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Determination of Heavy Metals and Bacterial Count of Some Frequently Consumed Spices in Jordan

Sati Y. Al-Dalain[1], Moawiya A. Haddad*[2], Jehad S. Al-Hawadi[3], Ahmad Freihat[4], Shereen Arabiat[5], Maisa M. A. Al-Qudah[6], Mazen A Ateyyat [7]

[1]Department of Medical Supports, Al-Karak University College, Al-Balqa Applied University, 19117 Al-Karak, Jordan
[2]Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University19117 Al-Salt, Jordan
[3]Ash-Shoubak University College, Al-Balqa Applied University, 19117 Al-Salt, Jordan
[4] Department of Applied Science, Ajloun University College, Al-Balqa Applied University, Ajloun, Jordan
[5]Medical Allied Sciences, Salt College, Al-Balqa Applied University, 19117 Al-Salt, Jordan
[6] Department of Medical Laboratory Sciences, Faculty of Science, Al-Balqa Applied University, Al-Salt, 19117, Jordan
[7]Faculty of Agricultural Technology, Plant Production and Protection Dept., Al Balqa Applied University, Salt, 19117, Jordan.
Corresponding Author: Moawiya A. Haddad **Email**: haddad@bau.edu.jo

ABSTRACT

Background: Many factors contribute to the contamination of spices with heavy metals and bacteria including fertilizers, soil contamination and source of irrigation water.

Methods: In this study, four spices already consumed in Jordan (cinnamon, black pepper, cubeb "allspice" and nutmeg) were investigated regarding heavy metals and bacterial contents. India and China (as Asian origin) as well as United States of America (as North American Origin) and Brazil (as South American origin) were considered as the main producers and exporters of spices for most countries of the world as Jordan.

Results: results indicated that (Fe) element was came in the first ordered as a major investigated one in Indian nutmeg. Black pepper came as a predominant one in Indian black pepper contrary to that of Chinese one which had (Mn) element as a predominant. (Mn) is the major element in Chinese cubeb, but Indian cubeb contained (Fe) as a major element. It could be detected iron metal (Fe) as the highest heavy metal in either Indian or Chinese cinnamon. By calculating the total heavy metals content, it could be ascendingly ordered the investigated Asian spices as: Chinese cubeb, Chinese nutmeg, Chinese black pepper, Indian cinnamon, Indian cubeb, Indian nutmeg, Indian black pepper and Chinese cinnamon. Three of Chinese spices, i.e., cubeb, nutmeg and black pepper came in the first order considering their total heavy metals content. Meanwhile, in the second order Indian spices (cinnamon, cubeb and nutmeg) was coming with moderate total heavy metals content. The highest level of total heavy metals (over 9 ppm) was detected in both of Indian black pepper and Chinese. Regarding to the total content of heavy metals in four investigated American spices, black pepper possesses the lowest level of total heavy metals and meanwhile the highest level was found in American cubeb. Cubeb and nutmeg contain iron with 1.9 times higher than that of cinnamon spice. In the second order (Mn) element showed a similar trend. It is interested to notice that all of spices in this study had approximately the same level of (Pb) metal. The total heavy metals content in investigated Brazilian spices, black pepper possessed the highest. While cinnamon had the lowest. Three of heavy metals (As, Ti and Li) were also not detected in Brazilian spices similar to those of American ones. As a general trend, black pepper spice either imported from Asian (China and India) or American (USA and Brazil) origin had the highest bacterial count, meanwhile, the lowest count was detected in nutmeg spice imported from the same origins.

Keywords: Allspice (Cubeb), Black pepper, Cinnamon, Heavy metals, Jordan spices.

Correspondence:
Moawiya A. Haddad
Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University19117 Al-Salt, Jordan
Email: haddad@bau.edu.jo

INTRODUCTION

As spices and herbs are widely consumed in the world, their heavy metals and/or bacterial contents are of great concern. Heavy metals have bio-importance as trace elements, instead the bio-toxic effects of many of them in human biochemistry should be considered as health concern. On contrary, mainly owing to its medicinal values consumption of spices was markedly increased in the world [1].

