hawthorn and mint teas. Similar

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values of Pb in mint tea were found by Maghrabi (2013), while Lozak et al. (2002) and Kalny et al. (2007) recorded higher concentrations.

On the other hand, Cd showed variations among the samples with elevated values for some. Nevertheless, some teas exceeded the maximum permissible limit of 0.3 mg/kg for Cd. Thyme, bearberry, and green tea showed slightly higher Cd values, while yarrow and St John's wort had significantly higher concentrations than established as maximum permissible levels. Dong et al. (2016) reported that the cadmium content in most samples was above the international safety limit. Elevated Cd in St John's wort may indicate that the plant was grown in a polluted environment (Razic and Kuntic 2013). The presence of phosphate fertilizer in agriculture land may be a source of Cd in soil (Karak and Bhagat 2010). In general many factors, including the soil pH and presence of other metals influence Cd absorption by plants. Sembratowicz and Rusinek-Prystupa (2014) reported that the presence of Zn, Cu, and Pb may influence the absorption of Cd by plants. For example, increased lead in soil stimulates the cadmium absorption. However, plants may accumulate metals from the environment (Gasser et al. 2009; Sembratowicz and Rusinek-Prystupa 2014) and *Hypericum perforatum* it is known to have high affinity for Cd (Đurović et al. 2013). The higher Cd in St John's wort was also found by Đukić-Ćosić et al. (2011) and these authors listed many studies where higher levels of Cd were documented. Baranowska et al. (2002) reported elevated levels of Cd in St John's wort similar with our values. However, plants with high Cd may not be health hazards; Cd in the infusion depends on the efficiency of extraction, which may be low (Korfali et al. 2013).

The differences in the total metals among the plants firstly depends on the species but also on other conditions (Kalny et al. 2007). The geological conditions, climate factors, air and soil pollution, altitude, rainfall, and age of the plant are also important (Street et al. 2006;

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Szymczycha-Madeja, Welna, and Pohl 2012; Maghrabi 2014). Pb and Cd are naturally found in air, water, and soil but they also represent contaminants released during industrial processes. Commercial teas may have elevated metals due to the production process and storage. Polechońska et al. (2015) reported that bagged teas contained significantly higher concentrations of trace metals than leaves of the same brand. This was due to processing methodology, as well as with tea quality, since tea bags often contain older plants and low-cost tea. Elevated concentrations of Mn and Pb are, for example, found in older plants. The elevated concentrations of Cr may be connected with the crush, tear, and curl process during tea manufacturing (Polechońska et al. 2015).

Medicinal plants are widely used for tea preparations. Therefore it is beneficial to determine total metals in these plants, as well as the extractable metals available to humans (Pytlakowska et al. 2012). The release of metals from the plant to water in infusions is influenced by the tea-water ratio, quality of water (hardness) (Gallaher et al. 2006), temperature, and pH (Street et al. 2006; Szymczycha-Madeja, Welna, and Pohl 2012). In this study, the focus was on the influence of the time for releasing metals and the pH.

The PCA of metal concentrations obtained from residues of tea samples after boiling for 5, 10 and 20 min are shown in **Figure 1**. High overlap of supplementary variables for boiling at 5, 10, and 20 min is observed. They are at the center of the ordination diagram, indicating that the total metals from residues after boiling were similar. Also, they were all similar to the total metals determined from tea samples as shown in **Table 3**. The arrangement of metal vectors and tea supplementary variables show that the highest content of metals is in the same samples as shown in **Table 3**. The variables for flower and leaf indicate that flower teas are on the left of the ordination diagram separate from the leaf teas on the right side. Most metals are oriented toward

the right and correlate with the leaf nominal variable. This result occurs because leaf teas, including as green and bearberry tea, had highest total content of the metals. Aziz et al. (2016) examined metals in leaves and flowers of *Catharanthus roseus* where higher concentrations of most metals were in the leaves.

The relationship between extracted metals, tea samples, and time of boiling is shown in **Figure 2**. Metal vectors are arranged so that sample of tea from which they were best extracted. For example, Mn, As, and Ni were more extracted from green tea, Cu from St John's wort, Fe from bearberry and elderflower. Since Pb was not present in any samples, the Pb vector is not shown on the ordination diagram. Most metals were best extracted from one sample, while the total metal content was highest in other samples (**Table 3**). Only Mn and Cd had both the highest totals and were extracted most efficiently from the same samples. For supplementary variables that refer to 5, 10, and 20 min of boiling, they are placed near the center of the ordination diagram. Their position shows that the significant differences were not observed in the concentrations of metals from the tea samples with changes in boiling time. Sembratowicz and Rusinek-Prystupa (2014) determined metals content after brewing for 5 and 10 min and reported slightly increases and decrease in some metal concentrations. However, significant variations were not found so the authors concluded that brewing time did not significantly affect elemental extraction. Although some elements increase with extraction time, others are efficiently extracted in the first 5 min (Szymczycha-Madeja, Welna, and Pohl 2012; Memić et al. 2014). Juranović Cindrić et al. (2015) reported that the concentrations of metals in the infusion after boiling for 5 min did not substantially increase. In first 5 min of extraction, 90% of the maximum concentrations were obtained.

