DOI 10.1007/s11094-022-02716-w
Pharmaceutical Chemistry Journal, Vol. 56, No. 6, September, 2022 (Russian Original Vol. 56, No. 6, June, 2022)

ASSESSMENT OF THE QUALITY OF HERBAL TEAS FROM ŠABAC, SERBIA IN TERMS OF THE CONTENT OF HEAVY METALS

Kosana Popović,[1] Mirjana Antonijević Nikolić,[1,*] Branka Dražić,[2] Dragoljub Jovanović,[3] and Slađana Tanasković[2]

Original article submitted August 25, 2021.

Chemical components of teas have received great interest because they are related to health. In this work, data on the determination of foreign matter, loss on drying/water content, total ash and ash insoluble in hydrochloric acid are presented. The content of seven heavy metals including Cu, Fe, Mn, Zn, Ni, Cd and Pb were determined by atomic absorption spectrometry in samples of several herbal teas: *Matricariae flos, Thymi herba, Menthae piperitae folium, Betulae folium, Quercus cortex, Gentianae radix, Frangulae cortex, Althaeae radix, Uvae ursi folium* and *Glycyrrhizae radix* collected from Šabac's market, Serbia. The sample preparation procedure involved dry digestion and dissolution of the ash in 6M HCl and then in 0.1 M HNO_3. Herbal teas showed the concentration of heavy metals Cu, Fe, Mn, Zn and Ni in the range: 2.9 ± 0.1 – 22.2 ± 0.9 mg/kg, 118.5 ± 1.1 – 755.5 ± 2.5 mg/kg, 19.0 ± 5.8 – 561.0 ± 1.9 mg/kg, 6.5 ± 0.4 – 242.5 ± 1.4 mg/kg and 2.5 ± 0.1 – 10 ± 1.1 mg/kg, respectively. The level of copper in all samples was uniform. The highest content of Fe was in *Thymi herba*, while Mn and Zn were at maximum in *Betulae folium*. The levels of toxic heavy metals Cd and Pb were below the detection limit. The obtained values were compared with data available from literature. The herbal tea samples analyzed contained essential heavy metals (Cu, Fe, Mn, Zn) and probably essential in trace (Ni), and could obey the daily dietary requirements. Noncancer health risk assessment detected that the herbal teas of *Betulae folium* and *Frangulae cortex* can manifest some health risk to consumers.

Keywords: heavy metals; herbal teas; atomic absorption spectrometry; health risk assessment.

1. INTRODUCTION

Medicinal plants have a long history of therapeutic use throughout the world and still represent an important part of traditional medicine [1]. A report of the World Health Organization (WHO) displayed that about 70 – 80% of the world population applies non-conventional medicine, mainly of herbal origin in their primary healthcare [2]. In recent times, medicinal herbs have obtained important attention from the pharmaceutical, health food and natural cosmetic industries, especially natural and/or organic grown medicinal herbs [3]. Also, the use of medicinal herbs proved to be significant especially now during the COVID-19 pandemic time, which was reported by China's Ministry of Science and Technology, according to which 87.5% of the total cases in China were treated by Chinese herbal medicines and had positive effects at all stages of the disease [4, 5].

The use of herbal teas have spread in Serbia as a complementary way to treat and prevent illnesses. According to the Rulebook on the quality of tea, herbal tea and their products, herbal tea is defined as an equally fragmented and dried part of the plant (fruit or parts of the fruit, seed, flower, parts of flower, leaf, herb, stem, cortex, root, rhizome, sachets or tuber), of which the beverage can be prepared in the usual way, which include fruit tea and a mixture of plant tea [6].

Herbal teas have complex chemical compositions, and chemical substances they contain can exhibit biological activity in humans [1]. Consequently, herbal teas represent sources of various organic and inorganic components that can influence the human health. Tea is a rich source of minerals and trace elements that are essential to human health [7 – 9] and drinking of teas can be an important source of some essential minerals [10].

[1] Academy of Applied Studies Šabac, Department for Medical, Business and Technological Studies, Šabac, Serbia.
[2] Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia.
[3] Faculty of Veterinary Medicine, University of Belgrade, Bul. Oslobodjenja 18, 11000 Belgrade, Serbia.
[*] e-mail: nikanto@ptt.rs; mantonijevicnikolic@vmpts.edu.rs

Essential minerals, including the trace elements, are inorganic elements that have a physiological function within the body. These must be provided in the diet and vary from grams per day for the major minerals through milligrams to micrograms per day for the trace elements [11].

Minerals, important for humans, have been divided into the next groups: (i) essential or major: calcium, magnesium, phosphorus, sodium, potassium, chlorine; (ii) essential in traces: iron, copper, chrome, zinc, iodine, selenium, fluorine, cobalt, manganese, molybdenum; (iii) probably essential in traces: silicon, nickel, tin, vanadium; and (iv) non-essential: arsenic, mercury, lead, aluminum, boron, gold, silver, titanium, lithium, strontium, germanium, cadmium, bismuth, rubidium, bromine [12]. In the WHO report [13], trace elements have been classified into three groups from the point of view of their nutritional importance in humans: (1) essential elements; (2) elements which are probably essential; and (3) potentially toxic elements, some of which may nevertheless have some essential functions at low levels.

A disadvantage, as well as elevated concentration of essential elements, can have a negative impact on human health [11]. Special attention is devoted to determining the content of heavy metals in teas. The surge in energy production and the increasing use of heavy metals in various industrial processes have led to the generation of large quantities of industrial waste containing heavy metals. Therefore, heavy metals are released into the environment including air, water, soil, and biosphere in excessive amounts on daily basis. Heavy metal ions have a high atomic weight and a density at least 5 times higher than that in water [14]. Heavy metals are typically toxic, persistent, non-biodegradable and bioaccumulative. Due to the high solubility, heavy metals are easily absorbed by plants and aquatic species and subsequently enter the food chains and then the human body. The presence of high levels of heavy metals in the human body may cause various health effects including skin irritations, stomach cramps, vomiting, multiple organ damage, birth defects, nerve system damage and development of autoimmunity. Teas contain both essential (Fe, Cu, Mn, Zn) and toxic elements (As, Cd, Hg, Pb). The biological effects of heavy metals in living systems strongly depend on their concentration [15] and thus should be carefully controlled, especially when herbal teas are used in human medicine.

The content of metals in food is defined in terms of maximum allowed concentrations, which represent the amount of toxic substance in the adult weighing 70 kg, consumed over all their lives, will not cause unwanted toxic effects [16]. The content of heavy metals in herbal teas depend on climatic factors, plant species, vegetation period, air pollution, by genetically-determined properties of a plant as well as by external factors, including geographic location, soil type and profile, fertilization, availability of water, pollution by pesticides or dusts, and gases, and other environmental factors [17]. Differences can also be a consequence of specific methods of the production of certain sorts of tea, including type, age of the herbal material, season of harvesting and manner of manuturing and storing [18]. The distribution of the heavy metals among plant organs is selective and dependent on the part of the plant, surface characteristics of the plant organ, and the element that is examined [19].

