

ANALYSIS OF RISK ELEMENTS IN HERBAL TEA SAMPLES

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ABSTRACT

Tea is one of the most popular drinks in the world. Other infusions and decoctions of various plant herbs are also known under this name. We refer to these infusions as herbal teas. Herbal teas are prepared mainly for the active substances contained in the given herb. They have curative or preventive effects. It is often a mixture of various meadow and forest herbs (flowers, leaves), but there are also herbal teas using the effects of only one plant. Many sources point to the possible presence of risk elements in herbal teas. These are risk elements that, at certain concentrations, have a harmful effect on human health and other biotic components of the ecosystem. Due to the development of industry, large amounts of these risk elements entered the biosphere. However, the problem is that they mainly get into the soil and water. The work describes the content of risk elements (copper, lead, nickel, arsenic) in herbal materials - herbal teas - alchemilla vulgaris (*Alchemilla vulgaris*), turnip (*Agrimonia eupatoria*), horsetail (*Equisetum arvense*), stinging nettle (*Urtica dioica* L.), chamomile (*Matricaria chamomilla*), peppermint (*Mentha piperita*). Three methods were used for the analysis of herbal materials: method I (based on the action of acid for 24 hours), method II (acid treatment for 30 minutes at 90 °C) and method III (acid treatment for 30 minutes at room temperature). Risk elements in individual herbal tea samples were measured using an atomic absorption spectrometer. The impact and importance on human health was also evaluated. The mean of risk elements concentration, using methods, data distribution, and samples, were applied to generate data from the articles. The results indicated that all commonly consumed herbal teas included risk elements.

Keywords: Herbal tea, Risk elements, Human health, Analysis

INTRODUCTION

By extracting the leaves of the tea plant in hot water, one of the most widespread drinks in the world is obtained - tea. Tea leaves are obtained primarily from the Chinese tea tree (*Camellia sinensis*), the Indian tea tree (*Camellia assamica*), the Irrawaddy tea tree (*Camellia irrawadiensis*) and the Tali tea tree (*Camellia taliensis*). Today, however, none of the mentioned types of tea trees are cultivated as a pure species. They are mostly multiple interspecies hybrids, bred mainly for the quality and yield of young shoots from which tea is made (Rop *et al.*, 2009). Medicinal plants are used in traditional and modern medicine for consumption to stabilise the state of health and treat and prevent diseases (Salmani *et al.*, 2024). Infusions and decoctions of various plants are called herbal teas. These are mainly prepared for the active substances contained in the given herb, which have curative or preventive effects (Deetae *et al.*, 2012). Herbal teas are mostly a mixture of various meadow and forest herbs and flowers. Of course, there are also herbal teas that are made from only one plant. Therefore, we divide these teas into single-type herbal teas and multi-type herbal teas. Only dried herbs are used for their production, without the addition of preservatives, dyes and flavours. Active substances are distributed either in the whole plant or only in some of its parts. Usually, the part that contains the most of them is collected. From above-ground parts of the plants, the clove, young top, herb, bud, leaf, wood, bark, fruit, stem, seed, glands and spores are collected. The underground plant parts include the root, rhizome, tuber, and bulb (Oh *et al.*, 2013). It is possible to use dried or fresh herbs to prepare tea. The temperature at which the tea is prepared is especially important because some volatile substances evaporate at a higher temperature, and the medicinal tea becomes ineffective. Thus, we distinguish three ways in which we can prepare tea. They are macerate, infusion and decoction (Pirbalouti *et al.*, 2014). The preparation of teas in the form of macerate, infusion, and decoction differs in the length of the infusion and the temperature of the water, as well as the parts of the plants used in the preparation. Preparing tea by maceration involves steeping the herbs or tea leaves in cold or room-temperature water for an extended period of time (usually several hours or overnight). When preparing tea by infusion, the tea leaves, flowers, or herbs are covered with hot water and left to infuse for a short time (usually 3 to 10 minutes, depending on the type of tea). Preparing tea as a decoction involves boiling the harder parts of the plant, such as the roots, bark, or seeds, in water for a longer time, usually 15 to 30 minutes. In Slovakia, the preparation of herbal infusions has a long history. As reported by