Since the levels of extracted metals in water medium after changes in boiling time were similar, further measurements used a 5 minute infusion. The infusions included boiling for 5 min, samples covered and left at room temperature for 5 min, and samples covered and left at room temperature for 5 min with added lemon juice. The goal was to evaluate how the tea preparation and pH affect the release of metals into water. The pH values for the infusions were 4.71 to 7.74, 4.79 to 7.09, and 2.96 to 3.75, respectively.

The relationship between extracted metals after these three infusions were examined using RDA (**Figure 3**). The results showed statistical significance, with $F = 5.1$ and $P = 0.0004$. The pH showed a positive correlation with the first RDA axis ($r = 0.6961$), so the right part of the ordination diagram corresponds to higher pH, while the left region refers to lower pH values. Supplementary variables that indicate the tea preparation also show differences: supplementary variables that indicate 5 min of boiling and 5 min of cooling at room temperature were placed on the right side of the ordination diagram, while the supplementary variable that represents samples covered and left at room temperature for 5 min with added lemon juice was placed on the left showing a negative correlation with pH. All vectors of the extracted metals were on the left of the ordination diagram, showing negative correlation with the pH vector, meaning that their extraction was more efficient in acidic conditions.

Pb was not detected in these three infusions, while As was detected only in mint tea in the infusion with added lemon juice. The metal extraction after boiling for 5 min and from the infusion left at room temperature for 5 min was similar, although slight differencies were observed. The extractions for most metals were lower in the infusion that was left at room temperature for 5 min for most samples. The exceptions were Fe in thyme and green tea; Zn in

hawthorn, mint and bearberry; Cu for bearberry and St John's worth; Mn for yarrow, elder and nettle; and Ni for yarrow, nettle and thyme.

In general, the metal levels were higher when lemon was added to the tea. Acidification of the tea positively influenced the metal extraction. Fe, Zn, Mn, and Ni had higher levels in acidified tea in all samples. **Figure 3** shows the highest negative correlation with the pH vector, meaning that their extraction increased as the pH decreased. Cu, Cr, and Cd showed lower negative correlations with pH. Cu showed slightly higher values in acidified samples except for nettle, mint, and green tea where values were lower than or equal to values from the other infusions. The highest variations were observed for Cr. In many samples that were acidified, lower Cr values were measured, including hawthorn, yarrow, elder, thyme, and green tea. These results explain why the Cr vector does not show large negative correlation swith pH, indicating that pH has little influence on the extraction of this metal. Cd was not detected in hawthorn, yarrow, and elderflower. In St John's worth and thyme, cadmium was measured only in infusions without the lemon juice. In nettle, mint, and green tea, cadmium was only detected after acidification . In bearberry, cadmium was detected in all infusions but the highest value was measured with acidification.

In general, lower pH positively influences the extraction of elements from tea (Szymczycha-Madeja, Welna, and Pohl 2012). Acidification with lemon juice or citric acid positively influenced lead and cadmium extraction in black tea (Sembratowicz and Rusinek-Prystupa 2014). Jeszka-Skowron, Krawczyk, and Zgoła-Grześkowiak (2015) reported that Mn, Cd, and Pb concentrations in most tea infusions with citric acid were significantly higher than in pure tea infusions ($P = 0.05$). These authors reported that cadmium was positively influenced with acidification, while in this study, Cd showed significant variations. This confirms that the

metal extraction from acidified samples may depend on the tea type, the fragmentation of sample, and the place of origin (Jeszka-Skowron, Krawczyk, and Zgoła-Grześkowiak 2015).

According to Szymczycha-Madeja, Welna, and Pohl (2012), extraction efficiencies of elements in tea infusions are classified as highly extractable (>55%) (e.g., Ni); moderately extractable (20 to 55%) (e.g., Cu, Mn, Zn); and poorly extractable (<20%) (e.g., Fe). As, Cd and Pb are considered poorly extractable and their concentrations are usually low or undetectable. According to the same authors, Cr is considerd to be either moderately poorly extractable. Extraction efficiencies of metals from this work are shown in **Table 4**. Ni was highly extractable only in St John's wort in two non-acidified infusions and in mint tea in the acidified infusion. Cu, Cr, Mn, and Zn showed variations. In the majority of samples, the extraction efficiencies for these metals were between 20 and 55%. Cr and Mn exceeded 55% in acidified samples: Cr in St John's wort and Mn in all samples except hawthorn, yarrow, and bearberry. However, in many samples, values of 20% were also observed, especially for Cr, even in acidified samples. Fe was poorly extractable, with values below 7% in unacidified samples and below 12% in acidified samples except for mint tea where 39.58% was recorded. Pb and As are poorly soluble and Pb has not been detected, while As was measured only in acidified mint.

