

## Original Research Article

### **EFFECT OF TEA AND COFFEE CONSUMPTION ON BLOOD LEVELS OF SOME HEAVY METALS AND TRACE METALS**

#### **ABSTRACT**

In the present study, the content of Cd, Pb, Hg, Zn, Cu, and Se in blood following extended consumption of tea and coffee was determined. By random sampling method, sixty participants were selected for this study and grouped into 20 tea consumers, 20 coffee consumers and 20 controls. They were administered tea and coffee beverages respectively daily for 30 days, after which venous blood samples were collected from each participant into lithium heparin container. Blood Pb, Cd, Hg, Cu, Zn and Se were determined by employing Flame Atomic Absorption Spectrophotometry. All data generated were used to perform statistical analysis employing SPSS version 21. Blood Cd, Pb, Hg and Se were significantly higher ( $p=0.005$ ,  $p=0.003$ ,  $p=0.001$  and  $p=0.048$ , respectively) in tea Consumers compared with controls, while there were no significant difference in blood levels of Cu and Zn ( $p=0.923$  and  $p=0.784$  respectively) in tea Consumers compared with controls. Blood Cd, Pb, Hg, Zn and Se were significantly higher ( $p=0.001$ ,  $p=0.000$ ,  $p=0.012$ ,  $p=0.037$  and  $p=0.006$  respectively) in Coffee Consumers compared with Controls. There was no significant difference in blood Cu ( $p=0.222$ ) in Coffee Consumers compared with Controls. There were no significant differences in blood levels of Cd, Pb, Hg, Cu, Zn and Se ( $p=0.154$ ,  $p=0.459$ ,  $p=0.662$ ,  $p=0.226$ ,  $p=0.080$  and  $p=0.705$  respectively) in Tea Consumers compared with Coffee Consumers. There were significant negative correlations of blood Pb with Hg and Cu ( $r=-0.451$ ,  $p=0.046$  and  $r=-0.697$ ,  $p=0.001$  respectively) in Tea Consumers. There were significant negative correlations of blood Pb with blood Cu and Zn ( $r=-0.656$ ,  $p=0.002$  and  $r=-0.690$ ,  $p=0.001$  respectively) and significant positive correlation of Pb with Hg ( $r=0.538$ ,  $p=0.017$ ) in Coffee Consumers. These findings have shown that Tea and Coffee Consumption may be associated with higher blood levels of Pb, Cd, Hg and Se; hence, caution should be applied considering the deleterious effects of heavy metals.

Keywords: Tea, Coffee, Heavy Metals, Trace Elements, Blood

## 1.0 INTRODUCTION

“Tea is one of the most consumed beverages in the world and is prepared from the leaves of the shrub, *Camellia sinensis*” [1]. Green and black teas are the two most popular types. Drying and roasting the leaves produces green tea; black tea is obtained after a fermentation process. “Tea, when prepared by infusion of leaves, flowers, or roots, has generated significant scientific interest due to its increased consumption, antioxidant activity, and presence of some micronutrients, such as minerals, flavonoids, and catechins” [2]. “Studies have shown a presence of potentially toxic and cumulative substances in herbs such as inorganic contaminants” [3]. “These contaminants originate from different aspects of the herbal tea manufacturing process and include sources such as soil and water, fertilizers, and airborne industrial emissions” [4].

“Tea leaves (*Camellia sinensis*) are source of such mineral elements as essential for health: zinc, manganese, iron, magnesium, copper, titanium, aluminum, bromine, sodium, potassium as well as nickel, chromium and also phosphorus” [5]. “Several attempts have been made to assess tea quality by chemical analysis usually with reference to pigmentation and the flavouring characteristics. Metallic constituents of tea leave is normally different according to the type of tea (green or black) and geological sources” [6]. “However, to date little work has been done to identify the metal containing components of tea due to the analytical difficulties associated with both the separation of such components and their quantitative measurement” [1].