Iron is an essential element for growth and development of animals, plants and for humans too and an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, proteins and fats to control body weight, which is a very important factor is diabetes management. Iron is necessary for the formation of hemoglobin and also plays an important role in oxygen and electron transfer in the human body. Low iron content causes gastrointestinal infection, nose bleeding and myocardial infection. Zinc is necessary for proper growth, blood clotting, thyroid function and protein and DNA synthesis. Manganese plays a vital role in the photosynthesis, nitrogen metabolism and in the formation of other compounds that are required for the plant metabolism. Manganese is necessary for plant, animal and human as enzyme cofactor [20]. However, the deficiency of manganese in humans may lead to immunodeficiency disorder, rheumatic arthritis in adults, disorder of bony cartilaginous growth in infants, as well as myocardial infarction and other cardiovascular diseases [21]. Copper is an essential enzymatic element and is necessary for normal biological activities of amino oxides and tyrosinase enzyme. Tyrosinase is the enzyme that is required for catalytic conversion of tyrosine to melanin, which is a vital pigment located beneath the skin, and thus protects the skin from dangerous radiations [22]. Copper is essential to the human body since it forms a component of many enzyme systems, such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood. Copper deficiency results in anemia and congenital inability to excrete copper resulting in Wilson's disease. However, copper could be toxic depending on the dose and duration of exposure [23, 24]. It is considered that Ni is an essential element for the body, although there is no shortage of nickel in humans. In a small concentration, Ni is also very important for plant growth, while in high concentrations it is toxic to plants [25].

Lead is the most frequently occurring and stable heavy metal in the nature. It is highly hazardous for plants, animals and micro-organisms. It is a non-essential element that can be introduced to human by inhalation, ingestion or cutaneous absorption. It is a serious cumulative body poison. Levels of lead beyond the permissible limits or long term use of these contaminated plants could lead to toxicity characterized by colic, anemia, chronic nephritis, headache, convulsions, brain damage, and central nervous system disorders [26].

Cadmium is a non-essential trace element with uncertain direct functions in both plants and humans and responsible for several cases of poisoning through food. Recently, it is gaining more attention due to wide occurrence in water, soil, milk, dietary and herbal medicinal products (Singh, et al., 2014). Small quantities of cadmium cause adverse changes in the arteries of human kidney leading to kidney failure. It accumulates in human body, replaces zinc biochemically and causes hypertension, liver and kidney damage [27].

Due to the importance of essential heavy metals in herbal teas, a number of studies have been carried out to determine their levels by using atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma-atomic emission spectrometry (ICP-AES) [9, 28 – 34]. These methods employ highly sensitive spectroscopic techniques and generally require destruction of the sample matrix to render a solution of the analyte ready for analysis.

The present study was designed to evaluate the levels of seven heavy metals (Cu, Fe, Zn, Mn, Ni, Cd and Pb) in ten medicinal plants (*Matricariae flos, Thymi herba, Menthae piperitae folium, Betulae folium, Quercus cortex, Gentianae radix, Frangulae cortex, Althaeae radix, Uvae ursi folium and Glycyrrhizae radix*) collected from local health food stores in Šabac, Serbia and analyzed using the AAS method. The content of heavy metals in these herbal teas was used to calculate the estimated daily intake (*EDI*) of metals, the target hazard quotients (*THQ*) values for analyzed heavy metals and hazard index (*HI*) for adults.

2. MATERIALS AND METHODS

2.1. Sampling and Sample Preparation

All samples used in this research represent herbal teas (Table 1) collected from local health food stores in Šabac, Serbia. The samples were herbal teas *Matricariae flos, Thymi herba, Menthae piperitae folium, Betulae folium, Uvae ursi folium, Quercus cortex, Gentianae radix, Frangulae cortex, Althaeae radix, and Glycyrrhizae radix* sold in bulk. The tested samples of herbal teas were standardized in accordance with regulations of the European Pharmacopoeias. Within the standardization of herbal teas, macroscopic identification was performed for each sample according to the standards of the valid monograph for a given herbal tea according to Ph. Eur 10 [35]. Also, within the standardization, an examination of the general quality of the tested herbal teas was performed according to the valid monographs to Ph. Eur. 3 [36] and Ph. Eur 10 [35].

The dried tea samples were ground using a grinder (A 11 BASIC IKA) and passed through 0.5 mm sieve (laboratory vibrating screen, WQS, Gramma Libero). The powdered tea samples were stored at ambient temperature and used for the analysis of metals in herbal tea and for determining water content, total ash, and ash insoluble in hydrochloric acid.

2.2. Analytical Measurements

Determination of the presence of foreign matter, loss of drying/water, total ash and ash insoluble in hydrochloric acid were made according to the procedures as given in the general chapters of the European Pharmacopoeia [35, 36]. The presence of foreign matter is determined by the prescribed quantity of the sample, by examination plant material with naked eye and with a magnifying glass (Ph. Eur. 10, 2.8.2) [35]. Loss by drying was determined gravimetrically from the difference in the mass of the drug before and after drying in the dryer at a temperature of 105 ° C for 2 h (Ph. Eur. 10, 2.2.32) [35]. Water was determined by distillation of toluene and calculated as water content present in the herbal teas as milliliters per kilogram (Ph. Eur. 10, 2.2.13) [35].

The total ash was determined from differences in drug mass before and after irradiation at 600°C ± 25°C (Ph. Eur.10, 2.4.16) [35]. The ash insoluble in hydrochloric acid was determined the treatment of total ash hydrochloric acid (Ph Eur 10, 2.8.1) [35]. Preparation of tea samples for analysis of heavy metals was performed by dry digestion, burning and dissolving the ash in a suitable acid. In dry digestion, about 3 g of the sample (exact mass measured to 4 decimal places) was calcined gradually by raising the temperature by 50°C/h, from room to 450° C, at which the sample was kept for 8 h. The resulting ash was dissolved in 5 mL hydrochloric acid (c = 6 M) and the solution was evaporated in water bath to dryness. The residual precipitate was dissolved in ~10.0 mL nitric acid (c = 0.1 mol/dm^3), filtered, and washed with deionized water. The final volume was 50 mL [37]. The blank sample was prepared in the same way with no tea.

The following reagents were used: standard solutions of Cu, Fe, Ni, Mn, Zn, Cd, and Pb at a concentration of 1 mg/kg (Accu TraceTM Reference Standard, USA), nitric acid (p.a. 65%, Merck, Germany), hydrochloric acid (p.a. 35%, Lach-Ner, Czech Republic), deionized water with conductivity < 2 μS/cm. All other chemicals were of p.a. grade and used as supplied.

2.3. Instruments and Equipment

Heavy metals (Cu, Fe, Ni, Mn, Zn, Cd, and Pb) concentrations were determined by the atomic absorption spectrometry (AAS) method. Atomic absorption measurements were made using a Perkin-Elmer spectrophotometer AAS-5100/PC with hollow cathode lamps and background correction. Air–acetylene flame was used for the determination of all elements.

2.4. Calculation of Health Risk Assessment for Herbal Tea Consumption

The content of heavy metals in herbal teas were used to evaluate the estimated daily intake (*EDI*)of heavy metals, target hazard quotients (*THQ*), and hazard index (*HI*) for adults.