Cd showed variations in efficiency. In most samples, this element was not detected or was poorly extracted except in acidified samples of mint and bearberry and after 5 min of boiling from St John's wort. Since many people consume tea several times a day, the determination of extraction efficiencies of metals from tea is of importance. Tea infusions may be a good source of essential metals for humans (Street et al. 2006), but also a source of toxic elements (Jeszka-Skowron, Krawczyk, and Zgoła-Grześkowiak 2015). The extraction of elements from tea depends on the total concentration of the element, as well as on the type of tea. The availability

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also depends on whether the metals are more soluble in solution or bound to the organic matrix through complexation with catechols, flavonols, tannins, and polyphenols (Szymczycha-Madeja, Welna, and Pohl 2012).

CONCLUSIONS

Fe, Zn, Mn, Cu, Cr, Ni, Pb, As, and Cd were determined in hawtorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea. The total content of Cu and Mn was highest in green tea, while Cr, Ni, As, Fe, and Zn were highest in bearberry, Pb in hawthorn and mint tea, and Cd in St John's wort and yarrow. As and Pb were below the maximum permissible levels in all samples, while Cd in St John's wort exceeded the maximum permissible limit of 0.3 mg/kg. PCA showed the concentrations of metals in infusions after 5, 10, and 20 min of boiling were similar. The total content of metals obtained from the ash after 5, 10 and 20 min of boiling indicated no significant differences in element extraction.

Metal concentrations determined in tea based on boiling for 5 min, sample left at room temperature for 5 min, sample left at room temperature for 5 min with lemon juice added were compared by RDA to characterize the influence of pH on metal release. Similar results were obtained for the first preparation methods. The addition of lemon juice enhanced the extraction of most metals, indicating more efficient dissolution in acidic conditions. Cu, Cr, and Cd varied in the their efficiency among the samples. Since tea is a popular beverage consumed by many individuals several times a day, the extraction efficiencies of metals were determined and the elements were classified as highly, moderately and poorly extractable with Cr showing highest variations.

Acknowledgment

References

Altıntıg, E., H. Altundag, and M. Tuzen. 2014. Determination of multi element levels in leaves and herbal teas from Turkey by ICP-OES. Bull. *Chem. Soc. Ethiop.* 28 (1):9–16. doi:10.4314/bcse.v28i1.2

Arpadjan, S., G. Çelik, S. Taskesen, and S. Güçer. 2008. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food Chem. Toxicol.* 46:2871–75. doi:10.1016/j.fct.2008.05.027

Aziz, S., K. Saha, N. Sultana, H. Parvin Nur, A. Ahsan, S. Ahmed, and K. Hossain. 2016. Comparative studies of elemental composition in leaves and flowersof *Catharanthus roseus* growing in Bangladesh. *Asian Pac. J. Trop. Biomed.* 6 (1):50–54. doi:10.1016/j.apjtb.2015.10.003

Balabanova, B., T. Stafilov, and K. Bačeva. 2015. Bioavailability and bioaccumulation characterization of essential and heavy metals contents in *R. acetosa*, *S. oleracea* and *U. dioica* from copper polluted and referent areas. *J. Environ. Health. Sci. Eng.* 13:2. doi:10.1186/s40201-015-0159-1

Baranowska, I., K. Srogi, A. Włochowicz, and K. Szczepanik. 2002. Determination of heavy metal contents in samples of medicinal herbs. *Pol. J. Environ. Stud.* 11 (5):467–71.

Brzezicha-Cirocka, J., M. Grembecka, and P. Szefer. 2016. Monitoring of essential and heavy metals in green tea from different geographical origins. *Environ. Monit. Assess.* 188:183. doi:10.1007/s10661-016-5157-y

Dghaim, R., S. Al Khatib, H. Rasool, and M. Ali Khan. 2015. Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. *J Environ Public Health* 2015:1–6. doi:10.1155/2015/973878

Dong, X-L., J. Zhang, Y-L. Zhao, Z-T. Zuo, Y-Z. Wang, and J-Y. Zhang. 2016. Multivariate analyses of major and trace elements in 19 species of herbs consumed in Yunnan, China. *Int. J. Food Prop.* 20:1666–76. doi:10.1080/10942912.2016.1217009

Đukić-Ćosić, D., A. Stanojević, M. Djekić-Ivanković, M. Ćurčić, Z. Plamenac-Bulat, B. Antonijević, and V. Matović. 2011. Sadržaj kadmijuma u *Hypericum perforatum* L. i *Thymus serpyllum* L.sa lokaliteta planina Rtnja i Ozrena (Cadmium content in *Hypericum perforatum* L. and *Thymus serpyllum* L. from localities of the mountains Rtanj and Ozren). *Vojnosanit. Pregl.* 68 (11):930–34. doi:10.2298/vsp1111930d

Đurović, D., Z. Bulat, A. Buha, and V. Matovic. 2013. Cadmium, Mercury and Lead in *Hypericum perforatum* L. collected in Western Serbia. E3S Web of Conferences 1:15009.