The heavy metals in Ghanaian markets were copper, zinc, cadmium, lead and iron. The corresponding contents as ppm were (0.9-19.4), (2.4-34.6), (0.01-0.9), (0.6-1.8) and (19.4-971.4) as found by Darko *et al.*[2]. The spice plant, part i.e., seed, rhizome, leaf as well as fruit was affected the heavy metals concentration in spices available in Erbil city at Iraq as given by Gulzar *et al.*[3]. They assured that in Iraqi market most of available spices are safe for the human consumption with the exception of lead and cadmium.

People with the current emphasis for eating more healthy diets were turning to various spices to enhance the flavor of soups, vegetables, pasta and fried dishes [4-6]. Some of essential metals (iron, manganese and zinc) are very useful for growing a healthy body throughout very high levels are in tolerable, meanwhile lead, cadmium and mercury at very low concentrations are toxic for human body. So, the addition of spices which any contaminate and accumulate in human organs lead to various health troubles as reported by Gulzar *et al.*[3]. On the other hand, **Ozores *et al.*[7] (1997)** assured that such heavy metals were found and contaminated plants and canned foods through irrigation water of soil and during industrial processing and packaging steps. It is of interest to notice that lead, cadmium and mercury have no bio-importance as given by Divirikli *et al.* [8].

Ozkutlu *et al.*[9] and Lenntech [10] explained that heavy metals are crucial because of its potential hazardous effect on human health due to its cumulative behavior toxicity. At the same time, Husain *et al.*[11] showed that monitoring the levels of heavy metal toxicity in spices would help the health impact of taking these spices as well as provide relevant data on such spices in the country and for studying their dangerous effect.

The contamination of plants then animals and humans are due to several sources, i.e., environmental pollution throughout air emissions (from automobile exhaust), wastes from mining and pesticides that leaching in water [12]. Spices contamination with heavy metals depends on cultivation soil, fertilizers as well as irrigation water source. Beside colorants that added to be flavor enhancers may contain lead as shown by **Ekpo & Jimmy** [13] **and Abdullahi *et al.*** [14].

Spices considered as inseparable components of daily life in different societies [15]. Cinnamon and black pepper are most famous spices that used for their enormous benefits for human health besides making flavor, colour and odor in food products [16]. For instance, cinnamon could regulate blood glucose as well as overweight, while black pepper considered as anti-constipation, for removing toothache, sunburns as well as abscess and eye problems as reported by Kalicanin & Velimirovie [17] and Belay *et al.*[16].

Many of spices had antimicrobial effects but handling and preparation processes can make them as food poisoning source [18]. So, this study throws the light on the level of heavy metals in some spices that commonly consumed in Jordan kingdom, i.e., cinnamon, black pepper, cubeb (allspice) and nutmeg. The effect of cultivation origin, i.e., importation countries (Asian or American one) on heavy metals level as well as its bacterial load was considered.

MATERIALS AND METHODS

Materials

Samples of dry spices named cinnamon (*Cinnamonum verum*), black pepper (*Peper nigrum*), cubeb (*Piper cubeba*) and nutmeg (*Myristica fragrans)* were collected from Jordanian markets. The collected samples were those imported from various origins, i.e., from India and China (Asian origin), from USA (North American origin) and from Brazil (South American origin) considering these Nations as main producers and exporters of spices for most Countries of the world. Samples were cleaned, oven-dried, powdered and sieved then kept dry until analyzed.

Methods

Proximate composition

Proximate composition of spices was carried out according to AOAC, 2007. [19].

