

# MEDICAL AND BIOLOGICAL ASPECTS RELATED TO ASSESSMENT OF IMPACTS EXERTED BY RISK FACTORS

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## STUDYING THE CONTAMINATION OF TEA AND HERBAL INFUSIONS WITH MOLD FUNGI AS POTENTIAL MYCOTOXIN PRODUCERS: THE FIRST STEP TO RISK ASSESSMENT (MESSAGE 1)

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*We analyzed microbe contamination of 54 tea samples (Camellia sp.), black and green one, including those with various additives, and tea infusions, including herbal ones. Tea that was not packed (semi-finished product) came from the following regions: India, Indonesia, Sri-Lanka, Vietnam, Kenya, China; packed tea was bought in retail outlets in the RF. Overall, 83.3 % samples of unpacked tea conformed to microbiological standards as per mold fungi; 16.7 % samples that didn't conform to them contained mold fungi in quantities equal to  $1.3-8.2 \cdot 10^3$  CFU/g. We detected discrepancies in quantities of mold fungi in samples with different fraction structure of tea (in average CFU/g): large-leaved tea contained  $2.3 \cdot 10^2$  CFU/g; middle-leaved,  $7.4 \cdot 10^2$ ; small-leaved (including tea dust),  $1.7 \cdot 10^3$ . All packed tea samples (Camellia sp.), including those with additives, conformed to the requirements fixed by the existing standards. Aspergillus niger mold fungi prevailed in examined tea (Camellia sp.). We revealed substantial microbe contamination in herbal teas; 55 % samples didn't conform to the existing standards and contained more than  $10^{4-6}$  CFU/g of mold fungi. Besides, 72.2 % of these samples contained more than  $10^{5-8}$  CFU/g of bacteria; 62.5 % samples of herbal teas that conformed to the standards were contaminated with great quantities of bacteria equal to  $8 \cdot 10^5 - 2 \cdot 10^8$  CFU/g. We detected Aspergillus, Penicillium, Alternaria, Fusarium in herbal teas microflora; they were producers of hazardous mycotoxins, including emergent ones, and it could potentially cause contamination of herbal teas with mycotoxins. These data will be applied in future to identify hazards caused by mycotoxic fungi in tea and tea infusions as well as to update existing standards.*

**Key words:** tea, Camellia sp., herbal tea, mold fungi, producers of mycotoxins, producers of emergent mycotoxins, mycotoxins, microbe contamination, bacteria.

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Microscopic mold fungi are global contaminants polluting food vegetable raw materials; their prevalence is primarily determined by natural factors, and only to a certain extent, by anthropogenic influence. They can penetrate vegetable raw materials at all the stages including vegetation, picking, transportation, and storage. Certain species of mold fungi are potentially able to synthesize mycotoxins (MT) as secondary metabolites, and special attention should be paid to their producers as they contaminate food products. The most significant fungi here belong to the following stems: *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* [1]. A MT range produced by these microscopic fungi includes both highly toxic ones such as zearalenone (ZEA), deoxynivalenol (DON), aflatoxins (AF B1), fumonisins (FB1 and FB2), ochratoxin A (OTA) and patulin, T-2 toxin, with their contents in food products being strictly standardized in the Customs Union countries (CU TR 021/2011)<sup>1</sup> and abroad [2-8], and emergent mycotoxins (EMT) that have not been studied enough: enniatins, bovericin, moniliformin, fuzproliferin, fusaric acid, sterigmatocystin, emodin, mycophenolic acid, alternariol and its monomethyl ether, tenuazonic acid, asperglaucid, and tentoxin, that can simultaneously occur in food products and make its toxic contribution into an end product [9-12]. Mycotoxins cause actual hazards for people as they exert carcinogenic, mutagenic, and teratogenic impacts, suppress the immune system, and can induce a number of diseases [13].