Ernst *et al.* (2004) most plants are sensitive to risk elements. If the plants also appear on the site with the presence of risk elements, they grow with minor or major damage. When harvesting herbs, there is a risk that they grow in areas that are polluted by fertilisers, insecticides or exhaust gases, especially if they grow near agricultural areas or roads. Various models of the transfer of harmful substances into the plant organism are known. Risk elements accumulated in plants intended for medicinal purposes can have a harmful effect on human health during long-term exposure. There are also known studies in the world where the results showed that all commonly consumed herbal teas contained risk elements. Based on the published results, we can conclude that it is important to prevent the contamination of herbal teas with risk elements by adjusting cultivation practices and also to prevent the consumption of low-quality herbal teas (Salmani *et al.*, 2024). Tea can be a valuable source of various essential elements for human health, including iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca), potassium (K), sodium (Na), and selenium (Se) (Gu *et al.*, 2018). At the same time, it can be concluded that it is safer to drink herbal tea with fewer ingredients in terms of the presence of risk elements, and the possibility of contamination increases in herbal teas. Medicinal plant extract plays a vital role in promoting the health of human society (Salmani *et al.*, 2024). Therefore, the use of herbal teas compared to chemical drugs has been increasing dramatically in recent years (Boltman-Binkowski, 2016). Medicinal plant extract plays an essential role in promoting the health of human society (Salmani *et al.*, 2024). The aim of the presented work was to determine the content of risk elements in selected plant samples of herbal teas.

MATERIAL AND METHODS

Used plant samples and chemicals

The analysed herbal teas (A-F) were sourced from Nitra region (Slovakia). Samples of commercially available herbal teas A- F were chosen for the analytical determination:

Sample A: common alchemilla (*Alchemilla vulgaris*),

Sample B: turnip (*Agrimonia eupatoria*),

Sample C: horsetail (*Equisetum arvense*),

Sample D: stinging nettle (*Urtica dioica*),

Sample E: chamomile (*Matricaria chamomilla*),

Sample F: peppermint (*Mentha piperita*).

Herbal teas (weight 30 g) were stored before and after handling in a dark, dry place at room temperature ($t = 20\text{--}22\text{ }^{\circ}\text{C}$). Before the analyses, the samples were dried again at room temperature ($t = 21.5\text{ }^{\circ}\text{C}$).

The chemicals used in the analyses were:

- nitric acid p.a. 67%, Analpure SD, Analytika, spol. s.r.o., Czech Republic;
- deionised water; in all experiments, the chemical reagents (ACS grade) were dissolved using distilled and deionised (DDI) water produced using a MilliporeSigma™ Synergy™ Ultrapure Water Purification System (Meck Millipore, Bedford, MA, USA).
- copper – standard, nickel – standard, arsenic – standard, lead - standard (all 1000 mg/L, 3 % HNO₃, USA).

Methods for determination of risk elements in plant samples

Three different methods (*I - III*) were used to prepare plant samples for the determination of risk elements:

Method I (Du Laing et al., 2003):

Add 1 cm³ of HNO₃ p.a. to 1 g of plant material, cover with glass and leave at room temperature for 24 hours. Then, put the solution in the dryer for 30 min. Let it react at a temperature of 90 °C. After the end of the reaction, filter and make up to a volume of V=100 cm³ distilled water. Store in a plastic container in the fridge (Exp. period: 1 month).

Method II (Huang et al., 2004), (Hseu, 2004):

Add 1 cm³ of HNO₃ p.a. to 1 g of plant material. Cover it with an hourglass and let it react at a temperature of 90 °C for 30 min. After the end of the reaction, filter the solution and add to the volume V=100 cm³ with distilled water. Store in a plastic container in the fridge (Exp. period: 1 month).