Determination of heavy metals

Digestion Procedure

A wet digestion method was used to extract the heavy metals from the spices powder samples. For each sample, exactly one gram was accurately measured and transferred to the pre cleaned Teflon digestion vessels. Then 10 mL of high purity nitric acid HNO_3 (65%) was added to each vessel, followed by addition of 2 ml of high purity analytical reagent grade Perchloric acid, $HClO_4$ (70%). Then the vessels were capped and transfer to the oven at 70 ºC overnight. After the digestion complete, the vessels were cooled, vented and disassembled under the fume hood then the samples extract was then quantitatively transferred to standard polyethylene volumetric flask and diluted to a final volume of 50 mL using deionized water. Then the diluted solution was filtrated using 0.7 µm online syringe micro filter. Finally, the cleared solution after filtration were stored in 50 ml polyethylene bottle and stored at 4 º C in the refrigerator until analysis time.

Analytical instrumentation and calibration:

Eight metals (Zn, Ni, Pb, Cu, Fe, Cr, Cd, and Mn) were analyzed quantitatively based on internal calibration curves for series of standard solutions range between 0.01 to 10 ppm (0.01, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.00, 3.00, 5.00 and 10.0 ppm) using Atomic Absorption spectroscopy (AAS) 6200, Perkin Elmer, Canada-USA. Working standards were prepared by a serial dilution of the stock standard solutions (1000 ppm for each metal) purchased from Merck, (Germany). The calibration curves for these elements were buildup by the instrument with best regression lines with correlation coefficient (r^2> 0.998) by measured into the AAS instrument 3 time and the average value was used to build up the calibration curve.

A Total Six digested samples solution and one blank were analyzed for eight heavy metals (Zn, Ni, Pb, Cu, Fe, Cr, Cd and Mn), using the above instrument by triplicated measured. The average absorbance value was used for calculating the concentration of each heavy metal with a relative standard deviation (RSD%) of less than 5% for each heavy metal in all samples.

The heavy metals of 16 elements were determined using ICP Instrument Prodigy 7 specified by Optical Design High Energy Echelle Polychromat connected with a CMOS detector [20].

Total bacterial count

The method reported by US-FDA, 2020 [21] was applied to determine the total bacterial count.

Statistical analysis

The obtained data were subjected to analysis of variance using proc. ANOVA in SAS [22]. Mean separation was conducted using LSD in the same program at significant level P ≤ 0.05.

RESULTS AND DISCUSSION

Determination of heavy metals in spices imported from Asian origins

The heavy metals level that detected in four investigated spices imported from two Asian origins was given in Table (1) and Fig. (1). The following important findings could be summarized in these points, taking into account that only iron, manganese, and lead were statistically significant in comparison with all remaining heavy metals (P ≤ 0.05):

- The (Fe) element was came in the first ordered as a major investigated one ranged between about 2 to 5.5 ppm. It was 2.3 ppm in Indian nutmeg, while it was 2.2 ppm in its corresponded Chinese one.
- Regarding the (Fe) content in second investigated Asian spice, i.e., black pepper, it was 4.2 ppm came as a predominant one in Indian black pepper contrary to that of Chinese one which had (Mn) element as a predominant (2.1 ppm).
- Similarly, to above finding, it was recorded in case of the third investigated Asian spice named cubeb that (Mn) is the major element by 1.4 ppm in Chinese cubeb, but Indian cubeb contained (Fe) as a major element with 2.9 ppm.
- Considering the fourth investigated Asian spice (cinnamon) it could be detected that iron metal (Fe) as the highest heavy metal in either Indian or Chinese cinnamon. It was 1.7 and 5.7 ppm, respectively. In general, Fe was 2.7 ppm (standard deviation: ± 1.4 ppm). (Mn) as ordered as the second element found

in Indian cinnamon (1.5 ppm), but on the other hand, (Pb) heavy metal was the second one in Chinese cinnamon with 0.99 ppm. In general, Mn and Pb were 1.6 and 0.9 ppm respectively (standard deviation: ± 0.4 and ± 0.3 ppm, respectively).

Table 1: Heavy metals content (ppm) in different spices imported from Asian origins.