Gallaher, R. N., K. Gallaher, A. J. Marshall, and A. C. Marshall. 2006. Mineral analysis of ten types of commercially available tea. *J. Food Compost. Anal.* 19:53–57. doi:10.1016/j.jfca.2006.02.006

Garba, Z. N., S. Ubam, A. A. Babando, and A. Galadima. 2015. Quantitative assessment of heavy metals from selected tea brands marketed in Zaria, Nigeria. *J. Phys. Sci.* 26 (1):43–51.

Gasser, U., B. Klier, A. V. Kühn, and B. Steinhoff. 2009. Current findings on the heavy metal content in herbal drugs. *Pharmeur. Sci. Notes* 1:37–50.

Gentscheva, G. D., T. Stafilov, and E. H. Ivanova. 2010. Determination of some essential and toxic elements in herbs from bulgaria and macedonia using atomic spectrometry. *Eurasian J. Anal. Chem.* 5 (2):104–11.

Hocker, N., C. Wang, J. Prochotsky, A. Eppurath, L. Rudd, and M. Perera. 2017. Quantification of Antioxidant Properties in Popular Leaf and Bottled Tea by High-Performance Liquid Chromatography (HPLC), Spectrophotometry, and Voltammetry. *Anal. Lett.* doi:10.1080/00032719.2016.1242008

Jeszka-Skowron, M., M. Krawczyk, and A. Zgoła-Grześkowiak. 2015. Determination of antioxidant activity, rutin, quercetin, phenolic acids and trace elements in tea infusions: Influence of citric acid addition on extraction of metals. *J. Food Comp. Anal.* 40:70–77. doi:10.1016/j.jfca.2014.12.015

Juranović Cindrić, I., M. Zeiner, D. Mihajlov Konanov, and G. Stingeder. 2015. Metal Characterization of White Hawthorn Organs and Infusions. *J. Agric. Food Chem.* 63:1798−1802. doi:10.1021/jf504474t

Kaličanin, B., and D. Velimirović. 2013. The content of lead in herbal drugs and tea samples Cent. *Eur. J. Biol.* 8 (2):178–85. doi:10.2478/s11535-013-0117-1

Kalny, P., Z. Fijałek, A. Daszczuk, and P. Ostapczuk. 2007. Determination of selected microelements in polish herbs and their infusions. *Sci. Total Environ.* 381:99–104. doi:10.1016/j.scitotenv.2007.03.026

Kara, G. 2009. Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. *Food Chem.* 114:347–54. doi:10.1016/j.foodchem.2008.09.054

Karadas, C., and D. Kara. 2012. Chemometric approach to evaluate trace metal concentrations in some spices and herbs. *Food Chem.* 130:196–202. doi:10.1016/j.foodchem.2011.07.006

Karak, T., and R. M. Bhagat. 2010. Trace elements in tea leaves, made tea and tea infusion: A review. *Food Res. Int.* 43 (9):2234–52. doi:10.1016/j.foodres.2010.08.010

Konieczynski, P., A. Arceusz, and M. Wesolowski. 2015. Relationships between flavonoids and selected elements in infusions of medicinal herbs. *Open Chem.* 13:68–74. doi:10.1515/chem-2015-0003

Korfali, S., M. Mroueh, M. Al-Zein, and R. Salem. 2013. Metal Concentration in Commonly Used Medicinal Herbs and Infusion by Lebanese Population: Health Impact. *J. Food Res.* 2 (2):70. doi:10.5539/jfr.v2n2p70

Łozak, A., K. Sołtyk, P. Ostapczuk, and Z. Fijałek. 2002. Determination of selected trace elements in herbs and their infusions. *Sci. Total Enviro.* 289:33–40. doi:10.1016/s0048-9697(01)01015-4

Maghrabi, I. A. 2013. Determination of some mineral and heavy metals in Saudi Arabia popular herbal drugs using modern techniques. *Afr. J Pharm. Pharmacol.* 8 (39):1000–05.

Malik, J., J. Szakova, O. Drabek, J. Balik, and L. Kokoska. 2008. Determination of certain micro and macro elements in plant stimulants and their infusions. *Food Chem.* 111 (2):520–25. doi:10.1016/j.foodchem.2008.04.009

Mehra, A., and C. L. Baker. 2007. Leaching and bioavailability of aluminium, copper and manganese from tea (Camellia sinensis). *Food Chem.* 100:1456–63. doi:10.1016/j.foodchem.2005.11.038

Memić, M., D. Mahić, S. Žero, and T. Muhić-Šarac. 2014. Comparison of different digestion methods of green and black tea at the Sarajevo market for the determination of the heavy metal content. *Food Measure.* 8:149–54. doi:10.1007/s11694-014-9175-6

Mihaljev, Ž., Ž.-B. Milica, Ć. Željko, and S. Jakšić. 2014. Levels of some microelements and essential heavy metals in herbal teas in Serbia. *Acta Pol. Pharm. – Drug. Res.* 71 (3):385–91.