Estimated daily intake (*EDI*) of heavy metals. The *EDI* value (mg/kg body weight/day) of heavy metals is a fundamental parameter for metal transfer from plant to human. It can be calculated using the following equation [38]:

$$EDI = \frac{C \cdot F_{IR} \cdot TR}{W_{AB} \cdot 1000}, (1)$$

where C is the heavy metal content (mg/kg) in herbal tea, F_{IR} is the herbal tea ingestion rate (g/person/day), TR is the trans-

TABLE 1. Name and Definition of Analyzed Herbal Teas According to Ph. Eur. 10 and Ph. Eur. 3, Number of Monographs in the Pharmacopoeias, and Limit Values for Determination [35, 36]

No.	Herbal tea (Ph. Eur. 10)	Latin name of herbal tea (Ph. Eur. 10)	Definition by Ph. Eur. 10	Foreign matter	Loss on drying/ water	Total ash	Ash insoluble in HCl
1	Matricaria flower	*Matricariae flos*	Dried capitula of *Matricaria recutita* L. (*Chamomilla recutita* (L.) Rauschert).	*2.8.2.* maximum 2.0% [36]	*2.2.32.* maximum 12.0% determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.16.* maximum 13.0%	-
2	Thyme	*Thymi herba*	Whole leaves and flowers separated from the previously dried stems of *Thymus vulgaris* L. or *Thymus zygis* L. or a mixture of both species.	*2.8.2.* maximum stems 10% and maximum 2% of other foreign matter	*2.2.13.* maximum 100 mL/kg, determined on 20.0 g of the powdered drug (10% v/m)	*2.4.16.* maximum 15.0%	*2.8.1.* maximum m 3.0%
3	Peppermint leaf	*Menthae piperitae folium*	Whole or cut dried leaves of *Mentha × piperita* L.	*2.8.2.* maximum 5% stems, maximum 2% foreign elements	*2.2.13.* maximum 110 mL/kg, determined on 20.0 g (11% v/m)	*2.4.16.* maximum 15.0%	*2.8.1.* maximum 1.5%
4	Bearberry leaf	*Uvae ursi folium*	Whole or cut, dried leaf of *Arctostaphylos uva-ursi* (L.) Spreng.	*2.8.2.* maximum 5% of stems and maximum 3 per cent of other foreign matter	*2.2.32.* maximum 10.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.16.* maximum 5.0%	-
5	Birch leaf	*Betulae folium*	Whole or fragmented dried leaves of *Betula pendula* Roth and/or *Betula pubescens* Ehrh. as well as hybrids of both species.	*2.8.2.* maximum 3% fragments of female catkins and maximum 3 per cent of other foreign matter	*2.2.32.* maximum 10.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.16.* maximum 5.0%	-
6	Marshmallow root	*Althaeae radix*	Peeled or unpeeled, whole or cut, dried root of *Althaea officinalis* L.	*2.8.2.* maximum 2% of brown deteriorated drug.	*2.2.32.* maximum 12.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.16.* maximum 6.0% for the peeled root and maximum 8.0% for the unpeeled root	-
7	Gentian root	*Gentianae radix*	Dried, fragmented underground organs of *Gentiana lutea* L.	*2.8.2.* maximum 2.0% [36]	*2.2.32*, maximum 8 – 12.0% [36]	*2.4.16*, maximum 6.0%	-
8	Liquorice root	*Liquiritiae radix*	Dried unpeeled or peeled, whole or cut root and stolons of *Glycyrrhiza glabra* L. and/or of *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch.	*2.8.2.* maximum 2.0% [36]	*2.2.32.* maximum 10.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.16.* maximum 10.0% for the unpeeled drug and maximum 6.0% for the peeled drug	*2.8.1.* maximum 2.0% for the unpeeled drug and maximum 0.5% for the peeled drug
9	Frangula bark	*Frangulae cortex*	Dried, whole or fragmented bark of the stems and branches of *Rhamnus frangula* L. (*Frangula alnus* Miller).	*2.8.2.* maximum 1%	*2.2.32.* maximum 10.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.*16. maximum 6.0%	-
10	Oak bark	*Quercus cortex*	Cut and dried bark from the fresh young branches of *Quercus robur* L., *Q. petraea* (Matt.) Liebl. and *Q. pubescens* Willd	*2.8.2.* maximum 2.0% [36]	*2.2.32.* maximum 10.0%, determined on 1.000 g of the powdered drug by drying in an oven at 105°C for 2 h	*2.4.*16. maximum 8.0%	-

ference rate of the heavy metal from herbal tea to herbal tea infusion, and W_{AB} is the average body weight.

Target hazard quotient (*THQ*). The targeted hazard quotient (*THQ*) was used to estimate the potential noncarcinogenic effects of individual heavy metals present in the herbal teas. The *THQ* value was calculated by the following formula:

$$THQ = \frac{EDI}{RfD}, \quad (2)$$

where the *THQ* is the dimensionless target hazard quotient, *EDI* is the estimated daily intake of heavy metals (mg/kg body weight/day), and *RfD* is the oral reference dose for the heavy metals (mg/kg body weight/day) [38, 39] which does not produce a lifetime harmful effect. The *RfD* values of heavy metals studied are shown in Table 2.

Hazard index (*HI*). The overall potential health risks may product from the influence of more than one metal. The cumulative potential noncarcinogenic effects of multiple heavy metals present in the herbal teas can be quantitatively evaluated by the hazard index (*HI*) [38, 39]. Different contaminants there are variety potential non-carcinogenic effects. Consequently, exposure to two or more heavy metals may result in additive and/or interactive effects [45]. If it is assumed that the toxic effects of heavy metals in herbal tea are additive rather than synergistic or antagonistic to human health, *HI* can be estimated by the sum of the target hazard quotients of all studied pollutants and can be calculated by the following equation [38, 39, 45, 46]:

$$HI = THQ_1 + THQ_2 + THQ_3 + \ldots + THQ_n, \quad (3)$$

where *THQn* is the *THQ* value of an individual metal and *HI* is the hazard index of multiple metals.

2.5. Statistical Data Analysis

All determinations were carried out in triplicate. Experimental data was subjected to ANOVA test and statistical significance was obtained at $p < 0.05$. Finally, the data was expressed as mean ± standard deviation (±SD).

3. RESULTS AND DISCUSSION

Medicinal plants are the raw material for many herbal formulations and popular supplements. The use of herbal medicines has been on the rise in recent years due to their low prices. The last few decades have witnessed a rapid development in the diet studies focused on the determination of trace elements, which reflect their role in human health and nutrition. Deficiency, excess or imbalance of trace element intake into human body may result in various diseases. In this work, the quality of ten herbal tea samples was analyzed. Herbal tea samples were collected from the local health food markets on the territory of Šabac, Serbia. Analyzed samples were in bulk, without adequate evidence of their quality.

Identification of samples of *Matricariae flos, Thymi herba, Menthae piperitae folium, Betulae folium, Uvae ursi folium, Quercus cortex, Gentianae radix, Frangulae cortex, Althaeae radix, and Glycyrrhizae radix* was done by macroscopic analysis according to monographs Ph. Eur. 10 [35]. All tested samples of herbal teas are in accordance with the regulations of identification by Ph. Eur. 10 [35].