“Coffee is a brewed drink prepared from roasted coffee beans, which are the seeds of berries from the *coffea* plant. The genus *Coffea* is native to tropical Africa (specifically having its origin in Ethiopia and Sudan) and Madagascar, the Comoros, Mauritius and Reunion in the Indian Ocean” [7]. The primary psychoactive chemical in coffee is caffeine [8]. “Coffee is the second

most popular drink after water in the world. Coffee consumption varies widely according to geographical location. The highest consumption has been observed in Northern Europe (Finland; 12.0 kg per capita/year) whereas in Southern Europe the highest consumption is in Bosnia and Herzegovina (6.1 kg per capita/year). According to the International Coffee Organization, in 2008 coffee was consumed at a rate of 2.5 billion cups per day (1 cup = 30 mL). This consumption is a model for addictive behavior.

Genetic investigations in twins suggest that the heritability of coffee intake can be estimated to be in the range of 39 to 56%” [9, 10]. “Coffee seems to have distinct acute and long-term effects on health. Interestingly, its consumption has been suggested to be beneficial in dementia, Alzheimer’s disease, Parkinson’s disease and diabetes mellitus type 2” [11, 12]. “However, other researchers have associated coffee drinking with an increased risk of developing coronary heart disease” [13].

“Many elements present in food at major, minor and trace levels are reported to be essential to man’s wellbeing. However, their ingestion in excessive amount can cause severe health problems” [14]. “Human body requires both metallic and non-metallic elements for healthy growth, development and the proper functioning of the body. The determination of these elements in beverages, water, food, plant and soil is thus of utmost importance and is currently the subject of studies by various researchers” [1]. In the present study, the content of Cd, Pb, Hg, Zn, Cu, and Se in blood following extended consumption of tea and coffee has been determined.

“Tea, when prepared by infusion of leaves, flowers, or roots, has generated significant scientific interest due to its increased consumption, antioxidant activity, and presence of some

micronutrients, such as minerals, flavonoids, and catechins” [2]. “Studies have shown a presence of potentially toxic and cumulative substances in herbs such as inorganic contaminants” [3]. “These contaminants originate from different aspects of the herbal tea manufacturing process and include sources such as soil and water, fertilizers, and airborne industrial emissions” [4]. Depending on the concentration, potentially toxic elements can cause damage to human health ranging from liver and kidney dysfunctions to carcinogenesis. However, not all contaminants remain in the tea infusion. Most residual toxins are present in very low levels, lowering the risk associated with tea ingestion. The health hazards associated with heavy metals such as lead, mercury and cadmium as well as toxicity of elevated levels of trace metals is well known. The scanty literature that exist as regards the effects of tea and coffee consumption on blood heavy metal and trace metal levels necessitate the study of the influence of these popular beverages in affecting heavy metal and trace metal levels in blood.

## **2.0. MATERIALS AND METHODS**

### **Pre-survey Contact**

Using Random Sampling method, a total of 60 randomly selected individuals aged between 17 to 30 years were allocated into four groups in a transversal analytic study: Study Group 1(SG1) - 20 individuals who participate in coffee consumption regularly; Study Group 2(SG2) - 20 individuals who partake in regular consumption of tea; Control Group (CG) – 20 controls chosen from the general population, who did not partake in tea or coffee consumption. The Groups 1 and 2 were given a cup of tea made of 1 Lipton yellow label tea bag and 1 sachet of Nescafe coffee sachet, respectively daily for 40 days. They were monitored to ensure that they took it at sight.

Additional demographic data were obtained using the study questionnaires during a structural interview. It included basic socioeconomic information, some medical health history, occupational and other exposure to heavy metals. Every participant signed an informative form thereby indicating their consent.

### **Sample Collection**

Blood Samples were collected using tourniquet, 5ml syringe, cotton wool, 70% alcohol, and labeling tapes, from the participants and dispensed into lithium heparin tubes using 5ml syringe.

### **Methods of Analysis of Serum Heavy Metals and Trace metals**

The individuals were referred for venous blood sampling to assess the levels of Pb, Hg, Cd, Zn, Cu and Se. Plasma concentrations of Pb, Hg, Cd, Zn, Cu and Se were determined by Atomic Absorption Spectrophotometry (AAS). “Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA (American Public Health Association)” [15]. The specific light source lamp to the lamp housing was fixed, and the instrument was switched on. The source lamp was lighted and the wave length dial of the spectroscope was adjusted to the wavelength of the analytical line specified and set at an appropriate current value and slit-width. Using the supporting gas and the combustible gas specified, the mixture of gases was ignited and the gas flow rate and pressure was adjusted, then the zero adjustment was made after neutralizing the solvent into the flame. Using wavelengths of 228.8nm, 217.3nm, 253nm, 213.9nm, 324.8nm and for cadmium, lead, mercury, zinc, copper and selenium respectively the metals were analyzed and the instrument calibrated before use.

