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INVESTIGATION OF HEAVY METALS CONTENT IN SELECTED TEA BRANDS MARKETED IN PODGORICA, MONTENEGRO

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ABSTRACT: Medicinal plants are sources of different chemical substances with different biological activities in living organism. Some of the chemical compounds so-called "heavy metals", can be dangerous because they tend to bioaccumulate and transported in all biota via the food chain. The aim of our study was to investigate the concentration of heavy metals in commercially available tea samples purchased in markets in Podgorica, Montenegro state. The samples preparation has been performed using microwave digestion. The content of metals concentrations was determined by ICP-OES, while the mercury content is determined by DMA 80 and Perkin-Elmer Analyst 300. Results obtained in the present study showed that analysed samples of the investigated medicinal plants contain heavy metals namely copper, cobalt, chromium, iron, manganese, nickel, and zinc that are considered essential elements; and arsenic, cadmium, lead and mercury which are classified as highly toxic metals. The concentration ($mg\ kg^{-1}$) of heavy metals in all the tea samples was found to be as follows: arsenic (0.0107 to 0.2495), cadmium (0.001 to 0.01), copper (9.18 to 38.44), cobalt (0.005 to 0.032), chromium (0.007 to 0.089), iron (69.36 to 741.55), manganese (20.08 to 1521.15), mercury (0.0035 to 0.0177), nickel (1.32 to 10.68), lead (0.22 to 2.24) and zinc (16.44 to 30.04). Significant variation exists in the concentration of heavy metals in tea samples. Overall, the results of this study give current insights into the metal levels in analysed samples of teas which are commonly consumed in Podgorica. The variations in metals concentrations of the commercially available samples were attributed to several factors.

INTRODUCTION: The use of herbs for medicinal purposes is as old as the humanity itself. The importance of medicinal plants from the beginning until today is great, which covers both conventional and traditional approaches. The knowledge that flora was a laboratory in which was produced a number of pharmacologically active compounds.

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Isolation of the chemical compounds and modification in their chemical structure was produced a large number of drugs for human use [1]. Moreover, development of pharmacognosy, phytochemistry and organic chemistry until now was isolated and identified nearly to 20,000 biologically active compounds [2].

An increasing number of side effects, contraindications and inadequate therapeutic response with synthetic drugs therapy, affect to the reusing of current herbal drugs [3]. According to the World Health Organization (WHO), almost 70-80% of the world's population relies on alternative medicine [4].

Also, growing interest in the therapeutic benefits of herbal products, there is a concern in terms of safety and toxicity of the herbal substance and herbal preparations. Recorded cases of poisoning and undesirable effects associated with the use of herbal substances and herbal preparations indicate in the misconception about the statement that herbs are absolutely safe for human use [5]. The toxicity of plants can be from metals, pesticides, microorganisms and other chemical agents, as well as the conditions during storage and transport of herbal products [6].

The greatest uses of herbal drugs are commonly prepared by infusion, which is also called "tea". Furthermore, tea is the second most popular and consumed beverage in the world, right after water. In fact, the consumption of tea has a long history. Analysis of the survey in the United Kingdom showed that 77% of the population consumes this type of beverage, with the average consumption of 2.3 cups (540 ml) per day [7]. In addition to considering that this type of beverages are usually consumed in order to improve health benefits, in the last times, these preparations were controlled by the content of metals in analysed samples [8].

Heavy metals defined as those metals which have the relative atomic weight greater than element sodium (Na), and density of the elements greater than 5 g cm^{-3}. Moreover, all these metals have an atomic number greater than 20. Therefore, the determination of heavy metals depends on the atomic number, atomic weight, density and position in the periodic table [9]. Heavy metals can be classified into four major groups based on their health importance. Essential metals are manganese (Mn), chromium (Cr), copper (Cu), cobalt (Co), iron (Fe) and zinc (Zn), also called micronutrients. Non-essential: aluminium (Al), barium (Ba), lithium (Li) and zirconium (Zr). Less toxic: aluminium (Al) and tin (Sn). Highly toxic: cadmium (Cd), lead (Pb) and mercury (Hg) [10]. The content of highly toxic metals such as arsenic, cadmium, lead and mercury, may cause undesirable effects, also in very low concentrations, because of the low rate of excretion by the kidneys.

According to the "Regulations on permitted amounts of heavy metals, mycotoxins and other substances in food" (Official Gazette of the Republic of Montenegro, No 55/215), some essential metals such as copper, cobalt, chromium, iron, manganese and zinc, represented in the permitted amounts can be essential for the physiological functions of the human body. Excess intake of these metals above levels recommended might cause toxic effects [8, 11].