Spice / Element	Nutmeg		Black pepper		Cubeb		Cinnamon	
	India	China	India	China	India	China	India	China
As	ND	ND	ND	ND	ND	ND	ND	ND
Ti	ND	ND	ND	ND	ND	ND	ND	ND
Li	ND	ND	ND	ND	ND	ND	ND	ND
Al	0.070	0.060	0.067	0.069	0.0661	0.069	0.0660	0.0659
Mo	0.175	0.125	0.126	0.175	0.1667	0.0187	0.1926	0.0595
B	0.026	0.025	0.036	0.0345	0.0191	0.0256	0.0117	0.0237
Pb	1.01	0.980	1.005	0.9791	0.996	0.100	1.003	0.9986
Cr	0.550	0.415	0.678	0.7966	0.199	0.0621	0.3792	0.0272
Cd	0.004	0.005	0.005	0.0016	0.0021	0.0026	0.0015	0.0015
Ni	0.004	0.005	0.005	0.0015	0.0021	0.0027	0.0015	0.0015
Mn	1.965	1.365	1.653	2.1166	1.7923	1.366	1.4946	0.7307
Zn	0.379	0.060	0.615	0.232	0.6763	0.009	0.6516	0.6740
Co	0.659	0.380	0.680	0.459	0.3873	0.228	0.5161	0.2102
Cu	0.312	0.080	0.094	0.172	0.2490	0.089	0.6008	0.9220
Ag	0.026	0.020	0.025	0.022	0.0278	0.026	0.0292	0.0245
Fe	2.326	2.166	4.198	1.5306	2.888	1.097	1.6900	5.6800
Total	7.500	5.600	9.187	6.432	7.4718	3.0957	6.6378	9.4193

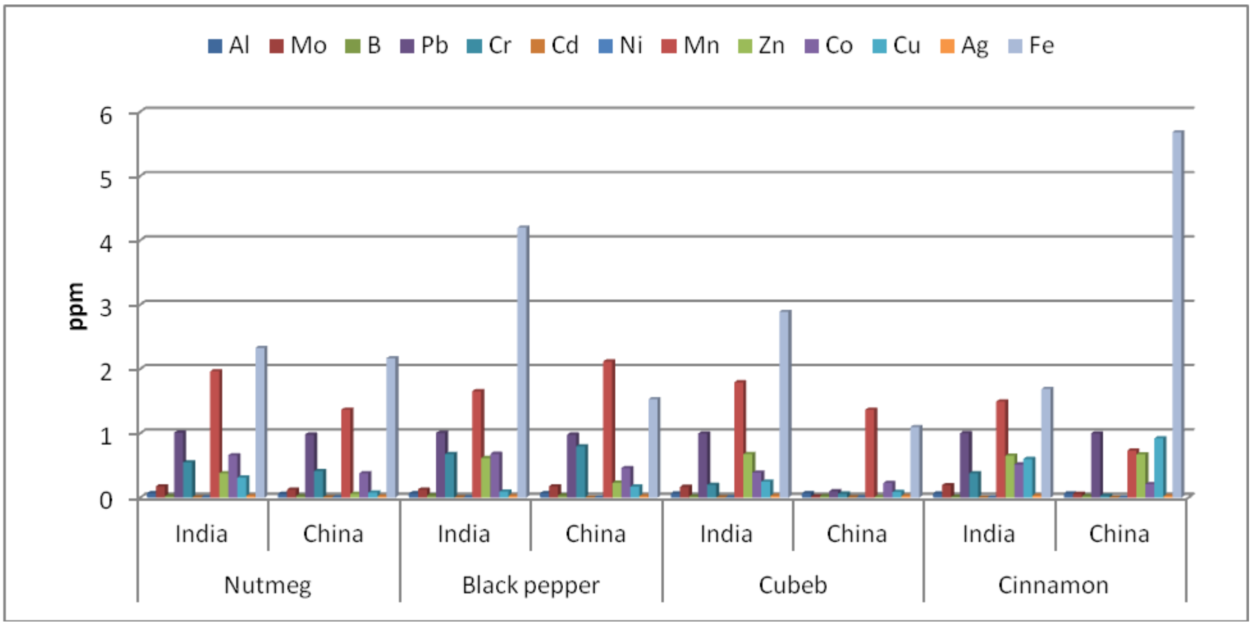

Fig. 1: Heavy metals content (ppm) in different spices imported from Asian origins.