### **Digestion and analysis of samples for lead, mercury, cadmium, zinc, selenium and copper**

Fresh whole blood collected in special lead-free tube containing lithium heparin was used for blood lead, mercury, cadmium, zinc, selenium and copper assay. 1 mL of nitric acid was added to 1 mL of sample and mixed properly. The mixture was boiled at 100 °C for 30min. Distilled water was added to make up to 10 mL for analysis. The contents were mixed and filtered using filter paper to get a clear solution. Then the mixture was transferred for analysis. Blood samples were analyzed using FS240AA agilent atomic absorption spectroscopy [16].

### **Preparation of reference solutions**

A series of standard metal solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

### **Calculation**

The concentration for blood heavy metals were calculated using the following formulas;

$$\frac{\text{Reading of sample}}{\text{Reading of standard}} \times \text{Concentration of standards} = R$$

$$R \times \text{Final volume after digestion of sample} = \text{concentration of sample } (\mu\text{g/dl})$$

### **Conversion factor**

$$\text{ng/dl} \div 10 = \mu\text{g/dl}$$

$$\text{g/l} \times 100 = \mu\text{g/l}$$

## Statistical Analysis

All data generated from this study were subjected to statistical analysis. Mean, standard deviation, student's t-test, and correlations were analyzed using SPSS. Results were expressed as Mean  $\pm$  SD. The 5% (0.05) level of significance was adopted for significance.

## RESULTS

### Blood Heavy Metals and Trace Metals in Tea Consumers versus Controls

Blood Cd, Pb, Hg and Se were significantly higher ( $p=0.005$ ,  $p=0.003$ ,  $p=0.001$  and  $p=0.048$  respectively) in tea Consumers compared with controls. There were no significant difference in blood levels of Cu and Zn ( $p=0.923$  and  $p=0.784$  respectively) in tea Consumers compared with controls (Table 1).

**Table 1: Blood Heavy Metals and Trace Metals in Tea Consumers versus Controls**

VARIABLES (MEAN $\pm$ SD)	TEA CONSUMERS (n=20)	CONTROLS (n=20)	t-value	p-value
<b>Cd (<math>\mu\text{g/dl}</math>)</b>	0.083 $\pm$ 0.043	0.053 $\pm$ 0.020	3.144	0.005
Lower 95% C.I	0.063	0.043		
Upper 95% C.I	0.103	0.063		
<b>Pb(<math>\mu\text{g/dl}</math>)</b>	12.43 $\pm$ 3.77	9.76 $\pm$ 2.61	3.399	0.003
Lower 95% C.I	10.66	8.56		
Upper 95% C.I	14.19	11.00		
<b>Hg(<math>\mu\text{g/dl}</math>)</b>	0.510 $\pm$ 0.197	0.358 $\pm$ 0.190	3.946	0.001
Lower 95% C.I	0.417	0.269		
Upper 95% C.I	0.602	0.447		
<b>Cu (<math>\mu\text{g/dl}</math>)</b>	120.7 $\pm$ 5.36	119.1 $\pm$ 5.47	0.098	0.923
Lower 95% C.I	95.60	93.5		
Upper 95% C.I	145.80	144.7		
<b>Zn (<math>\mu\text{g/dl}</math>)</b>	69.55 $\pm$ 29.90	71.12 $\pm$ 10.14	-0.273	0.784

Lower 95% C.I	55.55	66.37		
Upper 95% C.I	83.55	75.86		
<b>Se (µg/dl)</b>	27.23±13.66	16.02±13.77	2.114	0.048
Lower 95% C.I	20.83	9.57		
Upper 95% C.I	33.62	22.48		

### Blood Heavy Metals and Trace Metals in Coffee Consumers versus Controls

Blood Cd, Pb, Hg, Zn and Se were significantly higher ( $p=0.001$ ,  $p=0.000$ ,  $p=0.012$ ,  $p=0.037$  and  $p=0.006$  respectively) in Coffee Consumers compared with Controls. There were no significant difference in blood Cu ( $p=0.222$ ) in Coffee Consumers compared with Controls (Table 2).