Milani, R. F., M. A. Morgano, and S. Cadore. 2016. Trace elements in *Camellia sinensis* marketed in southeastern Brazil: Extraction from tea leaves to beverages and dietary exposure. *LWT - Food Sci. Technol.* 68:491–98. doi:10.1016/j.lwt.2015.12.041

Muntean, N, E. Muntean, C. Creta, and M. Duda. 2013. Heavy metals in some commercial herbal teas. *ProEnvironment* 6:591–94.

Özcan, M. M., A. Unver, T. Ucar, and D. Arslan. 2008. Mineral content of some herbs and herbal teas by infusion and decoction. *Food Chem.* 106:1120–27. doi:10.1016/j.foodchem.2007.07.042

Pantelić, A., S. Popović, B. Gavrilović, M. Ćirić, M. Vidović, M. Ivezić, and Ž. Gotovina. 2015. Evaluation of the heavy metals content in selected teas using FAAS. *Ecologica* 23 (81):95–99. doi:10.1515/ohs-2015-0041

Pękal, A., M. Biesaga, and K. Pyrzynska. 2013. Trace Metals and Flavonoids in Different Types of Tea. *Food Sci. Biotechnol.* 22 (4):925–30. doi:10.1007/s10068-013-0165-y

Pohl, P., A. Dzimitrowicz, D. Jedryczko, A. Szymczycha-Madeja, M. Welna, and P. Jamroz. 2016. The determination of elements in herbal teas and medicinal plant formulations and their artisans. *J Pharm. Biomed. Anal.* 130:326–35. doi:10.1016/j.jpba.2016.01.042

Polechońska, L., M. Dambiec, A. Klink, and A. Rudecki. 2015. Concentrations and solubility of selected trace metals in leaf and bagged black teas commercialized in Poland. *J. Food. Drug. Anal.* 23:486–92. doi:10.1016/j.jfda.2014.08.003

Prkić, A., J. Giljanović, S. Petričević, M. Brkljača, and M. Bralić. 2013. Determination of Cadmium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Potassium, and Zinc in Mint Tea Leaves by Electrothermal Atomizer Atomic Absorption Spectrometry in Samples Purchased at Local Supermarkets and Marketplaces. *Anal. Lett.* 46 (2):367–78. doi:10.1080/00032719.2012.718825

Pytlakowska, K., A. Kita, P. Janoska, M. Połowniak, and A. Kozik. 2012. Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions. *Food. Chem.* 135:494–501. doi:10.1016/j.foodchem.2012.05.002

Razic, S., and V. Kuntic. 2013. Diverse elements in herbal tea products consumed in Serbia using inductively coupled plasma mass spectrometry. *Int. J. Food Prop.* 16:1–8. doi:10.1080/10942912.2010.526273

Ražić, S., A. Onjia, S. Đogoa, L. Slavković, and A. Popović. 2005. Determination of metal content in some herbal drugs—Empirical and chemometric approach. *Talanta* 67:233–39. doi:10.1016/j.talanta.2005.03.023

Rubio, C., J. R. D. Lucas, A. J. Gutiérrez, D. Glez-Weller, B. Pérez Marrero, J. M. Caballero, C. Revert, and A. Hardisson. 2012. Evaluation of metal concentrations in mentha herbal teas (Mentha piperita, Mentha pulegium and Mentha species) by inductively coupled plasma spectrometry. *J. Pharm. Biomed. Anal.* 71:11–17. doi:10.1016/j.jpba.2012.07.015

Samolińska, W., B. Kiczorowska, M. Kwiecień, and E. Rusinek-Prystupa. 2017. Determination of minerals in herbal infusions promoting weight loss. *Biol. Trace Elem. Res.* 175:495–502. doi:10.1007/s12011-016-0790-4

Santos, L. M. G., P. Duboc, J. M. Goncalves, and S. C. Jacob. 2015. Determination of arsenic, cadmium and lead concentration in teas, commercialized in Rio De Janeiro, Brazil, and their transfer to tea infusion. *J. Biol. Chem. Res.* 2015:179–86.

Sembratowicz, I., and E. Rusinek-Prystupa. 2014. Effects of brewing time on the content of minerals in infusions of medicinal herbs. *Pol. J. Environ. Stud.* 23 (1):177–86.

Song, M., Q. Li, X. Guan, T. Wang, and K. Bi. 2013. Novel HPLC method to evaluate the quality and identify the origins of longjing green tea. *Anal. Lett.* 46 (1):60–73. doi:10.1080/00032719.2012.704532

Street, R., J. Száková, O. Drábek, and L. Mládková. 2006. The status of micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *Czech J. Food Sci.* 24 (2):62–71.

Szymczycha-Madeja, A., M. Welna, and P. Pohl. 2012. Elemental analysis of teas and their infusions by spectrometric methods. *Trends Analyt. Chem.* 35:165–81. doi:10.1016/j.trac.2011.12.005

Ter Braak, C. J. F., and P. Šmilauer. 2012. *Canoco reference manual and user's guide: Software for ordination, version 5.0*. Ithaca, USA: Microcomputer Power.

