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SELECTED PARAMETERS OF QUALITY AND SAFETY OF HERBAL TEA

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ABSTRACT

The aim of this work was to assess the heavy metal presence and possible microbiological contamination in herbal teas. Evaluation of selected tea products was performed from Nitra locality during years 2009 – 2013. Microscopic filamentous fungi detection, bacteria such as *Escherichia coli* and *Salmonella* spp. were compared to requirements given in the Codex Alimentarius of Slovakia. The highest permissible limit for microscopic filamentous fungi was not exceeded (in 32 observed herbal tea samples). For incidence of *Escherichia coli,* 93 samples were investigated and for *Salmonella* spp.*,* 91 herbal tea samples. No sample showed the presence of *Salmonella* spp., and at *E. coli* maximum permitted presence was detected below limit. Among chemical parameters, cadmium, lead and mercury content were monitored. The highest amount of lead and mercury was found in year 2012. In 2009, the highest cadmium content was found. The average content of lead in all 100 inspected herbal tea samples was 0.784 $mg.kg^{-1}$ so all the samples met requirements defined in the legislation. The mean content of mercury (98 investigated herbal tea samples) was 0.0161 $mg.kg^{-1}$ so all samples met the requirements as well. Average cadmium content was 0.1702 $mg.kg^{-1}$ while the highest permitted limit for cadmium is 1.0 $mg.kg^{-1}$. All herbal tea samples were in accordance with the legislation except one (white willow bark tea) with a very high content of cadmium (4.36 $mg.kg^{-1}$).

Keywords: tea; cadmium; mercury; lead; microbiological quality

INTRODUCTION

The tea is a product of plant origin, which is intended for preparing a beverage or prepared from such product. It is necessary to meet the legislative requirements for the production and import of tea, tea extracts and preparations thereof, for handling them and placing them on the market as is defined by the legislation. The composition of tea varies by variety, season, leaf age, climate and practices at harvest. Its chemical composition is a complex including carbohydrates, amino acids, proteins, alkaloids (caffeine, theophylline and theobromine), volatile compounds, polyphenols, minerals and trace elements **(Bansal et al., 2013)**.

The results of several studies show potential health benefits of tea, due to the consumption of tea. Multiple benefits are described, such as prevention of cancer and heart disease **(Sang et al., 2011)**.

However, known are risks associated with the microbial contamination and subsequent adverse events. The reasons of these adverse effects are common microbial contaminants that produce toxins and it is therefore important to identify them in herbal teas **(Omogbai and Ikenebomeh, 2013)**.

Biological contamination of medicinal herbs, their preparations and products may include living organisms such as bacteria and their spores, yeasts, microscopic filamentous fungi, viruses, protozoa, insects and other organisms. There are known also chemical contaminants, which are the products of microbial metabolism, toxic metabolites from microscopic filamentous fungi **(Koslaec et al., 2010)**.

Microscopic filamentous fungi are prevalent contaminants of herbs; most of their microbial population survive the drying and storage **(Gonzaga and Bauab, 2012)**.

Omogbai and Ikenebomeh (2013) at analyzing the microbiological quality of various herbal teas isolated bacteria identified as *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae, Serratia marcencens, Salmonella typhimurium, Pseudomonas fluorescens and Escherichia coli.* Among microscopic filamentous fungi they identified *Aspergillus niger, Aspergillus flavus, Penicilium expansum, Rhizopus stolonifer* and *Fusarium solani.*

Exceeding the threshold of microbial contamination is the most common reason to reject herbal plant material from growers **(Heindl and Müller, 2006)**.

Keeping good manufacturing and hygiene practices with the application of HACCP at cultivation, harvesting and processing of material is essential. To reduce contamination can be used various decontamination procedures **(Zweifel and Stephan, 2012)**. Between years 2003 and 2004 in Germany children received *Salmonella enterica* serotype Agona because of the herbal tea with aniseed from Turkey contained species.

Since it is a natural product, all parts of the plants can be contaminated with bacteria and microscopic filamentous fungi. Improper methods of cultivation and storage, improper collection and treatment, inadequate transportation, long-term drying and storage, poor hygiene at producing and natural climatic conditions cause that the raw plant materials are susceptible to microbial contaminants. Materials are often degraded by microorganisms prior to harvesting, handling, and after prolonged storage **(Stevic et al., 2012)**.