**Table 2: Blood Heavy Metals and Trace Metals in Coffee Consumers versus Controls**

VARIABLES (MEAN±SD)	COFFEE CONSUMERS (n=20)	CONTROLS (n=20)	t-value	p-value
<b>Cd (µg/dl)</b>	0.116±0.066	0.053±0.020	3.848	0.001
Lower 95% C.I	0.078	0.043		
Upper 95% C.I	0.141	0.063		
<b>Pb (µg/dl)</b>	12.28±2.83	9.78±2.61	14.231	0.000
Lower 95% C.I	10.96	8.56		
Upper 95% C.I	13.51	11.00		
<b>Hg (µg/dl)</b>	0.480±0.184	0.358±0.190	2.772	0.012
Lower 95% C.I	0.393	0.269		
Upper 95% C.I	0.566	0.447		
<b>Cu (µg/dl)</b>	106.80±29.77	119.10±54.76	-1.262	0.222
Lower 95% C.I	92.90	93.50		
Upper 95% C.I	120.70	144.70		
<b>Zn (µg/dl)</b>	91.61±40.09	71.12±10.14	2.238	0.037
Lower 95% C.I	72.84	66.37		
Upper 95% C.I	110.38	75.86		
<b>Se (µg/dl)</b>	25.57±13.28	16.02±13.77	3.076	0.006
Lower 95% C.I	19.35	9.57		
Upper 95% C.I	31.78	22.47		

### 4.3 Blood Heavy Metals and Trace Metals in Tea Consumers versus Coffee Consumers

There were no significant differences in blood levels of Cd, Pb, Hg, Cu, Zn and Se ( $p=0.154$ ,  $p=0.459$ ,  $p=0.662$ ,  $p=0.226$ ,  $p=0.080$  and  $p=0.705$  respectively) in Tea Consumers compared with Coffee Consumers (Table 3).

**Table 3: Blood Heavy Metals and Trace Metals in Tea Consumers versus Coffee Consumers**

<b>VARIABLES (MEAN±SD)</b>	<b>TEA CONSUMERS (n=20)</b>	<b>COFFEE CONSUMERS (n=20)</b>	<b>t-value</b>	<b>p-value</b>
<b>Cd (µg/dl)</b>	0.084± 0.043	0.110±0.066	-1.484	0.154
Lower 95% C.I	0.063	0.078		
Upper 95% C.I	0.104	0.141		
<b>Pb (µg/dl)</b>	12.43±3.77	12.28±2.83	0.193	0.459
Lower 95% C.I	10.66	10.96		
Upper 95% C.I	14.19	13.61		
<b>Hg (µg/dl)</b>	0.510±0.197	0.480±0.184	0.444	0.662
Lower 95% C.I	0.417	0.393		
Upper 95% C.I	0.602	0.566		
<b>Cu (µg/dl)</b>	120.70±53.66	106.80±29.77	1.246	0.228
Lower 95% C.I	95.60	92.90		
Upper 95% C.I	145.80	120.70		
<b>Zn (µg/dl)</b>	69.55±29.90	91.61±40.09	-1.849	0.080
Lower 95% C.I	55.55	72.84		
Upper 95% C.I	83.55	110.38		
<b>Se (µg/dl)</b>	27.23±13.66	25.57±13.28	0.384	0.705
Lower 95% C.I	20.83	19.35		
Upper 95% C.I	33.62	31.78		

#### 4.4 Correlation of Blood Pb with Heavy Metals and Trace Metals in Tea Consumers

There were significant negative correlations of blood Pb with Hg and Cu ( $r=-0.451$ ,  $p=0.046$  and  $r=-0.697$ ,  $p=0.001$  respectively) and no significant correlations of blood Pb with Cd, Zn and

Se( $r=-0.209$ ,  $p=0.377$ ;  $r=0.067$ ,  $p=0.778$  and  $r=0.246$ ,  $p=0.296$  respectively) in Tea Consumers (Table 4).