Method III (Narin et al., 2004):

Add 10 cm³ of HNO₃ to 1g of plant material. Cover with an hourglass and leave at room temperature for 30 minutes. After the end of the reaction, filter the solution and add to the volume V=100 cm³ with distilled water. Store in a plastic container in the fridge (Exp. period: 1 month).

The selection of As, Cu, Ni, and Pb for analysis was based on their known toxicity, prevalence in environmental contamination, and potential health risks associated with their accumulation in food and beverages. For the determination of the content of trace elements (As, Cu, Ni, Pb), we used atomic absorption spectroscopy on the equipment GT AAS; Agilent Technologies, GTA 120 Graphite Tube Atomizer (Hermes LabSystems, Ltd.; Bratislava, Slovakia). The instrument provides excellent performance with a graphite cuvette, no matter how small the sample. It has extremely fast atomisation and low operating costs. Its advantages include, for example, extremely fast atomisation even with difficult sample matrices. High sensitivity, low detection limits, and reduced gas operating costs.

RESULTS AND DISCUSSION

Determination of the concentration of selected risk elements (copper, lead, nickel, arsenic) in plant samples A-F

Methods *I - III* were chosen for the preparation of plant samples to determine the content of risk elements in herbal teas. Several acids (HNO₃, H₂SO₄, HCl) are used to decompose organic samples, which also include herbal parts of plants. However, the literature does not specify which acid is the most advantageous or most suitable to use (Rashid et al., 2016). An AAS Model GTA 120 atomic absorption spectrometer was used to analyse trace amounts of elements. Experimentally measured values of the concentration of risk elements by methods I-III are listed in Tab. 1.

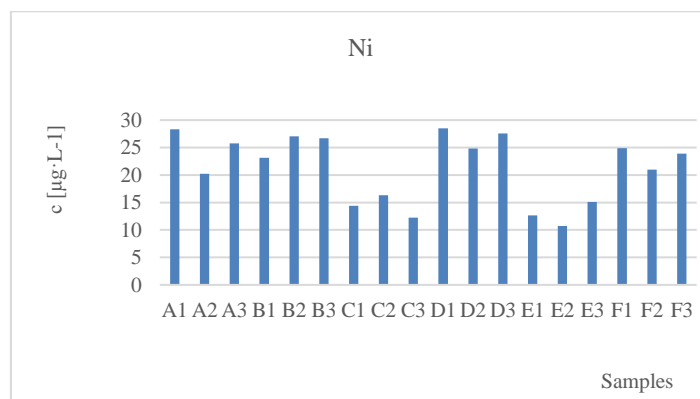
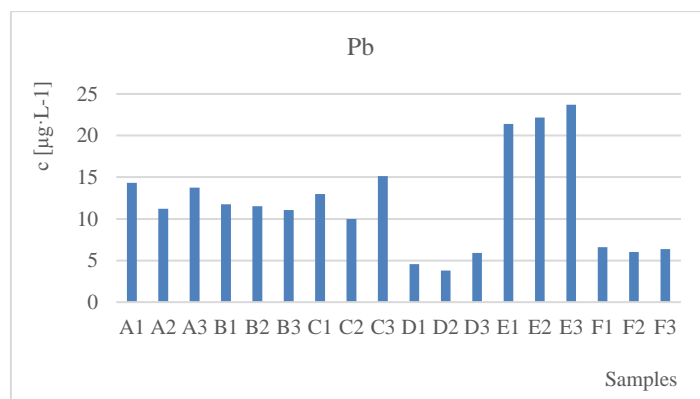
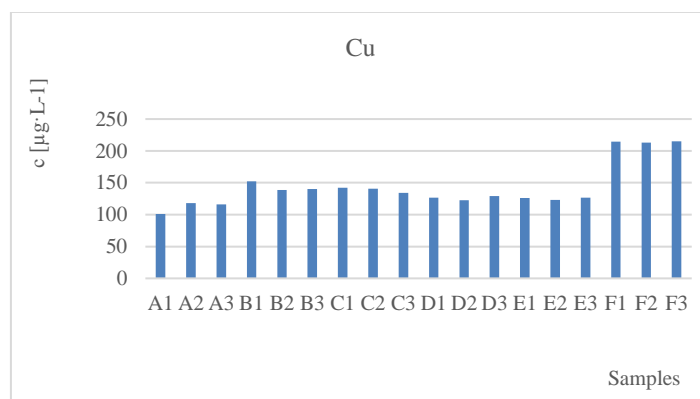
The measured values of risk element concentrations by individual methods in herbal samples determined on a GT AAS instrument (Agilent Technologies GTA 120) ranged in the intervals that we present for individual methods:

- Method 1: for copper 101.06 – 214.44 μg·L⁻¹; for plumb 4.58 – 21.38 μg·L⁻¹; for nickel 12.65 –29.51 μg·L⁻¹; for arsenic 0.51 – 6.68 μg·L⁻¹.
 - Method 2: for copper 118.22 – 213.18 μg·L⁻¹; for plumb 3.80- 22.15 μg·L⁻¹; for nickel 10.72 –27.05 μg·L⁻¹; for arsenic 0.17 – 4.90 μg·L⁻¹.
 - Method 3: for copper 115.86 - 214.83 μg·L⁻¹; for plumb 5.91 – 23.71 μg·L⁻¹; for nickel 12.23 –27.59 μg·L⁻¹; for arsenic 0.34 – 6.78 μg·L⁻¹.
- Methods *I-III* gave similar results; that is, the experimentally obtained concentration values are comparable.

The highest permitted amount of risk element concentrations stated in the Decree of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic No. 414/2003 – 100 and No. 18558/2006-100 are given in mg·kg⁻¹ (Cu: 150 mg·kg⁻¹; Pb: 5 mg·kg⁻¹; Ni: 2 mg·kg⁻¹; As: 1 mg·kg⁻¹). We experimentally determined the concentrations of risk elements in μg·L⁻¹. Figure 1 shows a comparison of the values of the risk elements detected by methods *I – III*.

Table 1 Method I-III. The concentration of selected risk elements (copper, lead, nickel, arsenic) in herbal tea samples A-F. Method *I* corresponds to samples ending in "1," method *II* to "2," and method *III* to "3."

Risk element Sample	Cu [μg·L ⁻¹]	Pb [μg·L ⁻¹]	Ni [μg·L ⁻¹]	As [μg·L ⁻¹]
A1	101.06	14.31	28.35	0.72
A2	118.22	11.22	20.23	0.17
A3	115.86	13.74	25.76	0.34
B1	152.38	11.76	23.12	5.81
B2	138.66	11.52	27.05	4.30
B3	140.34	11.05	26.69	6.78
C1	142.14	12.99	14.40	0.91
C2	140.90	10.00	16.32	0.57
C3	134.00	15.13	12.23	0.60
D1	126.59	4.58	29.51	6.68
D2	122.73	3.80	24.85	4.90
D3	128.99	5.91	27.59	5.28
E1	126.04	21.38	12.65	0.51
E2	123.19	22.15	10.72	0.48
E3	126.75	23.71	15.12	0.68
F1	214.44	6.58	24.88	0.89
F2	213.18	6.00	20.96	0.61
F3	214.83	6.35	23.92	0.86



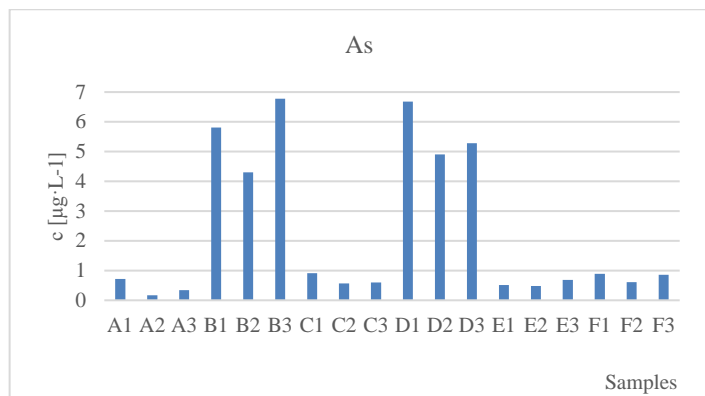


Figure 1 Measured values of risk elements Cu, Pb, Ni, As in samples A-F

As we can see in the graphs shown in figure 1, the highest measured values of the concentration of risk elements are among the samples:

Cu: sample F3; tea from stinging nettle (*Urtica dioica*), value 214.83 µg·L⁻¹.

Pb: sample E3; chamomile tea (*Matricaria chamomilla*), value 23.71 µg·L⁻¹.

Ni: sample D1; tea from stinging nettle (*Urtica dioica*), value 29.51 µg·L⁻¹.

As: sample B3; turnip (*Agrimonia eupatoria*) tea, value 6.78 µg·L⁻¹.

Characterisation of risk elements analysis

Copper has a high affinity for the formation of complex compounds. It is among the essential elements for plants and animals. Copper accumulates more significantly on the surface horizon of soils due to its high affinity to organic matter and bioaccumulation. The stability of organic copper complexes increases with increasing pH value, which is associated with conformational changes in the humic acid molecule. Copper contamination can be caused by fungicides, pesticides, and chemical fertilisers. Copper in the soil is uptake by plant roots and stored in its living organs (Karimi et al., 2008). Many copper compounds are potentially toxic, especially soluble copper salts – e.g., copper sulphate pentahydrate and copper chloride- part of plant treatment preparations (Samešová et al., 2012). Although copper is essential for normal plant growth and development, increased concentration in the soil can lead to symptoms such as stunted growth (Lin et al., 2013). Copper is one of the important biogenic elements, and its daily intake should be in the range of 0.5 mg to 2.5 mg, which is significantly higher than that of the other determined elements. In the case of copper, we recorded the highest concentrations, which, however, are within the norm if we take into account the highest permissible amount in tea. The highest concentration was measured in sample F3 the lowest in sample A1.

Lead is known to cause a range of toxic effects on living organisms, which are of morphological, physiological, and biochemical origin. Among other things, it also disrupts, for example, plant growth, root elongation, seed germination, seedling development, transpiration, chlorophyll production or cell division (Pourrut et al., 2011). Lead compounds affect vegetation mainly in the form of aerosols. They are

caught on the surface of plants. 60-70% of lead in plants comes from a polluted environment and is captured on their surface by atmospheric deposition. It is least abundant in fruits and seeds. The most lead is found on the leaves, less in the stems and the least inside the plant tissues. Suppose uptake by roots predominates for lead. For example, in greenhouses, 90% of the lead is concentrated in the roots. The high content of lead was described in grain, tea, and mushrooms (Dadová et al., 2015). We did not notice an increased lead content in our herbal samples, which is very positive for Slovak consumers. The highest concentration was measured in sample E3 the lowest in sample D2.

Compared to other risk elements, nickel is less sorbed in the soil. Adsorption of nickel to the soil decreases with complexation and an increase in the organic content in the soil. The mobility of nickel increases with a decrease in pH. Plants receive nickel from the soil mainly through the roots and have the ability to accumulate it. Nickel has a positive effect on plant growth, but its high content limits growth and suppresses photosynthesis and transpiration (Samešová et al., 2012). Nickel is necessary to produce red blood cells, but in concentrations above the permissible level, it is slightly toxic, so it causes damage to the heart and liver (Randelović et al., 2013). In terms of the content of risk elements, high demands are placed especially on the productive parts of plants that are used in human nutrition as plant products. According to Tóth, Dombos and Hornung (2023) who determined that nickel content in crops is very low, not even reaching 10% of the limit value. From his comparison, it follows that the nickel content is a predetermined value in all variants, just like in our case. The highest concentration was measured in sample D1 the lowest in sample E2.