In the framework of general testing of the samples, the presence of foreign matter was examined, a loss of drying/water content, and the total ash and ash insoluble in HCl (Table 3) in according with standards of Ph. Eur. 10 and Ph. Eur. 3 [35, 36] (Table 1). The content of metals (Fe, Cu, Zn, Mn, Ni, Cd, Pb) in herbal tea samples was analyzed by atomic absorption spectroscopy (AAS) method. Table 4 shows the concentrations of selected metals in analyzed samples.

3.1. Results of General Testing

Foreign substances. Foreign substances in herbal drugs are considered: (a) unprocessed parts of the same plant, (b) parts of other plants, and (c) substances of mineral origin. The amount of foreign substances is defined for each of the drugs within the appropriate box monographs in Pharmacopoeia (Table 1). However, in the samples 5 and 6 the content of foreign substances is greater than prescribed values (Table 3). In addition, in plant drugs, molds, insects and other contamination of animal origin must be completely absent [35, 36].

TABLE 2. Oral Reference Dose (*RfD*), Upper Tolerable Daily Intake (*UL)* and Recommended Daily Intake (*RDI*) Values for Investigated Heavy Metals

Heavy metal	Cu	Fe	Ni	Mn	Zn
The oral reference dose (*RfD*) (mg/kg body weight/day)	0.04 [5, 40 – 42]	0.7 [5, 40 – 43]	0.02 [41, 42]	0.014 [41, 42]	0.3 [5, 40 – 43]
Upper tolerable daily intakes (*UL*)* (mg/day/person) for adults [41, 44]	10	45	1	11	40
Recommended daily intakes (*RDI*)** (mg/day/person) for men (women) [41, 44]	0.900	8 (18)	0.500	2.3 (1.8)	11 (8)

* The tolerable upper intake level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the specified life stage group [44].

Loss of drying/water content. Excessive moisture in the drug can be a consequence insufficient drying of the drug or absorption of moisture from the air of the following adequate conditions storage. Increased moisture content in the drug can cause deterioration plant material and degradation of active principles [47]. Loss of drying/water was determined for all samples according to corresponding monographic boxes in the Pharmacopoeia (Table 1) [35, 36]. In all samples, the loss of drying/water is within the prescribed limits (Table 3).

Total ash and ash insoluble in hydrochloric acid. Total ash represents non-volatile mineral substances present in the drug, if they have in the plant significant role [47]. In total, the determination of ash insoluble in hydrochloric acid is prescribed which represents silicon-dioxide. The excess of ash above the allowed limit is an indicator the malfunction of the drug, that is, points to insufficiently clean drugs, which are either contaminated inorganic additives (soil, plaster, sand, brick, chalk, etc.) or have grown on the field which it is rich in mineral substances. In the tested samples, total ash and ash insoluble in hydrochloric acid are in according with the requirements prescribed by the Pharmacopoeia (Table 1 and Table 3) [35, 36]. The exception is sample 4 which has a slightly higher total ash content than the allowable value (Table 1 and Table 3). Samples 4, 5 and 6 (Table 3) according to the Pharmacopoeia do not meet the general quality standards for herbal teas due to deviations from the prescribed values (Table 1) [35, 36].

3.2. Content of Heavy Metals

In this work, the content of essential (Fe, Zn, Cu and Mn), probably essential in trace (Ni), and toxic elements (Pb and Cd), was determined in the ten investigated herbal teas by using the AAS method. The content of each metal is expressed in mg/kg of dry matter. Table 4 shows the contents of elements in marketed herbal teas studied in this work. All herbal tea samples contain significant concentrations of essential elements (Fe, Cu, Mn, Zn and Ni) that differ from the plant species. The content of toxic elements (Pb and Cd) is below the maximum allowed limit (according to the rules of the World Health Organization (2011), the maximum permitted values in dried plant materials are 10.0 and 0.3 mg/kg, respectively) [47].

Among the essential elements, Fe and Mn are the most abundant. The herbal tea samples also contain significant amounts of Zn. Threshold values of Cu, Fe, Mn, Ni, Zn and Cd are not proposed in the current standards of tea in Serbia [6]. The quality of the analyzed herbal teas was evaluated by comparing with the literature data (Table 5). Among the investigated metals Fe was the highest (118.0 ± 1.1 – 775.5 ± 2.5 mg/kg). Manganese was the second highest element found in all tea samples (19.0 ± 5.8 – 561.0 ± 1.9 mg/kg). The concentrations of Cu, Ni and Zn in the analyzed samples were in the range of 2.9 ± 0.1 – 22.2 ± 0.9, 2.5 ± 0.1–10.0 ± 1.1 mg/kg and 6.5 ± 0.4 – 242.5 ± 1.4 mg/kg, respectively. The content of Pb and Cd in all samples has been below the detection limit (Pb less than 2.5 mg/kg and Cd less than 0.07 mg/kg).

TABLE 3. Determination of Foreign Matter, Loss on Drying/Water Content, Total Ash, Ash Insoluble in Hydrochloric Acid, and Sulfate Residue (Mean Values ± SD).

No.	Herbal tea	Foreign matter [%]	Loss on drying [m/m%]/ water [v/m%]	Total ash [%]	Ash insoluble in HCl [%]
1	*Matricariae flos*	1.43 ± 0.09	8.54 ± 0.06	7.50 ± 0.08	-
2	*Thymi herba*	3.77 ± 0.15 (stems)	6.49 ± 0.03	9.00 ± 0.22	0.96 ± 0.02
3	*Menthae piperitae folium*	1.67 ± 0.05 (foreign elements)	9.23 ± 0.12	8.93 ± 0.05	0.63 ± 0.02
4	*Uvae ursi folium*	1.20 ± 0.03 (stems)	8.88 ± 0.20	6.64 ± 0.03	-
5	*Betulae folium*	4.62 ± 0.02 (other foreign matter)	9.82 ± 0.15	4.72 ± 0.08	-
6	*Althaeae radix*	7.13 ± 0.09	9.09 ± 0.09	4.99 ± 0.10 (the peeled root)	-
7	*Gentianae radix*	1.15 ± 0.04	8.94 ± 0.7	4.02 ± 0.03	-
8	*Glycyrrhizae radix*	1.54 ± 0.05	8.79 ± 0.16	5.94 ± 0.06 (the unpeeled drug)	0.96 ± 0.05 (the unpeeled drug)
9	*Frangulae cortex*	0.73 ± 0.03	8.99 ± 0.08	5.23 ± 0.05	-
10	*Quercus cortex*	0.27 ± 0.02	9.45 ± 0.11	7.43 ± 0.15	-

Iron. Herbs are particularly rich in iron because polyphenol compounds, especially flavonoids, are bound in plants by metals such as Al, Fe and Zn. Its deficiency can hinder metabolism. The analyzed herbal tea samples contain large amounts of Fe from 118.0 ± 1.1 to 755.5 ± 2.5 mg/kg. The lowest value of Fe was found in samples of *Frangulae cortex* and the highest was found in *Thymi herba* samples. Results of the current study show a wide variation of iron in different herb samples, which agrees with results in the literature (Table 5). These levels are relatively high compared to iron in some medicinal plants for the treatment of anemia. A conducted study on selected medical herbs indicated iron concentrations within the range 6.67 – 223 mg/kg [48]. Based on these investigations and the total content of Fe in the analyzed herbal teas, it can be concluded that these herbal teas can contribute to the daily dietary requirements (8 (18) mg/day, Table 2) [30, 41, 44]. Especially, it can be recommended herbal tea *Thymi herba* to use for increased daily intake of Fe.