**Table 4: Correlation of Blood Pb with Heavy Metals and Trace Metals in Tea Consumers**

<b>Dependent Variables</b>	<b>N</b>	<b>r-value</b>	<b>p-value</b>
<b>Cd</b>	20	-0.209	0.377
<b>Hg</b>	20	-0.451	0.046
<b>Cu</b>	20	-0.697	0.001
<b>Zn</b>	20	0.067	0.778
<b>Se</b>	20	0.246	0.296

**Correlation of Blood Pb with Heavy Metals and Trace Metals in Coffee Consumers**

There were significant negative correlations of blood Pb with blood Cu and Zn ( $r=-0.656$ ,  $p=0.002$  and  $r=-0.690$ ,  $p=0.001$  respectively) and significant positive correlation of Pb with Hg ( $r=0.538$ ,  $p=0.017$ ) in Coffee Consumers. There were no significant correlation of blood Pb with Cd and Se ( $r=-0.204$ ,  $p=0.389$  and  $r=0.380$ ,  $p=0.099$  respectively) in Coffee Consumers (Table 5).

**Table 5: Correlation of Blood Pb with Heavy Metals and Trace Metals in Coffee Consumers**

<b>Dependent Variables</b>	<b>N</b>	<b>r-value</b>	<b>p-value</b>
<b>Cd</b>	20	-0.204	0.389
<b>Hg</b>	20	0.528	0.017
<b>Cu</b>	20	-0.656	0.002
<b>Zn</b>	20	-0.690	0.001
<b>Se</b>	20	0.380	0.099

### Correlation of Blood Cu with Heavy Metals and Trace Metals in Tea Consumers

There were significant negative correlation of blood Cu with Pb ( $r=-0.697$ ,  $p=0.001$ ), but significant positive correlation of blood Cu with Hg and Zn ( $r=0.662$ ,  $p=0.001$  and  $r=0.579$ ,  $p=0.007$  respectively) in Tea Consumers. There was no significant correlation of blood Cu with Cd ( $r=-0.104$ ,  $p=0.663$ ) in Tea Consumers (Table 6).

**Table 6: Correlation of Blood Cu with Heavy Metals and Trace Metals in Tea Consumers**

Dependent Variables	N	r-value	p-value
Cd	20	-0.104	0.663
Pb	20	-0.697	0.001
Hg	20	0.662	0.001
Zn	20	0.579	0.007
Se	20	-0.086	0.717

### 4.7 Correlation of Blood Cu with Heavy Metals and Trace Metals in Coffee Consumers

There were significant negative correlations of blood Cu with Pb and Hg ( $r=-0.656$ ,  $p=0.002$  and  $r=-0.590$ ,  $p=0.006$  respectively) and no significant correlations of blood Cu with Cd, Zn and Se ( $r=0.268$ ,  $p=0.254$ ;  $r=0.472$ ,  $p=0.036$  and  $r=-0.087$ ,  $p=0.715$  respectively) in Coffee Consumers (Table 7).

**Table 7: Correlation of Blood Cu with Heavy Metals and Trace Metals in Coffee Consumers**

<b>Dependent Variables</b>	<b>N</b>	<b>r-value</b>	<b>p-value</b>
<b>Cd</b>	20	0.268	0.253
<b>Pb</b>	20	-0.656	0.002
<b>Hg</b>	20	-0.590	0.006
<b>Zn</b>	20	0.472	0.036
<b>Se</b>	20	-0.087	0.715

## **DISCUSSION**

“After water, tea and coffee, respectively, ranked the 1st and 2nd most widely consumed beverage in the world” [17]. Coffee is planted in several countries [18], including Nigeria [19]. Coffee consumption varies widely according to geographical location, and seems to have distinct acute and long-term effects on health. Tea and coffee contains a number of beneficial health ingredients, such as trace elements, also contains undesired substances including heavy metals which can pose serious problems to human health because they are not biodegradable, remain in the environment and can become part of the food chain. The total metal concentration of the tea leaves and coffee beans depends on the influence of other factors, including the properties of the soil. Despite the limited extent of metal migration from these plants to their prepared beverage forms, their pollution with metals such as lead, cadmium or mercury is not indifferent to human health.

From the present study, tea consumption may cause an increase in blood cadmium, lead, mercury and selenium (table 1). Also, coffee consumption may lead to increase in blood cadmium, lead, mercury, zinc and selenium (table 2). Differences in the extent to which these

beverages influence blood heavy and trace metal levels are subtle according to data from the present research (table 3).