- By calculating the total heavy metals content in the investigated Asian spices, it could be ascendingly ordered as: Chinese cubeb (3.1 ppm), Chinese nutmeg (5.6 ppm), Chinese black pepper (6.4 ppm), Indian cinnamon (6.6 ppm) Indian cubeb (7.5 ppm); Indian nutmeg (7.5 ppm), Indian black pepper (9.2 ppm) and Chinese cinnamon (9.4 ppm).
- It was clearly that three of Chinese spices, i.e., cubeb, nutmeg and black pepper came in the first order considering their total heavy metals content. Meanwhile, in the second order Indian spices (cinnamon, cubeb and nutmeg) was coming with moderate total heavy metals content. On the other hand, the highest level of total heavy metals (over 9 ppm) was detected in both of Indian black pepper (9.2 ppm) and Chinese cinnamon (9.4 ppm).
- There are three of none detected heavy metals (As, Ti and Li) in all of different investigated Asian spices.

Determination of heavy metals in spices imported from North America

From Table (2) and Fig. (2), and taking into account that only iron, manganese, and lead were statistically significant in comparison with all remaining heavy metals ($P \leq 0.05$), it could be detected iron metal as predominant heavy metal in either nutmeg (3.3 ppm) or cubeb (2.8 ppm) spices that imported from North America (USA origin). In general, Fe was 1.8 ppm (standard deviation: ± 1.1 ppm). While, other two investigated spices (black pepper and cinnamon) had (Mn) metal as a predominant one with 2.3 and 2.4 ppm, respectively. In general, Mn had 2.2 ppm (standard deviation: ± 0.2 ppm).

Table 2: Heavy metals concentration (ppm) of spices imported from USA (as North American origin)

Element \ Spice	Nutmeg	Black pepper	Cubeb	Cinnamon
As	ND	ND	ND	ND
Ti	ND	ND	ND	ND
Li	ND	ND	ND	ND
Al	0.071	0.080	0.070	0.069
Mo	0.125	0.150	0.159	0.169
B	0.030	0.062	0.033	0.050
Pb	1.081	0.988	1.020	0.994
Cr	0.370	1.512	0.654	0.686
Cd	0.005	0.007	0.613	0.004
Ni	0.003	0.005	0.007	0.002
Mn	1.912	2.273	1.531	2.392
Zn	0.501	0.111	0.350	0.750
Co	0.082	0.132	0.460	0.531
Cu	0.248	0.062	0.962	0.455
Ag	0.032	0.031	0.028	0.033
Fe	3.310	0.512	2.814	1.672
Total	7.770	5.925	8.701	7.807