Arsenic is a toxic element that is present in air, water and soil. Inorganic arsenic tends to be more toxic than organic arsenic (O'Day, 2006). Due to its toxic properties, arsenic is one of the intensively monitored elements in biological material as well as in various components of the environment. According to Malik et al. (2012), arsenic exposure to plants is toxic, causing growth inhibition, physiological disturbances, and eventually death. Various analytical methods can be used for its determination, and atomic absorption spectrometry (AAS) techniques are among the most used. Many different decomposition procedures have been proposed for the determination of arsenic in environmental samples. Many of them use dangerous combinations of acids, such as H₂SO₄ and HClO₄, in conjunction with sample heating, which can lead to losses of volatile analyte compounds (Krachler et al., 2001). The content of arsenic in plant material was also investigated by Hagárová et al. (2006), who also used nitric acid. The total arsenic contents determined in its plant samples ranged between 0.20-0.90 µg·g⁻¹. Vithanage et al. (2012) measured arsenic values in plants occurring in non-contaminated places after dumping contaminated sludge in the range from 0.1 mg·kg⁻¹ to 0.6 mg·kg⁻¹. In our study, the highest concentration was measured in sample B3, while the lowest was observed in sample A2.

Medicinal plants have played an important role in human healthcare through their diverse use as food, spices, and traditional medicines, as well as an important source for new drug discovery (Atanasov et al., 2015). The properties, use of the studied herbal teas and their impact on human health are mentioned in Tab. 2.

Table 2 The properties and use of the studied herbal teas in the present work

Name of herbal tea	Properties and use	References
Alchemilla vulgaris (<i>Alchemilla vulgaris</i>)	Alchemilla has been commonly used in folk medicine to heal inflammations in the mouth, bleeding of the nose, furuncles, and gynaecological (menorrhagia and dysmenorrhoea). It is known for its astringent and anti-inflammatory properties; it is traditionally used to heal gastrointestinal diseases. Despite its folkloric use in wound healing, there is a lack of scientific data to support this therapeutic application.	(Tadić et al., 2020; Tasić-Kostov et al., 2019)
Turnip (<i>Agrimonia eupatoria</i>)	Turnip is a medicinal plant which possess cardiogenic, cardioprotective and antioxidant or positive effects on the heart. It also has he antioxidant properties	(Mirzaie et al., 2012; Alinia-Ahandani et al., 2023)
Horsetail (<i>Equisetum arvense</i>)	Horsetail has been used for centuries as a traditional, folklore medicinal plant for various types of aches and pains. Current research has confirmed that Horsetail exhibits a lot of pharmacological applications as a pain-relieving agent, hepatoprotective activity, treatment of anaemia, antidiuretic activity, antimicrobial activity, herbal treatment for nail disorders, skin and hair remedy, relieving rheumatism pain, beneficial for cardiovascular problems, as well as its usefulness to promote the growth and stability of the skeletal structure.	(Oh et al., 2004; Radulović, Stojanović, & Palić, 2006; Soleiman et al., 2007; Sandhu et al., 2010; Asgarpanah and Roohi, 2012)
Stinging nettle (<i>Urtica dioica L.</i>)	Stinging nettle is used as a diuretic to treat cough, cold, cuts, and wounds. It has biological and pharmacological potential against cancer, tumours, bacterial, viral or fungal infections significantly. This plant is gaining attention as a highly nutritious food, where fresh leaves are dried and used as powder or in other forms. The leaves are rich in many bioactive compounds, such as flavonoids, phenolic acids, and amino acids.	(Grauso et al., 2020; Sushma and Sharma, 2021; Bhusal et al., 2022; Devkota et al., 2022)
Chamomile (<i>Matricaria chamomilla</i>)	Chamomile's efficacy is due to its antioxidant, anti-inflammatory, antimicrobial, antinociceptive, analgesic, anxiolytic, sedative, and antispasmodic properties. Also, it treats gastrointestinal complaints such as cold symptoms, minor ulcers, superficial wounds, small boils, inflammation of the mouth, throat, and skin, anxiety, and insomnia, along with other complaints and illnesses.	(Gupta Lall and Srivastava, 2021)
Peppermint (<i>Mentha piperita</i>)	Peppermint may likewise go about as a carminative, disinfectant, and pectolytic, having soothing activity. M. piperita has been employed for myalgia and neuralgia. In addition to its medicinal and flavouring properties, peppermint is widely used in cosmetics, pharmaceuticals, and perfumery. Peppermint oil is greatly employed as an antispasmodic in the stomach and intestine in bile channel and for the treatment of peevish entrails disorder, inflammation of the respiratory tract, and aggravation of the oral mucosa.	(Mughal, 2022; Sepehri et al., 2023)