Storage in high humidity can promote growth of microscopic filamentous fungi. Storage of the tea requires a dry, cool, dark and inert spaces **(Mishra et al., 2006)**.

Heavy metals are a group of harmful inorganic chemical risks. Heavy metal pollution can be of anthropogenic origin, of natural origin and they can accumulate in the soil. It may be contaminated through emissions from emerging industrial areas of the landfill metal, leaded gasoline and paint, mine tailings, sewage sludge, pesticides, fertilizer use, irrigation wastewater, coal combustion, from petrochemicals, and atmospheric deposition **(Nagajyoti et al., 2010; Wuana and Okieimen, 2011)**. Those which are most commonly found in high concentrations in the contaminated areas are chromium, copper, zinc, cadmium, mercury and lead **(Cao et al., 2011)**.

Lead, cadmium and mercury are toxic substances of great interest for fear of danger posed. This is mainly a result of their environmental stability and potential bioaccumulation. These chemicals are widespread through the ecosystem and cause problems for all life forms. Different plants have the possibility to accumulate these contaminants when grown under natural conditions and are often used to assess environmental contamination **(Storelli, 2014)**.

The aim of this study was to evaluate quality parameters in different herbal teas available on the market in Nitra region, focused on assessment of possible microbiological contamination of samples and analysis of selected heavy metals content such as lead, cadmium and mercury.

MATERIAL AND METHODOLOGY

Chemical and microbiological parameters of herbal teas were evaluated under the requirements of current legislation, in cooperation with the Regional Veterinary and Food Administration in Nitra, which is responsible for the safety control of these products. For the quality of selected herbal teas are responsible manufacturers, importers and sellers of teas. Sampling was performed during the period 2009 – 2013, for the control of microbiological and chemical parameters of the herbal teas were selected 100 samples of tea placed on the Slovak market by known producers (Table 1).

Investigated samples originated from different kinds of herbal teas and their mixtures (plantain, agrimony, sage, anise, tea with smartweed and ladders, tea for women, urological tea, tea for intestines, herbal tea, detox tea, tea for kidneys, virgin tea, prostate, stomach tea, herbal tea from Liquorice root, nettle leaves, elderberry, flower tea for nursing mothers, horsetail tea, cleansing tea, white willow (bark), ginkgo biloba, chicory, small-leaved lime and small-flowered, St. John's wort, Goldenrod plain, yellow-green lady's – tops, horsetail, Stinging Nettle – leaves, tea for blood pressure, *Acorus calamus*, lemon balm, fennel, heart tea, weight loss, *Plantago lanceolata*, valerian, gentian, heather ordinary, dandelion, *Centaurium erythraea*, mallow tea, hops common, yarrow, stinging nettle, marigold, herbal blend for breathing, chamomile, herbal tea for thyroid, *Polygonum aviculare* etc.), which in some years were repeated. Total amount of analyzed samples of herbal teas was 100 kinds.

Samplings were performed within the common controls, or preventive controls in Nitra region. Investigated samples originated from different kinds of herbal teas.

Monitored parameters of herbal teas were as follows:

Microbiological indicators determined by following methods:

- *Escherichia coli by* STN ISO 16649-2. A certain amount of initial suspension was inoculated in parallel on two media with tryptone, bile salts, glucuronide (TBX). In the same way was prepared by tens dilution initial suspension spread on two agar plates for each dilution. The plates were incubated for 18 – 24 hours at 44 °C, then the presence of colonies that on the basis of their characteristics were considered as β- glucuronidasepositive *Escherichia coli,* were assessed. CFU of β-glucuronidasepositive colony forming units of *Escherichia coli* in a millilitre of sample was determined then.
- Number of microscopic filamentous fungi by STN ISO 21527-2. Microscopic filamentous fungi were assessed by horizontal method for the enumeration of viable yeasts and xerophilous microscopic filamentous fungi by Colony-count technique at 25 °C ±1 °C cultivation for products having a water activity of less than 0.95, or equal to 0.95.
- *Salmonella spp.*by STN EN 6579-2. To prove the presence of *Salmonella spp.* the sample was cultivated in non-selective liquid medium, followed by propagation in selective liquid media, from which we spread them on the surface of solid agar selective media. Suspected colonies were confirmed by appropriate biochemical and serological tests.