The aim of our study was to determine the quality of commercially available samples of different tea brands collected from different markets. Dried plant material was used for determining the content of heavy metals in analysed samples. The analysed metals and obtained results were compared with both World Health Organization and available scientific literature to provide researchers and tea consumers information about the content of heavy metals in different tea samples in Podgorica, the capital city of Montenegro.

MATERIAL AND METHODS:

Samples: Twelve tea leaves samples of different plants were purchased from three different markets in Podgorica, Montenegro state. The most citizens of Podgorica, purchase their herbs from these three shops. The most popular tea samples were randomly collected. Of all twelve tea leaves samples, three samples were of green and three of black tea from the same plant species (*Theae folium*, *Thea sinensis* L., *Theaceae*), three of chamomile tea (*Chamomillae flos*, *Chamomilla recutita* (L.) Rausch., *Asteraceae*) and three of mint tea (*Menthae piperitae folium*, *Mentha* × *piperita* L., *Lamiaceae*).

Herb samples preparation and metal analysis: The concentration levels of metals, As, Cd, Cu, Co, Cr, Fe, Mn, Hg, Ni, Pb and Zn in tea leaves samples were evaluated in this study. Samples were crushed, homogenised and sieved to 2mm and 1.0±0.01 g were measured for analysis, after drying at room temperature. Microwave acid digestion was used for sample preparation with ETHOS 1 in accordance with manufacturer's instruction (5ml of HNO_3 + 2ml of H_2O_2). After digestion metals concentration (As, Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb and Zn) was determinated by Inducted Coupled Plasma-Optical Emission Spectrometry (ICP/OES) (AMETEC-Spectro Arcos, Germany). The content of Hg was determined by direct mercury analyser (DMA 80) in dried tea leaves samples and as by hydride technique on Perkin Elmer A Analyst 300, after microwave digestion. As a guideline, we used the data from the scientific literature that defined the normal, toxic and permissible limits of the heavy metals in the medicinal plants (**Table 1**).

Reagents and standards: Analytical grades chemicals were used through the study. All used chemical reagents were of analytical grade. There was no further purification of chemicals which we used for the preparation of all reagents and calibration standards. Deionised ultrapure water was used with conductivity < 1 µS cm^{-1}. The mix of As, Cd, Cu, Co, Cr, Fe, Mn, Hg, Ni, Pb and Zn working analytical solutions were prepared after serial dilution of stock reference solution containing 1000 mg L^{-1} of each element (LGC-ICP-OES stock solution) in a gradient as needed. Certified reference material National Institute of Standards and Technology (NIST) standard reference material (SRM) tomato leaves 1573a was used for checking the obtained data by determination of accuracy and precision. The recovery of all metals As, Cd, Cu, Co, Cr, Fe, Mn, Hg, Ni, Pb and Zn ranged between 91-110 %. Precision as a relative standard deviation from triplicate measurement was less than 5 % for all investigated elements.

TABLE 1: TYPES OF HEAVY METALS AND THEIR NORMAL, TOXIC AND PERMISSIBLE LIMITS IN PLANTS (mg kg^{-1})

Heavy metals in plants (mg kg^{-1})					
Element	**Normal concentration**	**Toxic concentration**	**Ref.***	**Permissible limit**	**Ref.***
As	10-60**	<2	12	1	13
Cd	-	-	-	0.3	13,14
Cu	3-15	20	12	10	15,16
Co	0.05-0.5	30-40	12	1.5	17
Cr	<0.1-1	2	12	1.3	15
Fe	50-250	>500	12	450	15
Mn	15-100	400	12	200	18
Hg	<0.1-0.5	5	12	0.2	19
Ni	0.1-5	30	12	10	15
Pb	1-5	20	12	10	13,14
Zn	15-150	200	12	50	15,16

*: Ref. – Reference; **: µg kg^{-1}.

RESULTS AND DISCUSSION: Twelve varieties of tea samples were purchased from the three markets in Podgorica, the capital and biggest city in Montenegro. The most citizens of Podgorica, purchase their herbs from these three shops.

The results analysis of heavy metals such as As, Cd, Cu, Co, Cr, Fe, Mn, Hg, Ni, Pb and Zn present in different tea brands is important because some of them are toxic and can be transported into the human body via the food chain. Heavy metals may cause a number of problems in the long run.