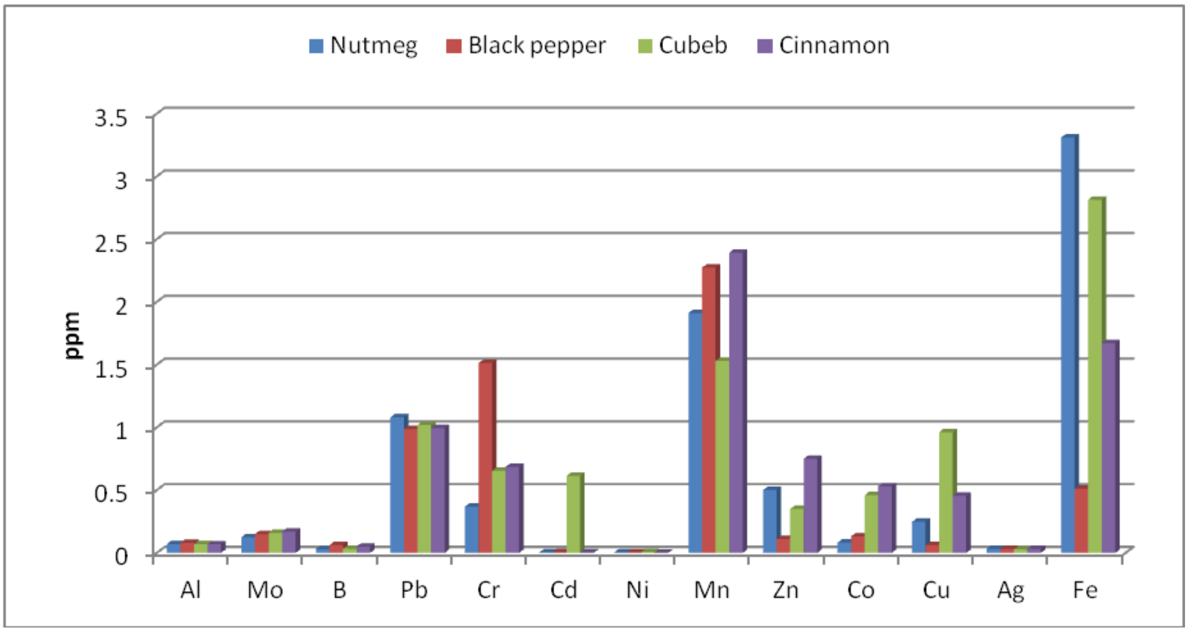

Fig. 2: Heavy metals concentration (ppm) of spices imported from USA (as North American origin)

Regarding to the total content of heavy metals in four investigated American spices, black pepper possesses the lowest level of total heavy metals, meanwhile the highest level was found in American cubeb that recorded 8.7 ppm.

Determination of heavy metals in spices important from South America

The heavy metals content of imported spices from South America (Brazil origin) was determined in four investigated spices and data were tabulated in Table (3) and figured as Fig. (3). From these data, and taking into account that only iron, manganese, and lead were statistically significant in comparison with all remaining heavy metals ($P \leq 0.05$), it could be detected the iron metal (Fe) in the first order with level ranged between 1.4 ppm (Brazilian cinnamon) to 7.5 ppm (Brazilian black pepper). It means that black pepper contains iron level with five times higher than that of cinnamon, while other two investigated Brazilian spices, i.e., cubeb and nutmeg contain iron with 1.9 times higher than that of cinnamon spice. In general, Fe was 3.8 ppm (standard deviation: ± 2.5 ppm). In the second order (Mn) element showed a similar trend. It is of interested to notice that all of spices in this study had approximately the same level of (Pb) metal (0.98–0.99 ppm). In general, Mn and Pb were 1.5 and 0.9 ppm respectively (standard deviation: ± 0.4 and ± 0.3 ppm, respectively), very similar to spices imported from North America.

Table 3: Heavy metals concentration (ppm) of spices imported from Brazilian (as South American origin)

Spice / Element	Nutmeg	Black pepper	Cubeb	Cinnamon
As	ND	ND	ND	ND
Ti	ND	ND	ND	ND
Li	ND	ND	ND	ND
Al	0.060	0.069	0.099	0.060
Mo	0.114	0.165	0.166	0.160
B	0.026	0.045	0.099	0.016
Pb	0.996	0.989	0.980	0.980
Cr	0.380	0.536	0.470	0.390
Cd	0.005	0.006	0.004	0.005
Ni	0.005	0.005	0.004	0.006
Mn	1.616	1.738	1.830	0.946
Zn	0.286	0.255	0.680	0.786
Co	0.230	0.215	0.390	0.056
Cu	0.169	0.960	0.486	0.156
Ag	0.029	0.026	0.026	0.036
Fe	2.735	7.500	2.780	1.440
Total	6.651	12.569	8.014	5.047

Regarding to the total heavy metals content in investigated Brazilian spices, black pepper possessed the highest (12.6 ppm), while cinnamon had the lowest (5.1 ppm). Three of heavy metals (As, Ti and Li) were also not detected in Brazilian spices similar to those of American ones.