As hygiene criteria were observed *Escherichia coli* and microscopic filamentous fungi which were evaluated by the *Codex Alimentarius* of the Slovak Republic (no. 06267/2006-SL, 06267/2006-SL) as well as *Salmonella* spp. as safety indicator (no. 06267/2006).

As heavy metal content for herbal teas were determined the content of lead, cadmium, mercury. These were determined by procedures given in the Slovak Technical Standards (STN 56 0076, STN 56 0065 a STN ISO 8288). Determination of lead and cadmium was performed by atomic absorption spectrometry with graphite cuvette in herbal teas and determination of mercury by device AMA 254 (Advanced Mercury Analyzer). The method of lead and cadmium determination is based on the quantification of mineralized samples in nitric acid. Metals present in solution are getting in to the atomic state and the specific optical density at a wavelength corresponding to the resonance line is proportional to the concentration of metal in the sample to be analyzed using a spectrometer. The results were expressed as the mass fraction of each metal in milligrams per kilogram of sample ($mg.kg^{-1}$). AMA 254

(Advanced Mercury Analyzer) is intended for direct determination of mercury in solid and liquid samples without the need of chemical pre-treatment.

Maximum levels for lead (10 $mg.kg^{-1}$), mercury (0.05 $mg.kg^{-1}$) and cadmium (1.0 $mg.kg^{-1}$) for herbal teas were evaluated by the *Codex Alimentarius* of the Slovak Republic (no. 18558/2006-SL; 608/3/2004-100).

RESULTS AND DISCUSSION

Herbal teas must meet requirements for maximum levels of microorganisms that are listed in the *Codex Alimentarius* of the Slovak Republic (no. 06267/2006-SL) and there are clearly defined contents for *Escherichia coli* as well. As safety criteria are given requirements for the absence of *Salmonella* spp. The maximum permitted levels were not exceeded in all our samples studied and thus meet the requirements and are suitable for use in the food industry.

There are some concerns about the safety of herbal preparations, a relatively high risk of contamination by pathogenic microorganisms, organic and inorganic residues, including heavy metals and non-metals, organic pollutants, mycotoxins, endotoxins and agrochemical residues **(Onyambu et al., 2013)**.

Evaluation of microbiological indicators in herbal teas

The results achieved in controlling the microbiological quality of herbal teas within the observed period (2009 – 2013) indicate that all samples of herbal teas meet the requirements for maximum levels of microorganisms that are listed in the *Codex Alimentarius* (no. 06267/2006-SL). No presence of *Salmonella* spp. was detected in accordance with the legislation, which demands zero its presence. The maximum permitted levels are not exceeded in all the samples studied and thus meet the requirements and are suitable for use in the food industry.

Indicator of *Escherichia coli* presence was evaluated in 93 samples, all samples complied with the legislation requirements, only three samples showed higher incidence of *E. coli* (0.2 x 10^2 $CFU.g^{-1}$; 0.2 x 10^2 $CFU.g^{-1}$; 2.3 × 10^2 $CFU.g^{-1}$. The highest amount was recorded in the "detoxification tea" (2.3 × 10^2 $CFU.g^{-1}$), while it also not exceeded the maximum permissible limit.

Permissible limits in the number of microscopic filamentous fungi were found, the highest amount was recorded in tea of *Equisetum arvense* (1.2 × 10^3 $CFU.g^{-1}$). All herbal teas also meet this requirement, and thus it can be concluded that all the samples comply with the legislation.