Bearing safety in mind, it is important to examine contamination with mold fungi, including EMT producers, that occurs in vegetable food products consumed by population in large volumes, for example, tea. As per data obtained in marketing research conducted in 2003 and 2018, 82-83% respondents drank tea every day [14, 15]. As there is a great variety of tea types distributed on the consumer market, such concepts as "tea" and "tea drinks" are often mixed up. According to the State Stan-

dard (GOST)<sup>2</sup> "tea" is made of tea leaves from *Camellia* stem plants, *Theaceae* family (*Camelliasp.*) and doesn't contain any other components; "tea with additives" should contain not less than 50% tea as well as include some other components; "tea drink" is made of vegetable raw materials with probably added tea (mass fraction is not more than 50%) and other components, and this concept covers herbals teas as well. Tea leaves that were at primary processing stages, including drying, are a semi-finished product.

As pace of life is becoming faster, people are switching from leaf teas to packed ones (tea in bags for brewing); thus, as per market analysis data obtained in 2016, packed teas had about 63% share of the overall consumption [18]. There are also changes in preferences of specific consumer groups as mature and elderly people tend to choose green or fruit tea; as per data collected in 2016 green tea had 23% share of the market [15], fruit and herbal teas, 3.5-5%, and these market segments were considered to be growing [15-16].

Most people tend to think that tea is a "useful", "healthy" and "safe" drink and it is due to both heat treatment involved in the process of making it and absence of any relations with food intoxications. CU TR fixes microbiological standards for tea and tea products that are to be met in the RF; these parameters are given in Table 1.

Concentration of mycotoxic aflatoxin B1 produced by *A. flavus*, *A. parasiticus*, *A. ochraceoroseus*, *A. pseudotamarii*, *A. bombycis*, *A. nomius* is regulated in tea and tea products and it shouldn't exceed 0.005 mg/kg [17].

Safety parameters regulated and standardized in other countries are determined by national legislation and can include either a single parameter or a whole set of them. Parameters recommended by Tea & Herbal Infusions Europe (THIE, 2018) [18-19], American Herbal Products Association (AHPA, 2016) [20] and by the WHO (2007) [21] are given in Table 2.

<sup>1</sup> The Customs Union Technical Regulations CU TR 021/2011 "On food products safety".

<sup>2</sup> GOST 32593-2013 Tea and tea products. Terms and definitions.

Table 1

Microbiological safety parameters for tea and tea products according to the requirements fixed in the CU TR 021/2011

A product	Amounts of mesophilic aerobic and facultatively anaerobic microorganisms (AMAFAnM)	Mold	Yeast	<i>B.cereus</i>	Coliform bacteria	<i>E. coli</i>	<i>S.aureus</i>	Pathogenic bacteria including salmonella
	CFU/g, not more				Mass of a product, g, where microorganisms are not allowed			
Tea	-	10 <sup>3</sup>	-	-	-	-	-	-
Mixtures of dried vegetable raw materials for making hot non-alcoholic drinks	5x10 <sup>5</sup>	100	100	-	1	-	-	25
BAD to food - mixtures of dried medicinal herbs (teas)	5x10 <sup>5</sup>	10 <sup>3</sup>	100	-	0.01	0.1	-	10
BAD-teas (dried mixtures for children)	5x10 <sup>3</sup>	50	50	200	0.1	1	1	25
- Herbal instant teas (made of plants) for pregnant and breast-feeding women; - Herbal drink for children (herbal tea)	5x10 <sup>3</sup>	50	50	100	1	-	-	25