In the present study, the results of total contents of the studied heavy metals in both tea and coffee (Table 1-7) show the ability of tea and coffee plants to accumulate heavy metals which is reflected in the blood levels of these metals. “The reason behind the influence of tea and coffee consumption on blood concentrations of these metals resides in the fact that these metals can get into the tea and coffee plants through anthropogenic sources such as metal-containing pesticides, metal-containing fertilizers, and irrigation water with high levels of these metals. These metal contaminants may also get to tea plants through atmospheric deposition” [20]. “They eventually bioaccumulate higher up in the food chain and this makes humans to be at the receiving end. While there is a growing concern for adequate dietary availability of these elements, there has also been a growing awareness that excess exposure to nutritionally elements can be toxic” [21]. “It is worrisome that tea and coffee plants sometimes contains some beneficial elements in concentrations that are higher than would naturally occur” [20]. When this happens, they are said to be contaminated. “The chemical composition of coffee is very complex and depends on the place of origin and species/cultivar of the coffee plant” [22]. “The technology used in the preparation and industrial processing of green beans, as well as the methods consumers use to prepare their coffee modify the concentrations of the substances in the final product. Additionally, potential contamination may derive from package and storage” [23]. “Some batches of tea can also be significantly contaminated with toxic metals, but even trace amounts of cadmium, arsenic and mercury are a threat for humans. Their provisional tolerable weekly intake from all sources (PTWI – Provisional Tolerable Weekly Intake) is determined by the Joint FAO/WHO Expert Committee” [24].

This study showed that there were no significant differences in blood levels of Cd, Pb, Hg, Cu, Zn and Se in Tea Consumers compared with Coffee Consumers (Table 3). It can be deduced that both Tea and coffee consumption affect the blood levels of these metals in similar pattern. It has been previously documented that these heavy metals and trace metals are contained in both tea and coffee. “However, there is large variation in the elemental composition of tea [25] due to differences in climate and agricultural practices, including soil, water and fertilizers” [26].

“It has been previously noted that exposure to heavy metals above the permissible level can cause high blood pressure, fatigue, as well as kidney and neurological disorders. Heavy metals are also known to have harmful reproductive effects” [27].

## **Conclusion**

This study concludes that Tea and Coffee consumption may be associated with higher blood levels of some heavy metals and trace metals. Cultivation of tea and coffee plants should be done under strict control to check irrigation practices, use of fertilizers and herbicides which are a significant source of toxic metal exposure. Also, tea and coffee farms should be cited far from industries and factories in general so as to eliminate or reduce contamination .

## **Ethical approval and consent**

Before commencement, ethical approval and the informed consents of the participants were obtained before enrollment into the study. The participants were assured of confidentiality of the information obtained from them during and after the study.

## REFERENCES

- [1] Saud AL-Oud S. Heavy metal contents in tea and herb leaves. *Pakistanian Journal of Biological Science*. 2003; 6: 208-212.
- [2] Bunkova R, Marova I, Nemeč M. Antimutagenic properties of green tea. *Plant Foods for Human Nutrition*. 2005; 60: 25-29.
- [3] Karak T, Bhagat RM. Trace elements in tea leaves, made tea and tea infusion: A review. *Food Research International*. 2010; 43: 2234 – 2252.
- [4] Han WY, Shi MA, Ruan JY. “Arsenic, cadmium, chromium, cobalt, and copper in different types of Chinese tea.” *Bulletins of Environmental Contamination and Toxicology*. 2005; 75: 272-277.
- [5] Gramza A, Wojciak RW, Korczak J, Hes M, Wisniewska J, Krejpcio Z. Influence of the Fe and Cu presence in tea extracts on antioxidant activity. *Food Sci. Tech*. 2005.
- [6] Fernandez-Caceres P, Martin MJ, Pablos M, Gonzalez AG. Differentiation of tea (*Camellia sinensis*) varieties and their geographical origin according to their metal content. *J. Agric. Food Chem*. 2001; 49: 4775-4779.
- [7] Maurin O, Davis AP, Chester M, Mvungi EF, JaufeerallyFakim Y, Fay M.F. Towards a phylogeny for *Coffea* (Rubiaceae): identifying well-supported lineages based on nuclear and plastid DNA sequences. *Annals of Botany*. 2007; 100: 1565-1583.
- [8] Herraiz T, Chaparro C. Human monoamine oxidase enzyme inhibition by coffee and beta-carbolines norharman and harman isolated from coffee. *Life Sci*. 2006; 78: 795-802