Tokalıoglu, S. 2012. Determination of trace elements in commonly consumed medicinal herbs by ICP-MS and multivariate analysis. *Food Chem.* 134:2504–08. doi:10.1016/j.foodchem.2012.04.093

Welna, M., A. Szymczycha-Madeja, and P. Pohl. 2013. A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. *Food Res. Int.* 53:922–30. doi:10.1016/j.foodres.2013.03.030

World Health Organization. 2007. World Health Organization guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva, 118 p.

Table 1. Atomic absorption conditions.

		Cr	Cd	Ni	As	Pb	Cu	Fe	Mn	Zn
GFAAS	Wavelength (nm)	357.87	228.80	232.00	193.70	283.31	324.75	248.33	279.48	/
	Step 1 (°C)	110	110	110	110	110	110	110	110	/
	Step 2 (°C)	130	130	130	130	130	130	140	130	/
	Step 3 (°C)	1500	500	1100	1200	850	1200	1200	1300	/
	Step 4 * (°C)	2300	1500	2300	2000	1600	2000	2100	1900	/
	Step 5 (°C)	2450	2450	2450	2450	2450	2450	2450	2450	/
FAAS	Wavelength (nm)	/	/	/	/	/	324.75	302.06	279.83	213.86
	Flame type	/	/	/	/	/	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2

*Atomization step.

Table 2. Limits of detection and quantification and analysis of certified reference material as mean ± standard deviation.

	Limit of detection		Limit of quantification		SRM Apple Leaves 1515		
	GFAAS (μg/L)	FAAS (mg/L)	GFAAS (μg/L)	FAAS (mg/L)	Certified value (μg/g)	Measured value (μg/g)	Recovery (%)
Cr	0.188	/	0.628	/	0.300	0.286 ± 0.020	95.3
Cd	0.110	/	0.367	/	0.013 ± 0.002	0.015 ± 0.009	115.4
Ni	0.387	/	1.290	/	0.910 ± 0.120	0.953 ± 0.154	104.7
As	0.452	/	1.507	/	0.038 ± 0.007	0.044 ± 0.021	115.9
Pb	0.484	/	1.614	/	0.470 ± 0.024	0.424 ± 0.038	90.2
Cu	0.422	0.012	1.406	0.039	5.640 ± 0.240	5.375 ± 0.153	95.3
Fe	0.173	0.057	0.575	0.189	83.00 ± 5.00	67.69 ± 1.90	81.6
Mn	0.092	0.012	0.306	0.041	54.00 ± 3.00	56.46 ± 3.45	104.5
Zn	/	0.005	/	0.015	12.50 ± 0.30	12.64 ± 0.25	101.1

Table 3. Total metals in dry tea (mean ± standard deviation).

	Cr (μg/g)	Cd (μg/g)	Ni (μg/g)	As (μg/g)	Pb (μg/g)	Cu (μg/g)	Fe (μg/g)	Mn (μg/g)	Zn (μg/g)
Hawtorn	0.680 ± 0.163	0.181 ± 0.047	1.04 ± 0.17	ND	0.804 ± 0.210	10.90 ± 0.48	192.0 ± 2.4	40.76 ± 1.27	30.58 ± 0.51
Yarrow	0.394 ± 0.089	0.702 ± 0.055	0.522 ± 0.103	0.041 ± 0.025	0.352 ± 0.111	6.77 ± 0.39	84.14 ± 2.90	35.99 ± 1.13	19.06 ± 0.33
St John's wort	0.219 ± 0.087	0.927 ± 0.074	0.518 ± 0.112	ND	0.330 ± 0.087	9.80 ± 0.41	66.07 ± 2.30	61.04 ± 2.52	27.75 ± 0.82
Elderflower	0.508 ± 0.094	0.254 ± 0.044	0.871 ± 0.230	ND	0.172 ± 0.094	9.73 ± 0.19	176.9 ± 4.1	65.60 ± 1.94	38.07 ± 1.15
Nettle	0.902 ± 0.205	0.104 ± 0.039	0.960 ± 0.171	ND	0.258 ± 0.088	9.22 ± 0.22	179.7 ± 3.4	41.62 ± 2.19	34.12 ± 1.07
Thyme	1.12 ± 0.199	0.379 ± 0.071	1.98 ± 0.36	0.059 ± 0.016	0.543 ± 0.108	9.90 ± 0.27	551.1 ± 8.7	106.0 ± 2.3	31.45 ± 0.88
Mint	2.36 ± 0.185	0.077 ± 0.025	2.42 ± 0.19	0.108 ± 0.061	0.748 ± 0.115	11.07 ± 0.57	133.3 ± 3.5	92.7 ± 1.7	26.70 ± 2.01
Bearberry	12.17 ± 1.08	0.389 ± 0.075	10.43 ± 0.71	0.555 ± 0.123	0.512 ± 0.174	7.45 ± 0.42	926.4 ± 9.9	230.1 ± 1.9	43.97 ± 1.81
Green tea	0.699 ± 0.097	0.344 ± 0.015	2.19 ± 0.19	0.049 ± 0.010	0.217 ± 0.084	15.83 ± 0.14	178.8 ± 4.3	845.4 ± 1.8	35.81 ± 0.79