Table 2

Microbiological parameters for tea, herbal drinks, and raw materials

A product	Aerobic bacteria quantity, CFU/g	Yeast, CFU/g	Mold, CFU/g	<i>Enterobacteriaceae</i> , CFU/g	<i>E. coli</i> , CFU/g	<i>Salmonella</i> , absence in a mass of product, g
<i>Tea &amp; Herbal Infusions Europe (THIE)</i>						
Tea ( <i>C. sinensis</i> )	≤ 10 <sup>7</sup>	≤ 10 <sup>6</sup>	≤ 10 <sup>5</sup>	-	≤ 10 <sup>2</sup>	125
Raw materials for herbal teas (dried)	≤ 10 <sup>8</sup>	≤ 10 <sup>6</sup>	≤ 10 <sup>6</sup>	-	≤ 10 <sup>4</sup>	125
Herbal teas (dried)	≤ 10 <sup>7</sup>	≤ 10 <sup>5</sup>	≤ 10 <sup>5</sup>	-	≤ 10 <sup>3</sup>	125
Drinks made of tea ( <i>C. sinensis</i> ) and herbal teas (cooled)	≤ 10 <sup>4</sup>	≤ 10 <sup>2</sup>	≤ 10 <sup>2</sup>	-	≤ 10 <sup>1</sup>	125
<i>American Herbal Products Association (AHPA)</i>						
Herbal additives (raw materials)	≤ 10 <sup>7</sup>	≤ 10 <sup>5</sup> , totally	≤ 10 <sup>4</sup>	≤ 10 <sup>4</sup>	≤ 10	25
<i>World Health Organization (BO3)</i>						
Herbal medications taken after being brewed with boiling water	≤ 10 <sup>7</sup>	≤ 10 <sup>4</sup> , totally	≤ 10 <sup>3</sup>	10	1	
	Not allowed in 1 g, <i>Clostridia</i> and <i>Shigella</i>					
Vegetable materials to be consumed as food	≤ 10 <sup>5</sup>	≤ 10 <sup>3</sup> , totally	≤ 10 <sup>3</sup>	10	1	
	Not allowed in 1 g, <i>Clostridia</i> and <i>Shigella</i>					

But still, there are no unified criteria in the international legislation. Low humidity of tea prevents microbe contamination development; however, when requirements to picking and processing are violated, or cross contamination occurs at a manufacturing plant, it can cause elevated risks of finished products being contaminated, and it is particularly true for herbal teas.

Research on microbe contamination that can be found in foreign literature primarily focuses on teas that underwent deep fermentation, for example, Pu'er teas; but such teas have rather insignificant share on the market, and as for mass consumption teas, there is very little research on them or such research is fragmentary [22-24]. The report on the results of the 23rd session held by the FAO Intergovernmental Group (IGG) on Tea in Hangzhou, China, on May 17-20, 2018 which was presented to the FAO Committee on Commodity Problems highlighted there was a necessity to promote scientific research in the sphere of natural contaminants and to reveal relationships that caused their occurrence in tea [25].

At present experts are being discussing an issue on microbiological parameters fixed for tea in the RF and possible alterations to them in order to harmonize the RF national legislation with international requirements recommended by the WHO. Given that, it seems vital to examine contamination of variable teas and tea products distributed in the Customs Union countries with mold fungi which are potential MT producers (including EMT); it is also necessary to assess existing microbiological standards as regards their efficiency for providing safety of tea and tea products for population.

**Our research goal** was to examine contamination of variable teas and tea products with mold fungi – mycotoxins producers, and also to determine bacterial contamination for further risk assessment and updating of hygienic standards existing for these products.

**Data and methods.** We performed experimental research on 54 samples that in-

cluded: unpacked traditional tea (*Camellia sp.*) (semi-finished product), green and black, totally 30 samples from 6 tea-producing regions (Vietnam, India, Indonesia, Kenya, China, Sri-Lanka); packed tea divided into three groups: traditional tea (*Camellia sp.*), tea with additives, 3 samples of each, and herbal teas, 18 samples overall (mono-component ones: Sudan rose, fermented sally-bloom, thyme, mint, camomile; and multi-component ones containing: echinacea, origanum, brandy mint, nettle, thyme, camomile, wild rose, sage, violet, licorice, St. John's wort, melilot, inula, hawthorn, everlasting, tansy, holy-thistle, marigold, sarcarolla, birch leaves, fermented sally-bloom etc.). Unpacked tea samples were provided by wholesale companies, and packed tea samples were bought in retail outlets. Prior to microbiological research all samples were kept unopened under room temperature.

Mycological inoculation was accomplished according to the State Standard GOST 10444.12-2013<sup>3</sup>, we applied microscopic fungi identifiers for taxonomic identification [27-29], and determined amounts of mesophilic aerobic and facultatively anaerobic microorganisms according to the State Standard GOST 10444.15-94<sup>4</sup>.