Similar issues were also tested by **Tournas and Katsoudas (2008),** who used 69 samples of various herbal tee. The teas such as peppermint, thyme, jasmine showed filamentous fungi contamination in 100% of their samples. For example, in tea of mint and papaya ranged from 1.0 x 10^3 $CFU.g^{-1}$ to 5.6 d 10^3 $CFU.g^{-1}$. The highest content of microscopical filamentous fungi was found in chamomile tea (5.8 x 10^5 $CFU.g^{-1}$) and the lowest in the tea of jasmine flowers (1.0 x 10^2 $CFU.g^{-1}$). The most common contaminants in herbal teas were: *Aspergillus niger, Penicillium* spp., *Eurotium rubrum, Eurotium chevalieri, Aspergillus flavus, Fusarium* spp., *Alternaria alternata* and yeasts. Contrary to these results, we did not determine any microbiological risk in observed samples from Nitra region.

Omogbai and Ikenebomeh (2013) isolated from 26 samples of herbal teas following bacteria and their percentage of occurrence in teas: *Staphylococcus aureus*

Table 1 Tea samples for microbiological and chemical examination.

Year	Number of samples	
	microbiological examination	**chemical examination**
2009	19	20
2010	10	17
2011	39	28
2012	13	13
2013	19	22

Table 2 The presence of microbiological indicators in samples of herbal teas for the monitored period (2009 – 2013).

year	*Salmonella* spp.		*Escherichia coli* $CFU.g^{-1}$		Microscopic filamentous fungi $CFU.g^{-1}$	
	max.	**min.**	**max.**	**min.**	**max.**	**min.**
2009	0 (no presence detected)		2.3 × 10^2	(< 10)	5.9 × 10^2	(< 10)
2010	0 (no presence detected)		(< 10)		1.2 × 10^2	0.5 × 10^2
2011	0 (no presence detected)		0.2 × 10^2	(< 10)	1.2 × 10^3	(< 10)
2012	0 (no presence detected)		(< 10)			(< 10)
2013	0 (no presence detected)		0.2 × 10^2	(< 10)		(< 10)

(90%), *Staphylococcus epidermidis* (60%), *Bacillus subtilis* (100%), *Pseudomonas aeruginosa* (40%), *Escherichia coli* (10%), *Klebsiella pneumoniae* (8%), *Serratia marcescens* (5 %), *Salmonella typhimurium* (3%), *Psudomonas fluorescens* (15%).

Koch et al. (2005) point out those factors such as the addition of sugar, storage temperature and time could affect the amount of *salmonella* at the time of consumption of tea.

Tournas and Katsoudas (2008) found by microbiological examination of herbal teas the number of microscopic filamentous fungi 5.8 × 10^5 CFU per gram of sample.

A lot of growers of medicinal plants do not have any adequate storage facilities (suitable relative humidity and circulation), and therefore there occur cross-contamination and the development of saprophytic and pathogenic microscopic filamentous fungi on medicinal herbal materials **(Stevic et al., 2012).**

Gonzaga and Bauaba (2012) deterioration of plant samples investigated that were stored for six to nine months after delivery. Some of the contaminated materials contained toxigenic strain of *A. flavus* and aflatoxin B1, which was higher than the permissible limit. In a study where the leaves were exposed to atmosphere with different relative humidity (75%, 45%, 0%), were found after 24 weeks chemical changes of important compounds. It was shown that microscopic filamentous fungi colonizing cause the largest loss of bioactive molecules of dried leaves by exposure with water.

Onyambu et al. (2013) in their study investigated unregistered herbal products for the presence of pathogenic bacterial contaminants. *E. coli*, which contaminated 75% of all samples is well known and is the most common enteropathogenic reason of childhood diarrhea of bacterial origin.

Evaluation of selected heavy metals in herbal teas

Heavy metals are a group of harmful inorganic chemical risks. Heavy metal pollution can be caused by anthropogenic origin, also of natural origin and can accumulate in the soil. It may be contaminated through emissions from emerging industrial areas, leaded gasoline and paint, mine tailings, sewage sludge, pesticides, fertilizer use, irrigation wastewater, coal combustion, from petrochemicals, or atmospheric deposition **(Nagajyoti et al., 2010, Wuana a Okieimen, 2011).** The most commonly found in high concentrations in contaminated areas are chromium, copper, zinc, cadmium, mercury and lead **(Cao et al., 2011)**.