Table 2 shows the concentration of the highly toxic metals in all analysed tea samples in our study. An examination of the data from **Table 2** shows that different tea samples in our study contain the elements such as As, Cd, Hg and Pb in various proportions. Therefore, the concentration of As, Cd, Hg and Pb were not detected above the permissible limit or their contents were very low. It can be concluded that all analysed samples of tea are not harmful and that they are safe for human consumption from the aspect of the content of highly toxic metals.

TABLE 2: THE CONCENTRATION OF THE HIGHLY TOXIC METALS IN ALL ANALYSED TEA SAMPLES (mg kg^{-1})

No*	TS**	M***	As	Cd	Hg	Pb
12	BT	V	0.0930	0.004	0.0045	2.06
11	CT	V	0.0770	0.002	0.0075	0.58
10	MT	V	0.2495	0.002	0.0135	0.92
9	GT	V	0.0317	0.001	0.0054	0.48
8	CT	K	0.0352	0.01	0.0069	0.22
7	MT	K	0.1209	<0.01	0.0177	0.76
6	BT	K	0.0107	0.001	0.0061	0.38
5	GT	K	0.0774	0.002	0.0053	1.42

4	BT	L	0.1149	0.003	0.0045	2.24
3	CT	L	0.0410	0.006	0.0035	0.26
2	MT	L	0.0987	0.001	0.0174	0.56
1	GT	L	0.0790	0.003	0.0084	1.44

*: No – Number;
**: TS – Tea samples (GT – Green tea; BT – Black tea; CT – Chamomile tea; MT – Mint tea);
***: M – Markets (L, K, V – Different markets in Podgorica).

Furthermore, **Table 3** displays the data on the concentration of available essential metals in all analysed tea samples. The concentrations of the analysed essential metals were within the allowed limits for elements like Co, Cr and Zn; while the concentration of Cu, Fe, Mn and Ni was recorded above the permissible limit according to the WHO. The high concentration of Cu, Fe, Mn and Ni in most tea samples probably depends on many factors responsible for variations in heavy metals contents in medicinal plants for human consumption.

TABLE 3: THE CONCENTRATION OF THE ESSENTIAL METALS IN ALL ANALYSED TEA SAMPLES (mg kg^{-1})

No*	TS**	M***	Cu	Co	Cr	Fe	Mn	Ni	Zn
12	BT	V	14.90	0.020	0.016	161.59	1521.15	5.38	21.72
11	CT	V	13.94	0.018	0.077	491.52	37.35	2.14	30.04
10	MT	V	11.98	0.021	0.079	741.55	80.83	1.70	19.06
9	GT	V	17.18	0.010	0.012	70.90	483.79	2.92	16.91
8	CT	K	9.18	0.007	0.007	74.42	33.06	2.10	22.36
7	MT	K	11.32	0.014	0.051	539.23	89.02	1.32	16.72
6	BT	K	15.06	0.01	0.007	69.36	458.91	2.26	16.44
5	GT	K	14.96	0.012	0.012	123.01	972.11	3.80	19.64
4	BT	L	38.44	0.032	0.089	633.96	1137.00	10.68	27.14
3	CT	L	12.46	0.005	0.013	92.70	20.08	1.54	29.10
2	MT	L	15.00	0.014	0.039	346.02	66.26	2.18	20.78
1	GT	L	13.36	0.022	0.020	230.02	1177.36	3.00	17.70

*: No – Number;
**: TS – Tea samples (GT – Green tea; BT – Black tea; CT – Chamomile tea; MT – Mint tea);
***: M – Markets (L, K, V – Different markets in Podgorica).

Toxic concentration of essential – heavy metals was found in most of the teas samples in our study. It can be seen from the above **Table 3.** Some tea samples are considered unsafe, for example, Sample-4 of black tea, purchased in the shop marked as “L”, shown the concentration of Cu, Fe, Mn and Ni, above the maximum permissible limit according to the WHO **Fig. 1**.

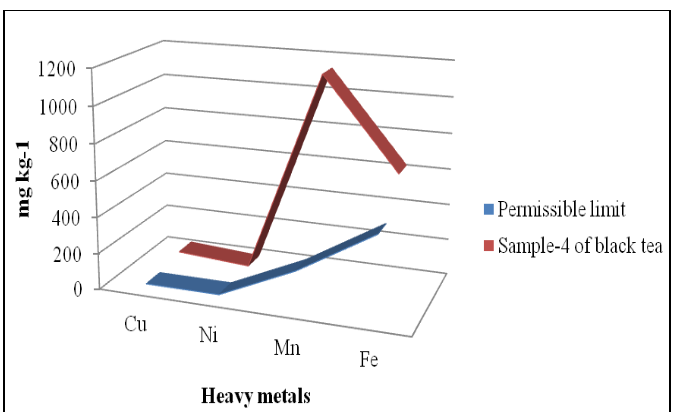

FIG. 1: CONTENT OF HEAVY METALS IN SAMPLE-4 OF BLACK TEA, mg kg-1.