**Results.** Safety of food products for consumers is provided by compliance with the fixed standards, and as data on new hazards and risk factors are accumulated or existing hazards are reduced somehow, these standards are to be updated. We examined microbe contamination of tea and tea products focusing on mold fungi as well as bacteria amounts of which in products allow to judge whether a production is hygienically clean or not.

**Determining contamination of tea samples with mold fungi.** The results of our research on contamination of unpacked tea (*Camellia sp.*) with mold fungi in 30 samples as per regions of origin are given in Table 3.

<sup>3</sup> GOST 10444.12-2013 Microbiology of food products and animal forage. Procedures for detecting and calculating amounts of yeast and mold fungi.

<sup>4</sup> GOST 10444.15-94 Food products. Procedures for determining amounts of mesophilic aerobic and facultatively anaerobic microorganisms.

Table 3

Distribution of unpacked tea samples as per levels of contamination with mold fungi obtained via mycological analysis

Region of origin	Number of samples	Mold contents, CFU/g		As per specific ranges, CFU/g (number of samples)			
		Average	Range of amounts	Less than 500	500-1,000	1,000-5,000	More than 5,000
<i>Black tea</i>							
Vietnam	5	56	18-150	5	-	-	-
India	5	1,577	173-5,850	3	-	1	1
Indonesia	5	2,364	110-8,250	2	1	1	1
Kenya	5	87	11-340	5	-	-	-
Sri-Lanka (Ceylon)	5	609	20-2,750	4	-	1	-
China	1	600	600	-	1	-	-
<i>Green tea</i>							
China	4	201	28-710	3	1	-	-
Overall, abs.	30	-	-	22	3	3	2
Overall, %	100	-	-	73.3%	10%	10%	6.7%

As we can see from the Table 3, contents of mold fungi in the examined samples of unpacked tea varied from 11 to 8,250 CFU/g in black tea and from 28 to 710 CFU/g in green tea. Standards were violated in 5 out of 26 (16.7%) black tea samples. As we examined all the samples as per regions of origin, we revealed that all the samples from Vietnam, Kenya, and China, as well as 4 samples from Sri-Lanka, 3 from Indonesia and 3 from India corresponded to the fixed standards but at the same time some samples from India and Indonesia (2 out of 5 for each country) and Sri-Lanka (1 out of 5) contained mold fungi in quantities higher than the standards. Therefore, most samples (83.3%) of unpacked tea (*Camellia sp.*) corresponded to the existing standards and mold fungi contents in them didn't exceed  $10^3$  CFU/g.

As we compared mold fungi contents in samples divided as per their fractional structure according to the State Standards GOST ISO 11286-2014<sup>5</sup>, GOST 32573-2013<sup>6</sup>, and GOST 32574-2013<sup>7</sup>, average data were distributed as follows: large-leaved teas (12 samples) contained  $1.6 \times 10^2$  CFU/g; average-leaved teas

(8 samples),  $8.8 \times 10^2$  CFU/g; small-leaved teas (including tea dust) (10 samples),  $1.7 \times 10^3$  CFU/g, and it means that small-leaved tea (including tea dust) that is usually used to make granulated and packed tea is the most contaminated one with mold fungi contents in it exceeding the fixed standards.

Having examined mold fungi species in tea inoculations, we revealed that *Aspergillus niger* prevailed and it was quite characteristic for traditional tea (*Camellia sp.*) [24].

Results of mycological inoculations obtained for packed tea samples are given in Table 4.

All the examined samples of packed traditional tea (*Camellia sp.*), including tea with additives corresponded to the standards as mold fungi contents in them didn't exceed  $10^3$  CFU/g.