In our work, we focused on determination of the chemical parameters, namely the determination of lead, cadmium and mercury in herbal teas and their mixtures for a period of five years. Totally 100 analyzed samples of herbal teas were observed.

The highest content of lead and mercury was measured in herbal teas in 2012 (tea for blood pressure). The highest cadmium content of herbal teas was found in 2009 (white willow - bark).

Ďurža (2003) states that the subjects of global monitoring are particular elements such as arsenic, cadmium, mercury and lead. These are generally considered as the most harmful to humans and animals.

Cadmium, lead and mercury are heavy metals, which have been identified in all environmental compartments. If we do not take into account the exposure by the inhalation route, food remains a major supply of heavy metals into living organisms; hence their monitoring in animal feed and human food is very important **(Golian et al., 2004)**.

Lead, cadmium and mercury are toxic substances of great interest for fear of danger posed. They are mainly a result of their environmental stability and potential for bioaccumulation. They are widespread throughout the ecosystem and cause problems for all life forms. Different plants accumulate these contaminants grown in natural conditions and are often used to assess environmental contamination **(Storelli, 2013)**.

By determination of lead content by AAS in herbal teas, we can see that during the analysed period all samples meet legislative requirements. Any sample of herbal teas does not exceed the maximum quantity for lead 10 mg.kg^{-1} (*Codex Alimentarius* of the Slovak Republic no. 18558/2006-SL; 608/3/2004-100). The mean lead content in individual years ranged from 0.476 to 1.08 mg.kg^{-1}. The highest lead content was measured in 2011 in the tea of nettle (3.69 mg.kg^{-1}).

In 2012, by the Public Health Authority of the Slovak Republic the content of lead was examined at 1001 food samples and in any of the examined samples was not detected exceeding limit for lead.

At analyzing the lead content of 650 food samples state **Golian et al. (2004)**, in tea samples has not been exceeded maximum approved content, the average amount of the 49 analyzed teas focused on lead content was 0.2217 mg.kg^{-1}.

At herbal teas that were during the period evaluated in

Table 3 The content of lead, mercury and cadmium in all samples of herbal teas for the monitored period (mg.kg^{-1}).

	Pb			Cd			Hg		
Maximum permitted limit	10 mg.kg^{-1}			1.0 mg.kg^{-1}			0.05 mg.kg^{-1}		
year	**max.**	**min.**	**mean**	**max.**	**min.**	**mean**	**max.**	**min.**	**mean**
2009	3.378	0.143	0.866	**4.36**	0.0055	0.31	0.0335	0.0065	0.0142
2010	0.485	0.399	0.476	0.257	0.0052	0.056	0.049	0.0085	0.0187
2011	3.6	0.083	0.85	0.45	0.00189	0.131	0.0181	0.0026	0.0147
2012	2.09	0.0193	1.08	0.347	0.0022	0.121	0.0344	0.0006	0.021
2013	2.06	0.022	0.65	0.77	0.0132	0.233	0.025	0.0006	0.012

relation to cadmium content is shown that all the samples analyzed, except for one sample in 2009 (white willow bark tea) comply with the requirements defined in legislation (*Codex Alimentarius* no. 18558/2006-SL; 608/3/2004-100) and cadmium content in them does not exceed the maximum amount which is set 1.0 $mg.kg^{-1}$.

In the sample of tea from white willow, cadmium content was measured 4.36 $mg.kg^{-1}$, thus it can be concluded that the maximum permissible quantity has been exceeded quadrupled. This sample is the only one exceeding the maximum level of all samples of tea over monitored period. The lowest cadmium content was determined in 2009 at tea intended for nursing mothers (0.0055 $mg.kg^{-1}$).

Bojňanská et al. (2002) investigated the cadmium content in different foods and among 61 samples exceeded the limit amount 11 of them, which is 16%. Tea showed cadmium content below the permissible limit (1.0 $mg.Cd.kg^{-1}$).

Besides white willow, higher content of cadmium was found in chamomile tea (0.74 $mg.kg^{-1}$) and (0.77 $mg.kg^{-1}$) at agrimony. The average content of cadmium, however, was 0.1702 $mg.kg^{-1}$, which is 83% less than the maximum level.