*ND – Not detected

Table 4. Extraction efficiencies of metals from examined tea samples (mean ± standard deviation)

	Boiling for 5 min	Room temperature 5 min	Room temperature 5 min with lemon	Boiling for 5 min	Room temperature 5 min	Room temperature 5 min with lemon	Boiling for 5 min	Room temperature 5 min	Room temperature 5 min with lemon
	Cr%			Cd%			Ni%		
Hawtorn	30.62 ± 4.10	6.37 ± 0.87	10.34 ± 1.02	ND	2.52 ± 0.50	ND	22.73 ± 2.04	14.49 ± 1.01	54.35 ± 1.51
Yarrow	45.33 ± 1.45	4.88 ± 0.52	11.25 ± 2.01	ND	0.60 ± 0.50	ND	29.03 ± 0.30	32.18 ± 0.20	55.55 ± 2.03
St John's Wort	70.14 ± 4.39	ND	83.25 ± 3.00	12.38 ± 1.03	6.54 ± 0.51	ND	91.14 ± 2.00	21.97 ± 0.92	98.5 ± 2.75
Elderflower	32.47 ± 2.02	16.58 ± 1.10	13.04 ± 1.00	ND	1.75 ± 0.70	ND	41.58 ± 1.50	26.14 ± 0.15	59.58 ± 1.13

Nettle	12.88 ± 0.68	2.28 ± 0.80	15.78 ± 1.37	ND	4.39 ± 0.51	11.38 ± 1.02	9.38 ± 0.40	10.15 ± 0.31	21.54 ± 0.53
Thyme	57.56 ± 1.05	5.5 ± 2.03	10.66 ± 0.70	4.96 ± 0.90	ND	ND	20.74 ± 1.70	22.97 ± 2.03	50.4 ± 2.01
Mint	5.97 ± 0.41	2.61 ± 0.72	6.37 ± 0.65	ND	ND	44.68 ± 1.07	35.64 ± 2.00	26.79 ± 0.20	74.74 ± 0.34
Bearberry	1.87 ± 0.54	0.27 ± 0.15	11.24 ± 2.00	12.55 ± 3.50	5.88 ± 1.00	71.97 ± 3.05	3.72 ± 0.85	2.33 ± 0.35	8.61 ± 0.61
Green tea	41.33 ± 1.02	8.07 ± 0.55	25.66 ± 0.73	ND	1.17 ± 0.15	11.02 ± 0.25	33.06 ± 0.11	31.58 ± 0.70	76.63 ± 0.42
	As%			Pb%			Cu%		
Hawtorn	ND	ND	ND	ND	ND	ND	16.09 ± 2.20	27.17 ± 1.85	27.24 ± 1.65
Yarrow	ND	ND	ND	ND	ND	ND	24.15 ± 1.70	25.55 ± 1.65	34.43 ± 2.56
St	ND	ND	ND	ND	ND	ND	29.4	28.55	39.68 ±

	Fe%			Mn%			Zn%		
John's Wort							7 ± 2.51	± 1.97	0.97
Eld erflower	ND	ND	ND	ND	ND	ND	25.41 ± 2.07	32.82 ± 1.15	40.06 ± 2.64
Nettle	ND	ND	ND	ND	ND	ND	22.65 ± 1.06	33.04 ± 0.87	31.43 ± 1.87
Thyme	ND	ND	ND	ND	ND	ND	12.04 ± 1.26	22.77 ± 1.54	25.83 ± 2.55
Mint	ND	ND	44.28 ± 6.32	ND	ND	ND	15 ± 0.97	35 ± 0.88	24.84 ± 0.89
Bea rberry	ND	ND	ND	ND	ND	ND	14.88 ± 2.16	ND	17.44 ± 1.24
Green tea	ND	ND	ND	ND	ND	ND	13.22 ± 0.67	32.93 ± 0.54	32.64 ± 0.67
	Fe%			Mn%			Zn%		