But only 8 out of 18 (44.4%) herbal teas met the fixed requirements as mold fungi contents in the remaining ten samples exceeded  $5 \times 10^3$  and even reached  $10^6$  CFU/g. Species identification revealed fungi from more than 5 stems in all the herbal tea samples including *Aspergillus sp.*, *Penicillium sp.*, *Alternaria sp.*, *Fusarium sp.*, *Cladosporium sp.* etc.; certain species from these stems produce toxins. We

<sup>5</sup> GOST ISO 11286-2014 Tea. Classification as per tea leaves sizes performed with grain-size analysis.

<sup>6</sup> GOST 32573-2013 Black tea. Specification.

<sup>7</sup> GOST 32574-2013 Green tea. Specification.

Table 4

Distribution of packed tea samples as per levels of contamination with mold fungi obtained via mycological analysis

Packed tea	Number of samples	Mold contents, CFU/g		As per specific ranges, CFU/g (number of samples)			
		Average	Range of amounts	< 500	500-1000	1000-5000	> 5000
Traditional (black and green)	3	$8.6 \times 10^2$	180 - 2200	2	1	-	-
With additives (black)	3	$3 \times 10^2$	< 5 - 900	2	1	-	-
Herbal	18	$9.7 \times 10^4$	<5 - $10^6$	7	1	-	10

should note that such a variable microflora is specific for wild meadow herbs [29]. We performed preliminary analysis of toxins formation *in vitro* by mold contaminants extracted from several examined samples of herbal teas; the results indicated these contaminants were able to synthesize fumonisin B1. Occurrence of fumonisin B1 in herbal teas and medicinal plants was detected in Turkey [30]. Extended results on toxin formation by extracted fungi strains will be obtained in subsequent research.

**Determining bacterial contamination of tea.** Spore-forming bacteria are natural antagonists of mold fungi and their ratio can determine microbe contamination of vegetable raw materials. Mycological analysis of herbal teas allowed to reveal that bacteria were growing actively in spite of antibiotics contents in a nutrient medium during mycological analysis. To assess hygienic state of variable tea and tea products, we analyzed their contamination with bacteria; results obtained for unpacked tea and given in Table 5; for packed tea, in Table 6.

Most samples of traditional packed tea, 29 out of 30 (96.7%), including both black and green tea, contained overall amounts of bacteria that varied from  $<1.5 \times 10^2$  to  $5 \times 10^3$  CFU/g, and only 1 sample (3.3%) which came from Sri-Lanka contained bacteria in amount that reached  $6.7 \times 10^3$  CFU/g.

As we can see from Table 6, amounts of bacteria were low in all the examined samples of packed traditional tea (*Camellia sp.*), namely in 5 out of 6 (83.3%), and in 2 samples

of tea with additives and didn't exceed  $5 \times 10^2$  CFU/g; it amounted to  $5 \times 10^4$  CFU/g only in 1 sample of tea with additives.

As for herbal teas, only 5 samples out of 18 (27.8%) contained bacteria in amounts within  $5 \times 10^2$  -  $5 \times 10^5$  CFU/g range, but most of them, 13 out of 18 (72.2%), contained bacteria in amounts higher than  $5 \times 10^5$  CFU/g and sometimes this parameter even reached  $2 \times 10^8$  CFU/g (leaves of fermented sally-bloom). It should be noted, that 10 samples (55.5%) in this group didn't correspond to microbiological standards as per mold fungi either and were considered "dirty" and 4 out of 8 "clean" samples contained bacteria in amounts varying from  $7 \times 10^5$  to  $2 \times 10^8$  CFU/g. We should also note that increased bacterial contamination which was on average equal to  $4 \times 10^7$  CFU/g was detected in 5 samples of herbal teas that contained leaves of fermented sally-bloom. It was apparently due to fermentation conditions favorable for development of spore-forming bacteria which, due to their antagonistic activity, are able to suppress mold fungi.

The obtained results indicate that microbiological standards for tea fixed in the CU TR 021/2011 don't provide bacterial cleanliness of herbal teas, and overall amount of detected bacteria is higher than standards fixed for products that contain vegetable raw materials (Table 1) as well as standards for overall amounts of bacteria recommended by the WHO, THIE, and AHPA (Table 2).