Zinc. The concentration of Zn in the analyzed plant samples was between 6.5 ± 0.4 – 242.5 ± 1.4 mg/kg. The highest concentration was recorded in *Betulae folium* (242.5 ± 1.4 mg/kg) while the remaining samples contents are much smaller (6.5 ± 0.4 – 39.0 ± 0.9 mg/kg) and in agreement with the results from the literature, (Table 5). The lowest value of Zn was found in sample herbal tea *Quercus cortex* (6.5 ± 0.4 mg/kg). Zinc intake beyond permissible limits produces toxic effects on the immune system of the body and disrupts copper levels [49]. It is the only metal which appears in all enzyme classes. In blood plasma, Zn is bound to and transported by albumin (60%) and transferrin (10%). Excessive iron can reduce zinc absorption, and vice versa. The concentration of zinc in blood plasma stays relatively constant regardless of zinc intake [50]. The obtained results indicate a significantly higher content of Zn in the medical plant *Betulae folium.* Whereas the recommended daily dietary intake of zinc stands at 11 (8) mg/day (Table 2) [30, 41, 44].

Manganese. According to the data in the literature, Mn is expected to be largely concentrated in leaves, decreasing in roots and stems [34], although its content may be very high in soil. The content of Mn in herbal teas samples is in the ranges from 19.0 ± 5.8 in the *Glycyrrhizae radix* to 561.0 ± 1.9 mg/kg in the *Betulae folium.* The adequate intake for adult men and women is 2.3 and 1.8 mg/day, respectively [41, 44]. A tolerable upper intake level of 11 mg/day was set for adults based on a no-observed-adverse-effect level [42, 45, 49]. In the present study, except for Mn levels of *Betulae folium* and *Frangulae cortex*, Mn concentration levels in the studied herbal teas were below the permissible limit set by FAO/WHO in medicinal plants [51]. The leaves of *Betulae folium* and *Frangulae cortex* contains higher levels than the permissible limits set for Mn in plants. Hence, they contain unsafe levels of Mn and might be detrimental to consumer's health.

Copper. The recommended daily intake for adults of Cu is 2 – 3 mg/day [30, 34,] or 0.900 mg/day [41, 44]. Copper is a necessary element in the human organism. It is one of the native metals found in tea, combined to polyphenol oxidase enzyme. The lowest value of copper was found in sample herbal tea *Frangulae cortex* (2.9 ± 0.1 mg/kg) and the highest in *Althaeae radix* (22.2 ± 0.9 mg/kg). The content of Cu in *Uvae ursi folium* is also low, while in the remaining samples of tea the Cu content is almost uniform (8.2 ± 0.4 – 13.4 ± 1.0 mg/kg). It is similar in the literature (Table 5). The results of total contents of studied heavy metals in *Althaeae radix* tea show the ability of this plant to accumulate heavy metals, particularly Cu (22.2 ± 0.9 mg/kg) and Fe (144.5 ± 0.8 mg/kg). The concentration of Cu in teas also depends on the dishes used for tea processing [18]. The

TABLE 4. Total Content of Heavy Metals (mg/kg dry weight) in Herbal Teas Collected in Šabac Markets and Determined by AAS (Mean Values ± SD)

No.	Herbal tea	Concentration (mg/kg)				
		Cu	Fe	Ni	Mn	Zn
1	*Matricariae flos*	11.1 ± 0.8	166.5 ± 2.3	5.0 ± 0.3	43.5 ± 1.4	26.5 ± 1.2
2	*Thymi herba*	13.4 ± 1.0	755.5 ± 2.5	8.5 ± 0.8	54.5 ± 1.5	29.5 ± 1.3
3	*Menthae piperitae folium*	11.0 ± 0.8	366.5 ± 1.9	7.5 ± 0.6	70.7 ± 1.7	16.5 ± 1.2
4	*Uvae ursi folium*	5.9 ± 0.4	489.0 ± 1.7	6.5 ± 0.7	20.5 ± 1.2	34.5 ± 1.8
5	*Betulae folium*	13.3 ± 0.7	193.5 ± 2.1	2.5 ± 0.1	561.0 ± 1.9	242.5 ± 1.4
6	*Althaeae radix*	22.2 ± 0.9	144.5 ± 0.8	5.5 ± 0.4	19.5 ± 0.9	20.0 ± 1.1
7	*Gentianae radix*	9.7 ± 0.6	269.0 ± 1.5	10.0 ± 1.1	111.0 ± 1.1	39.0 ± 0.9
8	*Glycyrrhizae radix*	8.9 ± 0.6	411.0 ± 1.2	2.7 ± 0.2	19.0 ± 5.8	11.5 ± 0.6
9	*Frangulae cortex*	2.9 ± 0.1	118.0 ± 1.1	3.3 ± 0.2	361.0 ± 1.5	8.0 ± 0.6
10	*Quercus cortex*	8.2 ± 0.4	238.0 ± 0.7	6.5 ± 0.5	150.0 ± 1.5	6.5 ± 0.4

TABLE 5. Content of Heavy Metals (mg/kg) in Herbal Teas (in Original Package) from Serbia Described in the Literature

Tea	Concentration, mg/kg				
	Cu	Fe	Mn	Zn	Ni
Matricariae flos [54]	14.25	130.26	76.14	34.67	—
Thymi herba [54]	8.94	445.78	127.06	44.26	2.51
Mentha x piperita [54]	17.15	443.90	111.97	26.86	—
Mentha piperita [16]	12.9 – 13.9	-	47.9 – 69.9	16.3 – 25.0	3.8 – 4.4
Chamomillae flos [16]	10.8 – 14.1	-	26.0 – 43.8	15.4 – 17.5	2.2 – 3.4
13 tea samples* [55]	5.77 – 10.63	58 – 2574	26 – 493	12.9 – 79.0	0.70 – 5.95
Echinacea purpurea [34]	4.4 – 15.9	32.2 – 292	8.80 – 67.6	3.9 – 18.6	0.50 – 9.30

* The sample preparation has been performed using wet digestion procedures.

Cu content of herbal tea samples (2.9 ± 0.1 – 13.4 ± 1.0 mg/kg), except *Althaeae radix,* were in the range concentration of Cu found in the same investigations, Table 5.

Nickel. The nickel concentration in all herbal tea samples was equally/lower than 10 mg/kg (range 2.5 ± 0.1 – 10.0 ± 1.1 mg/kg) and the usual range is from 2.9 to 22.6 mg /kg [52]. It can be seen that only in *Gentianae radix* content of Ni is greater compared to the literature data from the market in Serbia (Table 5).

Contents of microelements in medicinal plants are influenced by genetically-determined properties of a plant as well as by external factors, including geographic location, soil type and profile, fertilization, availability of water, pollution by pesticides or dusts, and gases. The differences can be a consequence the specific methods of the production of certain sorts of tea, including type, age of the herbal material, season of harvesting and manner of maturing and storing [31, 53].