Order of trace elements in the herbal substances for all samples:

(1) For *Alchemilla vulgaris* (*Alchemilla vulgaris*), Turnip (*Agrimonia eupatoria*), Horsetail (*Equisetum arvense*), Stinging nettle (*Urtica dioica* L.), and Peppermint (*Mentha piperita*) the order of trace elements was for all herbal teas:

Cu > Ni > Pb > As

(2) For Chamomile (*Matricaria chamomilla*), the order of trace elements was for all herbal teas:

Cu > Pb > Ni > As

The presence of risk elements was detected in all samples A-F; however, after studying the literature and regulations, we can evaluate that the measured data are within the norm.

According to the guidelines of the World Health Organisation (WHO) and the European Food Safety Authority (EFSA), the maximum permissible concentration of copper in drinking water is 2000 µg·L⁻¹, while the limit for lead is 10 µg·L⁻¹ for nickel 70 µg·L⁻¹ and for arsenic 10 µg·L⁻¹. In the context of these values, some of the measured concentrations, in particular for lead in *Matricaria chamomilla* tea and nickel in *Alchemilla vulgaris* tea, exceed the recommended limits for drinking water. These values point to potential health risks, especially with long-term consumption of these products.

CONCLUSION

The work deals with the determination of risk elements (copper, lead, nickel, arsenic) in samples of commercially available herbal teas *Alchemilla vulgaris* (*Alchemilla vulgaris*), turnip (*Agrimonia eupatoria*), horsetail (*Equisetum arvense*), stinging nettle (*Urtica dioica*), chamomile (*Matricaria chamomilla*), peppermint (*Mentha piperita*). Three methods using reaction with nitric acid were used for the analysis of herbal materials. Risk elements in individual herbal tea samples were analysed using GT AAS. In the case of method I, the concentration range for copper was determined to be 101.06 – 214.44 µg·L⁻¹; lead 4.58 – 21.38 µg·L⁻¹; nickel 12.65 – 29.51 µg·L⁻¹; arsenic 0.51 – 6.68 µg·L⁻¹. In method II for copper 118.22 – 213.18 µg·L⁻¹; lead 3.80– 22.15 µg·L⁻¹; nickel 10.72 – 27.05 µg·L⁻¹; arsenic 0.17 – 4.90 µg·L⁻¹ and in method III for copper 115.86 – 214.83 µg·L⁻¹; lead 5.91 – 23.71 µg·L⁻¹; nickel 12.23 – 27.59 µg·L⁻¹; arsenic 0.34 – 6.78 µg·L⁻¹. Thus, the experimental work confirmed the presence of risk elements such as copper, lead, nickel and arsenic in commercially available herbal teas. The determined concentrations of the analysed risk elements may have a potential impact on human health if they exceed the limit values set by the relevant health authorities. It is, therefore, important that consumers pay attention to the possible risk element content of herbal teas, especially if they consume them regularly and in large quantities. It may be concluded that it is safer to drink herbal tea with less number of ingredients as far as the presence of risk elements is concerned. According to the review results, it is important to prevent risk element contamination of herbal teas by modifying cultivation patterns and also to prevent the consumption of low-quality herbal teas. Health authorities and manufacturers should ensure regular testing and monitoring of these products in order to minimise potential risks to consumer health.

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