Bacterial contamination in all the examined tea samples with high contents of bacteria was caused by spore-forming bacilli *Bacillus sp.*

Table 5

Distribution of unpacked tea samples as per contamination with bacteria obtained via microbiological analysis

Region	Number of samples	AMAFAnM, CFU/g		As per specific ranges, CFU/g (number of samples)			
		Average	Range of amounts	$<5 \times 10^2$	$>5 \times 10^2 - 5 \times 10^3$	$>5 \times 10^3 - 5 \times 10^5$	$>5 \times 10^5$
Black tea							
Vietnam	5	470	$<1.5 \times 10^2 - 1.3 \times 10^3$	4	1	-	-
India	5	232	$<1.5 \times 10^2 - 8 \times 10^2$	4	1	-	-
Indonesia	5	13	$<1.5 \times 10^2$	5	-	-	-
Kenya	5	1530	$2 \times 10^2 - 3.3 \times 10^3$	2	3	-	-
Sri-Lanka	5	1365	$<1.5 \times 10^2 - 6.7 \times 10^3$	4	-	1	-
China	1	600	600	-	1	-	-
Green tea							
China	4	98	$<1.5 \times 10^2 - 2.5 \times 10^2$	4	-	-	-
Totally, abs.	30	-	-	24	5	1	0
Totally, %	100	-	-	76.7	20	3.3	0

Table 6

Distribution of unpacked tea samples as per contamination with bacteria obtained via microbiological analysis

Packed tea	Number of samples	AMAFAnM, CFU/g		As per specific ranges, CFU/g (number of samples)			
		Average	Range of amounts	$<5 \times 10^2$	$>5 \times 10^2 - 5 \times 10^3$	$>5 \times 10^3 - 5 \times 10^5$	$>5 \times 10^5$
Traditional	3	77	$< 1.5 \times 10^2 - 1.8 \times 10^2$	3	-	-	-
With additives	3	$1.6 \times 10^4$	$<1.5 \times 10^2 - 5 \times 10^4$	2	-	1	-
Herbal	18	$1.3 \times 10^7$	$4.7 \times 10^3 - 2 \times 10^8$	-	1	4	13

**Determining microbe contamination of tea after heat treatment.** To assess whether tea is safe when it is ready for consumption, it is necessary to examine how heat treatment during brewing influences microbe contamination of tea with mold fungi and bacteria. In order to do that, we took 6 samples with different microbe contamination (3 out of each group: traditional unpacked tea and herbal tea), poured them with boiling water (in a ratio 10 g tea per 90 ml of water), then brewed them for 10 minutes (just as any ordinary tea); infusions were then cooled and inoculated, and results recalculated as per dry tea are given in Table 7.

As we can see from the Table 7, heat treatment results in considerable fall in amounts of mold fungi in all tea samples from its initial level that was  $>10^2 - 10^6$  CFU/g to a level below test sensitivity which was  $< 5$  CFU/g. However, we

Table 7

Comparative analysis of mold fungi and bacteria contents in inoculations before and after heat treatment: results obtained for traditional teas and herbal teas

Sample	Mold fungi, CFU/g		AMAFAnM, CFU/g	
	Initial product	After brewing	Initial product	After brewing
Traditional (unpacked) tea				
1	$5.8 \times 10^3$	$< 5$	$8 \times 10^2$	$< 1.5 \times 10^2$
2	$8.2 \times 10^3$	20	$< 1.5 \times 10^2$	$< 1.5 \times 10^2$
3	$6 \times 10^2$	$< 5$	$1.3 \times 10^3$	$< 1.5 \times 10^2$
Herbal (packed) tea				
4	Less than 5	$< 5$	$2 \times 10^8$	$6.1 \times 10^4$
5	$10^6$	$< 5$	$5 \times 10^6$	$2.7 \times 10^5$
6	$2.2 \times 10^4$	$< 5$	$9 \times 10^6$	$4 \times 10^6$

didn't observe a similar fall as regards bacteria: their amount dropped to less than  $1.5 \times 10^2$  CFU/g in all 3 samples of traditional tea regardless of their levels in initial products while as for herbal teas, the greatest reduction, more than by 3,000 times, was detected in sample No. 4; bacteria amount dropped by 18 times in sample No. 5; and it fell by only 2.25 times in sample No. 6. And we should also note that amounts of bacteria remained rather high in all three samples of herbal tea even after heat treatment, from  $6.1 \times 10^4$  to  $4 \times 10^6$  CFU/g (recalculated as per dry product). These differences in inhibition under heat treatment are apparently caused by prevalence of spore-forming bacteria which are heat-resistant over vegetative ones.