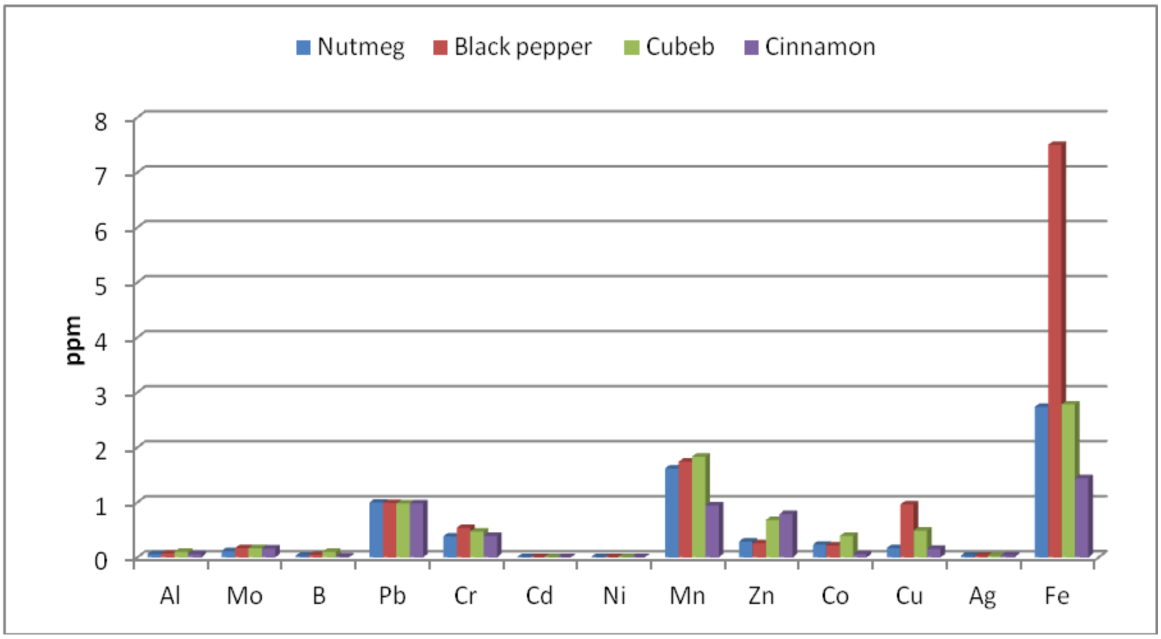

Fig. 3: Heavy metals concentration (ppm) of spices imported from Brazil (as South American origin).

Maximum permissible level (MPL) of heavy metals in Indian standard (ppm) were 5, 0.2, 0.4, 3,5, 2, and 1.6 for pb, Cd, Co, Cu, Fe, Mn, and Ni respectively. All samples of Asian origin spices (Table 1) were within MPL except: Mn and Co were higher than MPL black pepper of china (2.1 ppm for both). In addition, Co was higher than MPL for Chinese nutmeg and Indian cinnamon (0.46, 0.52 ppm respectively). Regarding to Fe, it was higher than MPL in Chinese cinnamon (5.7 ppm).

In spices of American origin (Table 2), all samples were within MPL regarding to heavy metals except: Mn was higher in black pepper and cinnamon (2.3, 2.4 respectively) and Co was higher in cubeb and cinnamon (0.46, 0.53 respectively). In Brazilian spices (Table 3) all heavy metals were within MPL except Fe in black pepper was 7.5 ppm. It is obvious that most of the spices have a good quality and within the MPL. It was expected that the contamination by heavy metals came from cultivation conditions (e.g water contamination), during storage and transportation, and grinding method.[23]

Bacterial load of imported spices

The total bacterial count of various investigated spices imported from either Asian or American origin were given in Fig. (4) and (5). As a general trend, black pepper spice either imported from Asian (China and India) or American (USA and Brazil) origin had the highest bacterial count (5.42 and 6.52 log_{10} cfu/ml) as well as (5.74 and 5.93 log_{10} cfu/ml), respectively. Meanwhile, the lowest count was detected in nutmeg spice imported from the same origins. The corresponding counts were (2.46 and 2.19 log_{10} cfu/ml) as well as (1.80 and 2.11 log_{10} cfu/ml) respectively.