Metals in plants:

Arsenic, As: Arsenic is widely common metalloid, very often classified as heavy metals. The general populations have exposure to arsenic compounds, mainly via intake of drinking water and food. It can be in two chemical forms such as organic or inorganic. Inorganic arsenic compounds are more toxic than organic arsenic compounds. The inorganic form of arsenic may cause cancers of the bladder, lungs, liver and skin. At lower levels of exposure, arsenic can cause vomiting and nausea. Also, severe disturbances of the central nervous and cardiovascular systems, and eventually death [20, 21].

According to the WHO, the maximum permissible limit of arsenic in plants is established as 1 mg kg^{-1} [13]. In all the collected plant samples, the concentration of arsenic was recorded in the range from 0.0107 to 0.2495 mg kg^{-1}.

Comparing the obtained results with the permissible limit, arsenic was in values which correspond with literature data.

Cadmium, Cd: Cadmium is known as one of the most toxic heavy metals. In fact, cadmium is a nonessential element for humans or plants. Also, cadmium can easily cause toxic effects on humans at low concentrations. It is classified as a human carcinogen. At very high levels, cadmium can cause vomiting and diarrhea. The effects of exposure to long-term at lower levels may cause toxic effects on the kidney, the respiratory and the skeletal systems [12, 21, 22]. According to the WHO, the permissible limit of cadmium in plants is 0.3 mg kg^{-1} [13, 14]. In all the tea samples in our study, the concentration of cadmium was recorded in the range from 0.001 to 0.01 mg kg^{-1}.

Comparing the obtained results with the permissible limit, cadmium was in values which correspond to the WHO requirements.

Copper, Cu: Copper is considered to be an essential element for every living organism. Although, copper is essential for normal growth and development of plants. It can be toxic to the high level of concentration. An excess of copper in humans can cause problems with skin, hair and nails. Toxicity of copper is a fundamental cause of chronic anaemia and Wilson's disease. In the human body, copper accumulates in the brain and liver [16, 23, 24]. According to the WHO, the permissible limit of copper in plants is 10 mg kg^{-1} [15, 16]. The concentration of copper in all the tea samples in our study was ranged between 9.18 to 38.44 mg kg^{-1}. In eleven of the twelve samples of tea samples, the concentration of copper was above the permissible limit. In Sample-4 of black tea, its concentration was up to 38.44 mg kg^{-1}. Although, copper is an essential element for all biota, however, it can be toxic if its concentration is higher than 20 mg kg^{-1} in plant material [24].

Cobalt, Co: Cobalt is considered to be an essential element necessary for the formation of vitamin B_{12}. The general population are exposed to cobalt primarily via diet. Exposure to this element alone produces an occupational asthma and skin reactions to patients [25]. On the other hand, trace amounts of cobalt are necessary for the normal activity of human body [12]. According to the WHO, the maximum permissible limit of cobalt is 1.5 mg kg^{-1} [17]. The concentration of cobalt in all the tea samples in our study was ranged from 0.005 to 0.032 mg kg^{-1}. By comparing results from our study with the maximum permissible limit in plants, cobalt was in values which correspond to the literature data.

Chromium, Cr: Chromium is an essential element involved in the normal metabolism of carbohydrate and lipid in humans. Furthermore, chromium is present in all plants, but it is unknown whether chromium is an important element for plants [26]. For the general population, chromium is particularly important as a trivalent chromium while the other forms of chromium are toxic and without of function in the body [24]. According to the WHO, the permissible limit of chromium in plants is 1.3 mg kg^{-1} [15, 16]. The value of chromium in all tea samples in our study was ranged between 0.007 to 0.089 mg kg^{-1}. In all the analysed samples concentration of chromium was recorded below the permissible limit set by WHO.

Iron, Fe: Iron is another essential element in human nutrition. Iron is present in two states of oxidation in nature as a divalent – ferrous ions and trivalent – ferric ions [27]. Overall, it is an essential trace element for humans, animals and plants [24]. Deficiency of iron in humans can cause problems with metabolism. The excess levels of iron in humans (>10 mg kg^{-1}) cause a rapid increase in coagulation of blood in blood vessels and pulse rate, hypertension and drowsiness [16, 24]. According to the WHO, the recommended level of iron in plants is 450 mg kg^{-1} [15]. The concentration of iron in analysed tea samples in our study was ranged from 69.36 to 741.55 mg kg^{-1}. In our study, the concentration of iron was recorded above the permissible limit in 4 tea samples, which was approximately one-third of all analysed samples.