**Conclusion.** We analyzed microbiological state of different teas (*Camelliasp.*): black and green tea (packed and unpacked), including tea with additives, and revealed that most samples corresponded to microbiological standards fixed in CU TR 021/2011 as amounts of mold fungi didn't exceed it in 83.3% of unpacked tea samples (semi-finished product). All the samples of packed tea, including those with additives, contained less than  $10^3$  CFU/g. We detected some differences in mold fungi quantities in tea samples with different fractional structure (in average CFU/g): they were less than  $10^3$  in large-leaved and average-leaved teas, and  $1.7 \times 10^3$  in small-leaved teas (including tea dust). *Aspergillusniger* prevailed among mold fungi detected in tea (*Camelliasp.*). After traditional tea (*Camelliasp.*) was brewed, the amount of mold fungi and bacteria in brewed drinks dropped from the initial level equal to  $10^{2-3}$  CFU/g to a level below test sensitivity and it made tea a safer drink for consumption. As we compared standards existing for tea in the Customs Union countries with standards recommended for tea (*C.sinensis*) by THIE association, we revealed that CU TR 021/2011 fixed stricter requirements to mold fungi contents, but a set of microbiological parameters fixed by THIE standards included 4 additional groups of microorganisms.

High microbe contamination was most frequently detected in herbal tea samples, both in terms of mold fungi and bacteria. 55.5% samples contained mold fungi in quantities higher than  $10^{4-6}$  CFU/g and it exceeded microbiological standards, and 72.2% samples contained overall quantity of bacteria that was higher than  $10^{5-8}$  CFU/g. And even when herbal tea samples corresponded to the fixed standards as per mold fungi contents, 62.5% of them still contained bacteria in quantity that was higher than  $5 \times 10^5$  CFU/g. After herbal teas were brewed, mold fungi contents went down from the initial  $10^{4-6}$  CFU/g to a level below test sensitivity, and bacteria quantity dropped from  $10^{6-8}$  CFU/g to more than  $10^{4-6}$  CFU/g, but it still remained rather high; that is, brewing doesn't provide microbiological safety of herbal tea as opposed to traditional tea (*Camelliasp.*).

The obtained results that allow to see hygienic state of herbal teas apparently indicate that when the existing microbiological standards for tea are applied to herbal teas, it doesn't provide their cleanness from bacteria. High bacteria quantities up to  $2 \times 10^8$  CFU/g exceed both AMAFAnM parameter fixed in the CU TR 021-2011 for all types of products that include herbal raw materials (Table 1) and quantities of bacteria recommended by the WHO and THIE and AHPA associations (Table 2). In order to make tea products safer for consumers, it is advisable to adjust "tea" category and make it "tea (*Camelliasp.*)", to single out "herbal tea" as a specific food product that is different from "tea (*Camelliasp.*)", and to specify a group of products "herbal tea" can be assigned into taking into account a wider list of parameters to be standardized.

We detected a great variety of microscopic fungi in herbal teas including *Aspergillus*sp., *Penicillium*sp., *Alternaria*sp., *Fusarium*sp., *Cladosporium*sp.; some of them are toxins producers. It allows us to assume there are mycotoxins produced by these fungi in herbal teas. To obtain a most comprehensive characteristics of tea samples, it is necessary to analyze mycotoxins occurrence in them with a multi-detection procedure aimed at detecting



a wider range of mycotoxins including emergent ones. When assessing safety of tea and tea products, it is advisable to take into account all the existing data obtained in microbiological and mycotoxicological research.

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