3.3. Health Risk Assessments

Estimated daily intake of heavy metals by consuming herbal tea. Health risk evaluation based on the estimated daily intake (*EDI*) of the heavy metal pollutant is one of the important health risk rating implements. The *EDI* concept takes into account the heavy metal content in herbal tea, the

TABLE 6. Estimated Daily Intake (*EDI*) (mg/kg bw/day) of Heavy Metals for Adults due to the Consumption of Herbal Teas from Serbia, the Mean Average *EDI* (mg/kg body weight/day), and the Recommended Daily Intake (*RDI*) Expressed for Body Weight (mg/kg body weight/day).

No.	Herbal tea	*EDI* (mg/kg body weight/day) for adults				
		Cu	Fe	Ni	Mn	Zn
1	*Matricariae flos*	6.04×10^{-4}	2.90×10^{-3}	3.46×10^{-4}	2.33×10^{-3}	9.49×10^{-4}
2	*Thymi herba*	7.29×10^{-4}	1.32×10^{-2}	5.88×10^{-4}	2.92×10^{-3}	1.06×10^{-3}
3	*Menthae piperitae folium*	5.99×10^{-4}	6.39×10^{-3}	5.19×10^{-4}	3.79×10^{-3}	5.91×10^{-4}
4	*Uvae ursi folium*	3.21×10^{-4}	8.53×10^{-3}	4.49×10^{-4}	1.10×10^{-3}	1.24×10^{-3}
5	*Betulae folium*	7.24×10^{-4}	3.38×10^{-3}	1.73×10^{-4}	3.01×10^{-2}	8.69×10^{-3}
6	*Althaeae radix*	1.21×10^{-3}	2.52×10^{-3}	3.80×10^{-4}	1.05×10^{-3}	7.17×10^{-4}
7	*Gentianae radix*	5.28×10^{-4}	4.69×10^{-3}	6.91×10^{-4}	5.95×10^{-3}	1.40×10^{-3}
8	*Glycyrrhizae radix*	4.84×10^{-4}	7.17×10^{-3}	1.87×10^{-4}	1.02×10^{-3}	4.12×10^{-4}
9	*Frangulae cortex*	1.58×10^{-4}	2.06×10^{-3}	2.28×10^{-4}	1.94×10^{-2}	2.87×10^{-4}
10	*Quercus cortex*	4.46×10^{-4}	4.15×10^{-3}	4.49×10^{-4}	8.05×10^{-3}	2.33×10^{-4}
The mean average of *EDI* (mg/kg body weight/day)		5.80×10^{-4}	5.50×10^{-3}	4.01×10^{-4}	7.57×10^{-3}	1.56×10^{-3}
Recommended daily intakes expressed by body weight (mg/kg body weight/day) which calculated for men (women) weighing 70 kg		1.29×10^{-2}	1.14×10^{-1} (2.57×10^{-1})	7.14×10^{-3}	3.29×10^{-2} (2.57×10^{-2})	1.57×10^{-1} (1.14×10^{-1})

herbal tea ingestion rate, the transference rate of the heavy metal from herbal tea to herbal tea infusion and the body weight of exposed persons.

Potential health risk from ingesting heavy metals from herbal teas was performed for adults. In this study the average body weight for adults was considered to be 70 kg [46, 56]. The heavy metals in herbal teas are not all ingested into the human body but already extracted into the herbal tea infusion, and then ingested into the human body. Consequently, the transference rates (*TR*) of the heavy metals from herbal tea to herbal tea infusion need to be included when determining the amount of heavy metals in herbal teas into human body. In the present study, the assessment of the concentrations of heavy metals in herbal tea infusion had not been perform. The transference rates of heavy metals from herbal tea to herbal tea infusion were cited from the results of certain earlier research. Accordingly, the transference rates of Cu, Fe, Ni, Mn and Zn were 38.10, 12.21, 48.40, 37.55 and 25.08%, respectively [57]. The transference rates of Cd and Pb are not listed, because their content in all samples was below the detection limit.

Based on the assumption that the average consumption of herbal tea infusion for single person is five cups a day with one packet of 2 g (for each), the daily consumption of dry herbal tea was 10 g/person/day (F_{IR}) [58].

According to Eq. (1), the estimated daily intake (*EDI*) values of heavy metals (Cu, Fe, Ni, Mn and Zn) in tested herbal teas from Serbia were calculated and the results are show in Table 6. In addition, Table 6 gives the values of recommended daily intake as expressed per body weight (mg/kg body weight/day) i.e., recommended daily intakes recalculated for adults weighing 70 kg.

The *EDI* values in tested herbal teas was the following: Mn > Fe > Zn > Cu > Ni because the mean average of *EDI* were 7.57×10^{-3}, 5.50×10^{-3}, 1.56×10^{-3}, 5.80×10^{-4}, 4.01×10^{-4} (mg/kg bw/day), respectively (Table 6). This means that the contribution of Mn was the largest among daily intakes of all analyzed heavy metals. Similarly, Zhang, et al. [38] also reported that *EDI* of Mn was the highest among investigated metals (Mn, Al, Zn, Cu, Ni, Cr, Pb, As, Hg and Cd) consumed through the use of tea.

The *EDI* for Cu, Fe, Ni, Mn and Zn in all tested herbal teas less than the recommended daily intakes expressed by body weight (Table 6). This indicates that the daily intake of these heavy metals surveyed as a five cups of the herbal tea infusion are much lower than human body needed. According, consuming herbal tea can supplement essential in traces elements (Cu, Fe, Mn and Zn) and probably essential in traces elements (Ni) [12] needed by human body, but their contents are much less than human body needs. The herbal tea of *Betulae folium* was an exception, because it has a slightly higher value the *RDI* expressed by body weight for women for Mn (Table 6). The *EDI* values in this research were all below the *RfD* values (Table 2) for all investigated heavy metals in the eight samples of herbal teas (1, 2, 3, 4, 6, 7, 8, 10) (Table 6), indicating that the drinking of the analyzed herbal teas did not appear to pose a health risk to consumers. On the other hand, for samples 5 and 9, the *EDI* values of the four heavy metals (Cu, Fe, Ni and Zn) the elements were lower than the appropriating *RfD* values, except for Mn. Also, the *EDI* values were significantly less than upper tolerable daily intakes (*UL*) values for all the five elements in the studied herbal teas (Tables 1 and 6).

By comparing the *EDI* and the *RDI* (Tables 2 and 6), we can determine the contribution to the daily intake of heavy metals (%) by consuming the tested herbal teas. The obtained calculated values are shown in Table 7.

Among the herbal teas tested, the greatest contribution to the daily intake of Cu (9.37%) is provided by drinking herbal tea of *Althaeae radix*; Fe (11.56% (for men) and 5.13% (for

TABLE 7. Calculated Contribution to Daily Intakes of Heavy Metals (%) by Consuming Analyzed Herbal Teas from Serbia for Adults (Men and Women).