Hawtorn	1.88 ± 0.8	1.34 ± 0.69	5.89 ± 0.90	26.06 ± 1.10	25.78 ± 1.28	52.51 ± 2.13	18.62 ± 1.60	19.12 ± 2.30	46.42 ± 2.69
Yarrow	3.51 ± 0.5	3.21 ± 0.72	7.97 ± 1.34	16.9 ± 0.91	18.67 ± 1.09	54.19 ± 1.22	16.24 ± 1.99	13.5 ± 0.93	34.19 ± 2.13
St John's Wort	6.83 ± 1.11	4.74 ± 0.85	11.58 ± 1.00	40.41 ± 1.00	22.65 ± 0.87	73.12 ± 1.64	24.7 ± 2.20	22.1 ± 2.08	53.24 ± 2.97
Elderflower	5.2 ± 0.81	2.88 ± 0.50	11.74 ± 1.23	27.63 ± 0.89	32.33 ± 1.01	62.87 ± 1.11	22.71 ± 1.90	16.4 ± 0.87	32.56 ± 1.60
Nettle	1.81 ± 0.60	1.82 ± 0.62	10.9 ± 1.15	6.04 ± 0.25	6.70 ± 0.39	59.99 ± 0.77	45.1 ± 1.50	29.34 ± 1.51	53.99 ± 1.49
Thyme	0.72 ± 0.30	0.94 ± 0.51	5.9 ± 0.98	32.93 ± 0.96	24.74 ± 0.96	76.87 ± 1.20	26.2 ± 0.91	15.06 ± 1.75	43.94 ± 1.60
Mint	2.99 ±	2.57 ± 0.70	39.58 ± 1.14	35.95 ± 0.60	30.13 ± 0.62	75.36 ± 0.69	14.88 ±	18.32 ± 0.89	53.64 ± 1.10

	0.71						1.30		
Bearberry	0.87 ± 0.25	0.31 ± 0.20	2.99 ± 0.50	29.81 ± 1.97	26.74 ± 0.89	48.37 ± 1.13	12.73 ± 2.64	16.57 ± 2.16	37.65 ± 1.97
Green tea	1.91 ± 0.51	2.06 ± 0.41	11.56 ± 0.49	37.08 ± 0.66	20.45 ± 0.54	64.83 ± 1.75	30.25 ± 1.00	21.99 ± 0.94	44.33 ± 1.11

*ND – Not detected

Figure 1. PCA of metals from tea residue following boiling for 5, 10, and 20 min. The supplementary variables include tea as green diamonds (hawtorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea); red squares refer to boiling time (5 min of boiling, 10 min of boiling, and 20 min of boiling); and yellow triangles represent the part of the plant from which the tea is primarily produced (leaves, flowers).
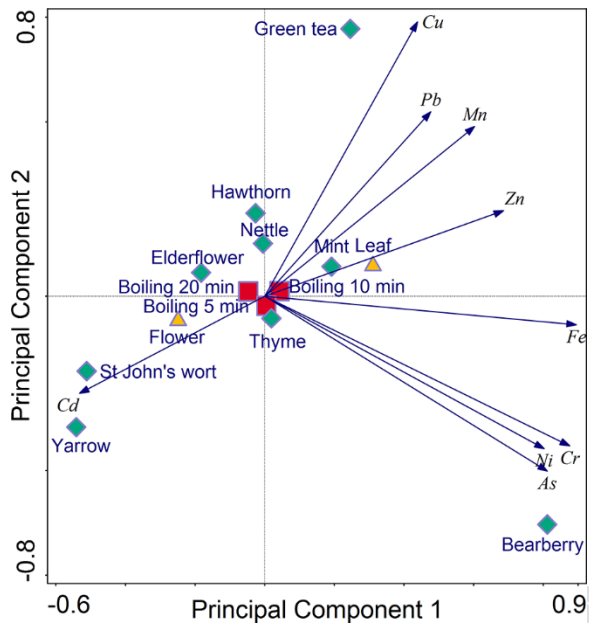
Figure 2. PCA of metals from water extract after boiling for 5, 10, and 20 min. The supplementary variables include tea as green diamonds (hawtorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea); red squares refers to boiling time (5 min of boiling, 10 min of boiling, and 20 min of boiling); and yellow triangles represent the part of the plant from which the tea is primarily produced (leaves, flowers).
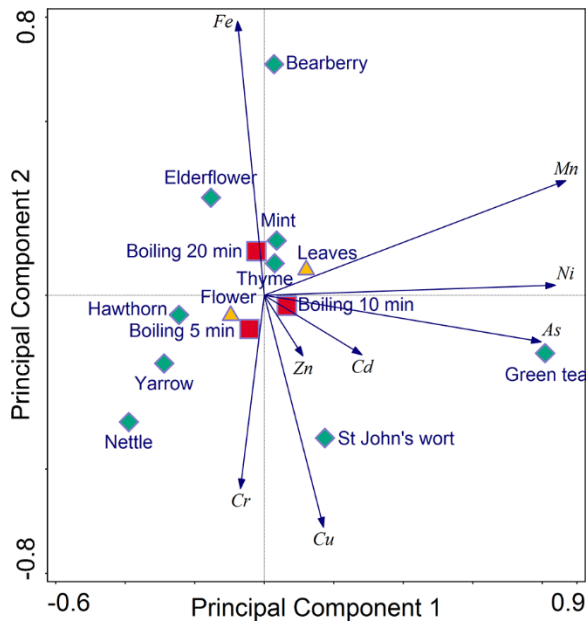
Figure 3. RDA of metals extracted from tea with pH as the explanatory variable. The conductivity, type of tea in green (hawtorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea), and preparation method in red (5 min of boiling, 5 min at room temperature, and 5 min at room temperature with addition of lemon juice) are included as supplementary variables.