Golian et al. (2004) measured among 48 analyzed samples of tea, the average cadmium content 0.0899 $mg.kg^{-1}$. While we analyzed mostly herbal teas, they focused on herbal, fruit, black and green teas.

The Public Health Authority of the Slovak Republic states that for the content of cadmium were examined 942 food samples in 2012, and just one sample of food supplement did not comply with legislation requirements. For the presence of mercury were examined 874 food samples, 2 samples of one food supplement did not comply with legislation requirements. The company distributing the product adopted a measure - removed product from Slovakian market **(ÚVZ SR, 2012)**.

Cadmium is a common part of the plant and can be absorbed through the leaves and roots. Plants do not have excrete mechanism for cadmium. High concentrations of cadmium may contain plants growing near sources of contamination from industry. The leaves and roots of plants generally have higher cadmium content than seeds, although oil seeds are high in cadmium content. The daily dose of cadmium is usually around 10–25 $\mu g.kg^{-1}$ **(Egyűdová and Šturdík, 2004)**.

The highest mercury content was observed in tea of *Cetraria islandica* /L./ Ach. (0.0335 $mg.kg^{-1}$). Determination of mercury in selected herbal teas showed that in 2010, the highest content of mercury was measured (0.049 $mg.kg^{-1}$) in one herbal tea, which was almost at the level of maximum limit (0.05 $mg.kg^{-1}$). It can thus be concluded that all the samples analyzed did not exceed the maximum level. The lowest mercury content was detected in 2013 in the tea named "Tea for hyperacidity" (0.0006 $mg. kg^{-1}$).

Limmatvapirat et al. (2012) concluded that all samples of teas purchased in Thailand are not safe for human consumption, particularly in terms of heavy metals content. They determined high levels of copper, iron, lead, zinc, aluminum, manganese, nickel and only at low concentrations of arsenic, cadmium, chromium and mercury.

Of all the elements that enter the food chain and cause contamination of food, are considered as the most important arsenic, cadmium, mercury and lead. Where soils are enriched with these elements, it is usually caused by industrial, agricultural and municipal human activities. The tendency for plants to accumulate these xenobiotics depends largely on climatic factors and plant genotype. The bioavailability of contaminants in general also depends on their physico-chemical properties and composition of the diet. It is a strong link among micro-nutrients of plants, animals and humans and the absorption and action of contaminants in these organisms **(McLaughlin and Singh, 1999)**.

If the heavy metals are taken in higher quantities, they show to all the living organisms adverse effects. Toxicity of heavy metals has caused various serious diseases and also led to widespread deaths **(Tasleem et al., 2013)**.

Some heavy metals such as cadmium, mercury and arsenic are highly toxic to some enzymes, which may result in inhibition of growth or death of organisms **(Nagajyoti et al., 2010)**.

Good manufacturing practice shall provide reducing the amount of heavy metals in the food into amounts that minimizes the estimated health risks **(Egyűdová and Šturdík, 2004)**.

Analysis of various parts of plants showed that higher content of metals generally contain roots, then the leaves and stems and the least seeds, fruits, tubers and bulbs. Monitoring of heavy metals in plants is especially important in terms of contamination of the food chain. The intensity of contamination of plants with heavy metals must be assessed according to the type of plants. Plants are able to accumulate trace elements, particularly heavy metals in their tissues due to the great ability to adapt to different chemical conditions in the environment. They become a reservoir of trace elements that can further pass to animal and human **(Ďurža, 2003)**. All herbal teas meet this requirement by legislation, so it can be stated that all samples meet microbiological and chemical parameters.

CONCLUSION

We can conclude that all observed herbal teas meet the requirements defined by the legislation among observed microbiological parameters. Within the monitored heavy metals only one sample of tea was not in accordance with legislation requirements, we recorded the cadmium content higher than the permissible limit (4.36 $mg.kg^{-1}$) while the maximum level for cadmium is 1.0 $mg.kg^{-1}$.

Based on our achievements, compared to the results of other authors dealing with similar issues, we can assume that the herbal teas available on the market in Nitra region during the period 2009-2013 are safe for the human health.

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