No.	Herbal tea	Contribution to daily intake of heavy metals (%) with herbal tea							
		Cu	Fe		Ni	Mn		Zn	
			for men	for women		for men	for women	for men	for women
1	*Matricariae flos*	4.68	2.55	1.13	4.84	7.09	9.08	0.60	0.83
2	*Thymi herba*	5.65	11.56	5.13	8.23	8.89	11.38	0.67	0.93
3	*Menthae piperitae folium*	4.64	5.61	2.49	7.26	11.53	14.76	0.38	0.52
4	*Uvae ursi folium*	2.49	7.48	3.32	6.29	3.34	4.28	0.79	1.08
5	*Betulae folium*	5.61	2.96	1.31	2.42	91.47	117.10	5.53	7.62
6	*Althaeae radix*	9.37	2.21	0.98	5.33	3.18	4.07	0.46	0.63
7	*Gentianae radix*	4.09	4.12	1.83	9.68	18.10	23.17	0.89	1.23
8	*Glycyrrhizae radix*	3.76	6.29	2.79	2.61	3.10	3.97	0.26	0.36
9	*Frangulae cortex*	1.22	1.81	0.80	3.20	58.86	75.35	0.18	0.25
10	*Quercus cortex*	3.46	3.64	1.62	6.29	24.46	31.31	0.15	0.20

TABLE 8. Target Hazard Quotient (*THQ*) and Hazard Index (*HI*) Values of Heavy Metals for Adults due to Consumption of Analyzed Herbal Teas from Serbia

No.	Herbal tea	*THQ* for adults					*HI* for adults
		Cu	Fe	Ni	Mn	Zn	
1	*Matricariae flos*	1.51×10^{-2}	4.15×10^{-3}	1.73×10^{-2}	1.67×10^{-1}	3.16×10^{-3}	0.2064
2	*Thymi herba*	1.82×10^{-2}	1.88×10^{-2}	2.94×10^{-2}	2.09×10^{-1}	3.52×10^{-3}	0.2788
3	*Menthae piperitae folium*	1.50×10^{-2}	9.13×10^{-3}	2.59×10^{-2}	2.71×10^{-1}	1.97×10^{-3}	0.3229
4	*Uvae ursi folium*	8.03×10^{-3}	1.22×10^{-2}	2.25×10^{-2}	7.85×10^{-2}	4.12×10^{-3}	0.1254
5	*Betulae folium*	1.81×10^{-2}	4.82×10^{-3}	8.64×10^{-3}	2.15	2.90×10^{-2}	2.2101
6	*Althaeae radix*	3.02×10^{-2}	3.60×10^{-3}	1.90×10^{-2}	7.47×10^{-2}	2.39×10^{-3}	0.1299
7	*Gentianae radix*	1.32×10^{-2}	6.70×10^{-3}	3.46×10^{-2}	4.25×10^{-1}	4.66×10^{-3}	0.4844
8	*Glycyrrhizae radix*	1.21×10^{-2}	1.02×10^{-2}	9.33×10^{-3}	7.28×10^{-2}	1.37×10^{-3}	0.1059
9	*Frangulae cortex*	3.95×10^{-3}	2.94×10^{-3}	1.14×10^{-2}	1.38	9.55×10^{-4}	1.4025
10	*Quercus cortex*	1.12×10^{-2}	5.93×10^{-3}	2.25×10^{-2}	5.75×10^{-1}	7.76×10^{-4}	0.6151

women)) *Thymi herba*; Ni (9.68%) *Gentianae radix*; Mn (91.47% (for men) and 117.1% (for women)) *Betulae folium* (58.86% (for men) and 75.35% (for women)) *Frangulae cortex*; and Zn (5.53% (for men) and 7.62% (for women)) *Betulae folium* (Table 7).

Risk of individual heavy metals by consuming herbal tea. The concept of the target hazard quotients were developed to evaluate potential health risks correlated with long term exposure to chemical pollutants. It is a complex parameter which includes the estimated daily intake of heavy metals as well as their oral reference dose [41]. If the value *THQ* is less than 1 indicate that there is no important risk of noncarcinogenic effects for the exposed consumers [38, 41, 45]. But if the value *THQ* is bigger is than 1 and less than 5, is considered as not safe for human health, therefore means that the exposed consumers is in a level of concern interval [41].

Values of the *THQ* for investigated heavy metals and herbal teas are presented in Table 8. The *THQ* for tested heavy metals (Cu, Fe, Ni, Mn and Zn) from the eight samples herbal teas (1, 2, 3, 4, 6, 7, 8, 10) were less than one, which is regarded as secure for human use. Values of the *THQ* for Mn for herbal teas of *Betulae folium* and *Frangulae cortex* is more than one i.e. 2.15 and 1.38, respectively. It makes these herbal teas unsafe for the consumer.

Combined risk of multiple heavy metals by consuming herbal tea. The combined noncarcinogenic influences of two or more heavy metals can be expressed by their *HI* values. Based on the *HI* values, no important hazard of noncarcinogenic consequences is expected if the value is less than one ($HI < 1$). But, if the *HI* is more than one ($HI > 1$), there is a possibility that noncarcinogenic risk effects may appear. It is very probability that the heavy metals have negative impacts on human health, which pretends to increase with the increment of *HI* value [43, 59]. If the *H*I value is higher than 10.0, there is a chronic toxic effect on human health [38].

The *HI* values of all investigated heavy metals via the consumption of herbal tea infusions are given in Table 8. It was discovered that *HI* values for all the tested heavy metals, in the eight samples herbal teas (1, 2, 3, 4, 6, 7, 8, 10), were below 1. This enabled us to conclude that there is actually no carcinogenic risk from the drinking of infusions of these herbal teas, in amounts and with the frequency assumed in this paper.

However, there are two herbal teas (*Betulae folium* and *Frangulae cortex*) having the *HI* values above unity. The *HI* value of the herbal tea of *Betulae folium* was 2.15, while the *HI* of the herbal tea of *Frangulae cortex* was 1.38. Consumption of these herbal teas might present potential health risks. The *HI* values of *Betulae folium* and *Frangulae cortex* were above unity, very likely due to a high daily intake of Mn with these herbal teas.

FUNDING

This work was partly supported by the Ministry of Science and Environmental Protection of the Republic of Serbia, Contract No. 451-03-68/2020-14/200161.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. F. Gil, A. F. Hernández and M. C. Martín-Domingo, Toxic contamination of nutraceuticals and food ingredients, in: *Nutraceuticals: Efficacy, Safety and Toxicity*, C. R. Gupta (Ed.), Elsevier Inc., Amsterdam (2016), Ch. 58, pp. 825 – 837.