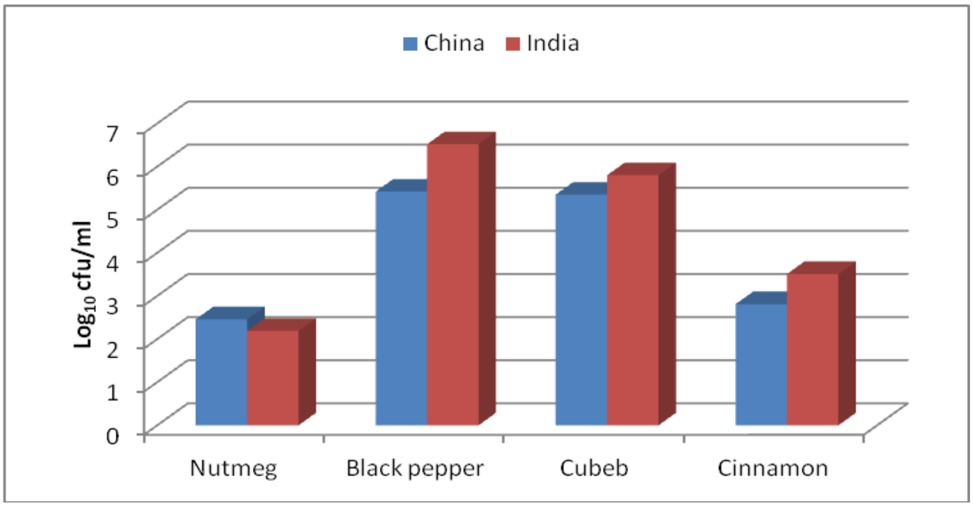

Fig. 4: Total bacterial count (log_{10} / cfu) of Asian spices.

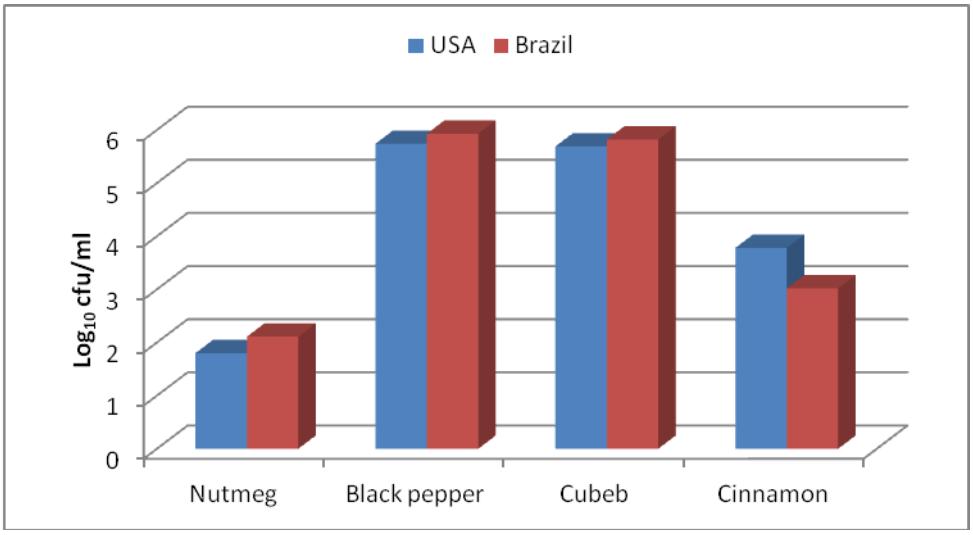

Fig. 5: Total bacterial count (log_{10} / cfu) of American spices.

CONCLUSION

Black pepper spice of Brazilian origin possessed the highest total heavy metals content in investigated samples while, cinnamon had the lowest. The As, Ti and Li heavy metals were not detected either in Brazilian or American spices. Pepper spice either imported from Asian (China and India) or American (USA and Brazil) origin had the highest total bacterial count, meanwhile, the lowest bacterial count was detected in nutmeg spice imported from the same origin.

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