Manganese, Mn: Manganese is an essential metal nutrient for humans and other living organisms. Moreover, manganese is normally present in many kinds of foods and deficiency of manganese in humans appears very rare. The population of tea drinkers may have an increased intake of manganese than the general population because tea is an important source of manganese. Side effects of manganese can be from both overexposure or deficiency of this element. It can cause neurotoxic side effects when exceeding the homeostatic range [28, 29]. According to Shah et al., the permissible limit of manganese in medicinal plants is 200 mg kg^{-1} [18]. The value of manganese in all tea samples in our study was ranged between 20.08 to 1521.15 mg kg^{-1}. In our study, the concentration of manganese was recorded above the permissible limit in 6 tea samples (50%).

Mercury, Hg: Mercury is one of the toxic – heavy metal with an unknown role in living organisms. In humans or animals, exposure to high level of mercury can cause toxic effects. Commonly reported side effects of mercury exposure can cause: anaemia, cardiovascular disease, neurobehavioral disorders, developmental abnormalities, cancer and damage to liver and kidney [30].

Additionally, the effects of high levels of mercury in humans above the allowable values have been associated with brain damage, inhibition of endogenous antioxidant enzymes, and male infertility [19]. According to Annan et al., the permissible values of mercury in medicinal plants are 0.2 µg g^{-1} [19]. The levels of mercury in all the tea samples in our study were ranged from 0.0035 to 0.0177 mg kg^{-1}. Overall, all values were below the permissible values of mercury.

Nickel, Ni: Nickel is another essential trace element with least several roles in animal species. It is not a cumulative toxin in humans or in animals but it can induce toxicity and carcinogenicity [31]. Nickel is known as the immunotoxic, hematotoxic, genotoxic, reproductive toxic, neurotoxic, nephrotoxic, hepatotoxic, pulmonary toxic and carcinogenic agent [32]. Also, it is the most common cause of metal allergy which is manifested with skin problems more often in women than men [33]. According to the WHO, the permissible limit of nickel in plants is 10 mg kg^{-1} [15, 16]. The concentration of nickel in all tea samples in our study was ranged between 1.32 to 10.68 mg kg^{-1}. In one of the tea; Sample-4 of black tea, the concentration of nickel was recorded above the permissible limit set by WHO.

Lead, Pb: Lead is highly toxic metal with the potential for causing irreversible health effects in humans. The biological function of lead in the body is still not known. In general, ingestion of lead can cause both acute and chronic toxicity. Nevertheless, lead can affect to almost of all the major organ systems of the body like renal, hematopoietic, nervous and cardiovascular systems [34]. According to the WHO, the permissible limit of lead in plants is 10 mg kg^{-1} [13, 14]. The concentration of lead in all tea samples in our study was ranged between 0.22 to 2.24 mg kg^{-1}. In all analysed samples in our study, the concentration of lead was recorded below the permissible limit according to the WHO.

Zinc, Zn: Zinc is a very important element for plants, animals and humans [35]. Deficiency in zinc affects many organ systems in humans, including the gastrointestinal, immune, integumentary, central nervous system, reproductive and skeletal systems [36]. Zinc is considered as a relatively non-toxic metal, but with extremely high-level of intakes can cause nausea, vomiting, fatigue, lethargy, and epigastric pain [37]. According to the WHO, the permissible limit of zinc in plants is 50 mg kg^{-1} [15, 16]. The concentration of zinc in all tea samples in our study was ranged between 16.44 to 30.04 mg kg^{-1}. In all the tea samples concentration of zinc was recorded below the permissible limit according to the WHO.

CONCLUSION: Overall, the results of this study give current insights into the metal levels in analysed samples of teas which are commonly consumed in Podgorica. The variations in the content of elements from sample to sample can be attributed to the differences in the botanical structure and mineral composition of the soil on which the plants are grown. Other factors responsible for variations in metals contents may be attributed to seasonal changes, geographical changes, chemical characteristics of the growing regions and cross-contamination during processing, packaging and storage these types of products. Therefore, it is very important that every medicinal plant should be checked for the concentration of heavy metals before of its processing for further pharmaceutical purposes or for human consumption.

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