2. *WHO, Traditional Medicine Strategy 2002–2005*, World Health Organization, Geneva (2002).
3. H. S. Kim, B. –H. Seo, J.-S. Bae, et al., *Ecotoxicol. Environ. Saf.*, **131**, 89 – 95 (2016).
4. L. Luo, B. Wang, J. Jiang, et al., *Front. Pharmacol*., **11**, 595335 (2021).
5. Y.-G. Chen, X. S. He, J. H. Huang, et al., *Ecotoxicol. Environ. Saf.*, **219**, 112336 (2021).
6. *Rulebook on the Quality of Tea, Herbal Tea and Their Products* [in Serbian], Official Gazette of RS, 4 / 2012, Republic of Serbia (2012).
7. J. Ruan and M. H. Wong, *Environ. Geochem. Health*, **23**(1), 53 – 63 (2001).
8. A. Mehra and C. L. Baker, *Food Chem*., **100**(4), 1456 – 1463 (2007).
9. S. Ražić and V. Kuntić, *Int. J. Food Prop*., **16**(1), 1 – 8 (2013).
10. M. Xie, A. von Bohlen, R. Klockenkämper, et al., *Z. Lebensm. Unters. Forsch.,* **207**, 31 – 38 (1998).
11. M. J. Gibney, H. V. Vorster and F. J. Kok, *Introduction to Human Nutrition*, Wiley-Blackwell, Oxford, United Kingdom (2009).
12. V. Daničić, *Vitaminologija, Sve o Vitaminima i Mineralima* [in Serbian], Tarifa, Beograd (2012).
13. *WHO, Trace Elements in Human Nutrition and Health*, World Health Organization, Geneva (1996).
14. P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, et al., *Exp Suppl*., **101**, 133 – 164 (2012).
15. D. A. Schuschke, J. T. Saari, F. N. Miller, et al., *J. Trace Elem. Exp. Med*., **9**, 63 – 72 (1996).
16. A. A. Perić-Grujić, V. V. Pocajt and M. Đ. Ristić, *Hem. Ind.*, **63**(5), 433 – 436 (2009).
17. R. Sovljanski, S. Lazic, V. Macko, et al., *Herba Hung.*, **29**(3), 59 – 65 (1990).
18. A. Szymczycha-Madeja, M. Welna and P. Pohl, *TrAC Trends Anal. Chem.*, **35**, 165 – 181, (2012).
19. V. Angelova, K. Ivanov and R. Ivanova, *J. Herbs Spices Med. Plants*, **11**(4), 37 (2005).
20. J. D. Crowley, D. A. Traynor and D. C. Weatherburn, *Met Ions Biol. Syst.*, **37**, 209 – 278 (2000).
21. S. Dey, A. Saxena, A. Dan, et al., *Arch. Environ. Occup. Health*, **64**(3), 164 – 167 (2009).
22. M. Z. Kirmani, S. Mohiuddin, F. Naz, et al., *J. Basic Appl. Sci.*, **7**(2), 89 – 95 (2011).
23. S. Martin and W. Griswold, *Environmental Science and Technology Briefs from Citizens*, Centre for Hazardous Substance Research, Kansas State University, Manhattan, 15, 1 – 6 (2009).
24. R. Ulla, J. A. Khader, I. Hussain, et al., *Afr. J. Pharm. Pharmacol*., **6**(25), 1829 – 1832 (2012).
25. N. C. Brady and R. Weilm, *The Nature and Properties of Soils*, Prentice Hall, New Jersey (1999) pp. 734 – 740.
26. C. D. Klaassen, L. J. Casarett and J. Doull, *Casarett and Doull's Toxicology: Basic Science of Poisons*, McGraw-Hill, New York (2001).
27. K. P. Singh, S. Bhattacharya and P. Sharma, *Am. Eurasian J. Agric. Environ. Sci*., **14**, 1125 – 1129 (2014).
28. M. M. O?zcan, A. U?nver, T. Uc?ar, et al., *Food Chem*., **106**, 1120 – 1127 (2008).
29. J. Malik, J. Szakova, O. Drabek, et al., *Food Chem*., **111**, 520 – 525 (2008).
30. S. Başgel and B. S. Erdemoğlu, *Sci. Total Environ*., **359**, 82 – 89 (2006).
31. N. Blagojević, B. Damjanović-Vratnica, V. Vukašinović-Pešić, et al., *Pol. J. Environ. Stud*., **18**(2), 167 – 173 (2009).
32. P. Pohl and B. Prusisz, *Food Chem*., **102**, 1415 – 1424 (2007).
33. N. S. Mokgalaka, R. I. McCrindle and B. M. Botha, *J. Anal. At. Spectrom*, **19**, 1375 – 1378 (2004).
34. S. Ražić, A. Onjia and B. Potkonjak, *J. Pharm. Biomed. Anal.*, **33**, 845 – 850 (2003).
35. *The European Pharmacopoeia, 10th edition*, Council of Europe, Strasbourg (2019).
36. *The European Pharmacopoeia, 3rd edition*, Council of Europe, Strasbourg (1996).
37. *Official Methods of Analysis of AOAC International*, 17th Ed. Official Method 999.11. Gaithersburg, MD, USA: AOAC International (2000).
38. J. Zhang, R. Yang, R. Chen, et al., *Int. J. Environ. Res. Public Health*, **15**(1), 133 – 155 (2018).
39. United States Environmental Protection Agency, Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document; EPA / 600 / R-06 / 013F, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH, USA (2007).
40. United States Environmental Protection Agency, Handbook for Non-Cancer Health Effects Evaluation. US Environmental Protection Agency, Washington (DC), (2000).
41. M. Harmanescu, L. M. Alda, D. M. Bordean, et al., *I. Chem. Cent. J.,* **2011** (5), 64 – 74 (2011).
42. J. Mehjbeen and U. Nazura, *SpringerPlus*, **5**, 776 – 784 (2016).
43. F. M. Kusin, N. N. M. Azani, S. N. M. Hasan, et al., *Catena,* **165**, 454 – 464 (2018).
44. FDA (Food and Drug Administration): Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Report of the Panel on Micronutrients. National AcademyPress, Washington, DC, Food and Drug Administration. Dietary supplements. Center for Food Safety and Applied Nutrition (2001).
45. F. Adusei-Mensah, D. K. Essumang, R. O. Agjei, et al., *J. Environ. Health Sci. Eng*., **17**(2), 609 – 618 (2019).
46. P. F. S. Tschinkel, E. S. P. Melo, H. S. Pereira, et al., *Biomed. Res. Int*., 1465051 (2020).
47. WHO*, Quality Control Methods for Herbal Materials*, World Health Organization, Geneva, (2011).
48. P. Konieczyñski, M. Weso³owski and P. Rafalski, *Herba Pol.,* **55**, 27 – 33 (2007).
49. G. J. Fosmire, *Am. J. Clin. Nutr.*, **51**(2), 225 – 227 (1990).
50. M. Valko, H. Morris, and M. T. D. Cronin, *Curr. Med. Chem***.**, **12**(10), 1161 – 1208 (2005).
51. WHO*, Quality Control Methods for Medicinal Plant Materials*, World Health Organization, Geneva, (1998).
52. A. Marcos, A. Fisher, G. Rea, et al., *J. Anal. At. Spectrom*., **13**, 521 – 525 (1998).
53. L. L. Nkuba and K. N. Mohammed, *Chem. Sci. Int. J.*, **19**(2), 1 – 11 (2017).
54. Z. Mihaljev, M. Zivkov-Balos, Z. Cupić, et al., *Acta Pol. Pharm*., **71**(3), 385 – 391 (2014).
55. N. J. Krstić, Author's Abstract of Cand. Sci. (Chem.) Disertation, [in Serbian], Niš (2017).
56. L. M. de Oliveira, S. Das, E. B. da Silva, et al., *Sci. Total. Environ*., **633**, 649 – 657 (2018).
57. S. S. L-Oud, *Pak. J. Biol. Sci,* **6** (3), 208 – 212 (2003).
58. S. S. Ranđelović, D. A. Kostić, A. R. Zarubica, et al., *Hem. Ind*., **67**(4), 585 – 591 (2013).
59. US Environmental Protection Agency, 2002. Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites, OSWER 9355. Office of Emergency and Remedial Response, Washington (2002).