- [9] Laitala VS, Kaprio J, Silventoinen K. Genetics of coffee consumption and its stability. *Addiction*. 2008; 103: 2054–2061.
- [10] Vink JM, Staphorsius AS, Boomsma DI. A genetic analysis of coffee consumption in a sample of Dutch twins. *Twin Research and Human Genetics*. 2009; 12: 127–131.
- [11] Gongora-Alfaro JL. Caffeine as a preventive drug for Parkinson's disease: epidemiologic evidence and experimental support. *Revista de neurologia*. 2010; 50: 221–229.
- [12] Hjellvik V, Tverdal A, Strøm H. Boiled coffee intake and subsequent risk for type 2 diabetes. *Epidemiology*. 2011; 22: 418–421.
- [13] Montagnana M, Favaloro EJ, Lippi G. Coffee intake and cardiovascular disease: virtue does not take center stage. *Seminars Thrombosis Hemostasis*. 2012; 38: 164–177.
- [14] Kumar A, Nair AGC, Reddly AVR, Garg AN. Availability of essential elements in India and US tea brands. *Food Chem*. 2005; 89: 441-448.
- [15] APHA (American Public Health Association). Standard methods for the examination of water and wastewater, 19th ed. Washington, DC, American Public Health Association/American Water Works Association/Water Pollution Control Federation. 1995.
- [16] Adrian WJ. A comparison of a wet pressure digestion method with other commonly used wet and dry-ashing methods. *Analyst*. 1973; 98, 213-216
- [17] Mcfarlane A, Mcfarlane I. *The Empire of tea*. The Overlook Press. 2004.
- [18] Greenop J. *The Lifestyle Food Index for South African Consumers*. Demeter Publications, Gauteng, South Africa. 1997.

- [19] Aroyeun SO, Odumbaku LO, Oloyede AO, Adebayo DA, Majolagbe IR. Effects of clonal variations on the chemical and organoleptic qualities of green tea processed on Mambilla highland, Nigeria. *Global Adv. Res. J. Fd. Sci. Tech.* 2012; 1(4):49-57.
- [20] Dawodu MO, Obimakinde O, Samuel O et al. Trace metal concentrations in some tea leaves consumed in Ibadan, Nigeria. *Afri J Agri Res.* 2013; 8(46): 5771-5775.
- [21] Goyer R, Golub M, Choudhury H, Hughes M, Kenyon E, Stifelman M. Issue paper on the human health effects of metals. U.S. Environmental Protection Agency, Risk Assessment Forum. 2004.
- [22] Farah A, de Paulis T, Trugo LC, Martin PR. Effect of roasting on the formation of chlorogenic acid lactones in coffee. *J Agric Food Chem.* 2005; 53(5):1505–1513. doi: 10.1021/jf048701t.
- [23] Arkadiusz N, Agnieszka T, Beata K, Jeremy SC, Maria L, Agnieszka K, Grażyna A. Concentrations of heavy metals (Mn, Co, Ni, Cr, Ag, Pb) in coffee. *ACTA BIOCHIMICA POLONICA.* 2013; 60(4): 623-627
- [24] Wojciechowska-Mazurek M, Starska K, Mania M, Rebeniak M, Karłowski K. Pierwi- astki szkodliwe dla zdrowia w herbacie - oce- na zagrożenia dla zdrowia. *Bromat. Chem. Toksykol.* 2010; 43, 233-239.
- [25] Costa LM, Gouveia ST, Nobrega JA. Comparing of heating extraction procedures for Al, Ca, Mg and Mn in Tea Samples. *Annal. Sci.* 2012; 18: 313-318.
- [26] Cabrera C, Gimenez R, Lopez MC. Determination of tea components with antioxidant activity. *J. Agric. Fd.* 2003; 51:4427-4435.

[27] Mubeen H, Naeem I, Taskeen A, Adiqe Z. Investigations of heavy metals in commercial spices brands. New York Science Journal. 2009; 2: 20-26.

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