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Appraisal of Selected Heavy Metals and Potential Health Risk in Commercial Tea Sold in South Western, Nigeria

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ABSTRACT

Tea is the most popular beverage all over the world and second to water. In recent decades, there has been an extensive increase in the level of heavy metal contamination in tea. The study aimed at evaluating the level of Cu, Co, Cr, Cd, Pb and Ni in some commercial green and black tea sold within Ibadan, Nigeria. Seven of the popular tea brands (Halmak green tea, Strong black tea, Lipton, Herb tea, Top tea, City tea and Kirkland green tea) among consumers were purchased in the open market. Heavy metals were quantified with Atomic Absorption spectrophotometer. Total Cu ranged between 11.02 and 24.12mgkg^{-1} while total Cr and Ni were within the range of 5.50-23.00 and 3.50-8.00 mgkg^{-1} respectively. After infusion of tea samples, 40.00, 37.73 and 16.25% of total Cu in Halmak (HM) green tea, Stong black tea (ST) and Lipton (LP) black tea was leached into tea drink respectively while 69.23, 16.22 and 15.20 % of total Ni in HM green tea, STG black tea and LP black tea was leached into tea drink respectively. The values of estimated daily intake and target hazard quotient obtained for the investigated commercial tea were within safe limit. Hence, regular consumers of the products are not likely to suffer health threat from metal contamination.

Keywords: Tea, heavy metal, health risk, contamination

INTRODUCTION

Tea beverage is obtained from the infusion of dried leaves of *Camellia sinensis* with boiled water. It is one of the most popular beverages all over the world. Types of tea that are regularly consumed in the world can be grouped into two major categories namely green and black tea which is based on the processing method. Green and black tea account for 20 and 80% respectively (Hussain *et al*. 2006).The black tea processing method involves leaf harvesting, withering, rolling, fermentation and drying whereas that for green tea includes leaf harvesting, roasting, rolling and drying (Sood *et al.*, 2004). Enzymatic oxidation of tea polyphenols occurs during fermentation, which produces theaflavin, thearubigins and some volatile organic compounds all of which are responsible for the characteristic odour and aroma of tea (Chen *et al.*, 2007). Black tea is much lower in catechins (10-12% dry weight) due to fermentation; however, it contains oxidative products, including theaflavins (3-6%, dry weight) and thearubigins (10-20%, dry weight) which gives black tea its dark colour (Haslam, 2003).

According to available reports, some of the health benefits of tea consumption includes reduction of cholesterol in the cardiovascular system, coronary artery disease, ameliorating hypertension and inflammation. Green tea has been reported to have reduced total and low density cholesterol significantly in a meta-analysis (Zheng *et al*. 2011). Green tea may reduce the risk of breast cancer development. The effect has been ascribed mostly to the phytochemicals which can modify the metabolism of estrogens (Fuhrman et al. 2013). The phytochemicals include polyphenols (Catechins) such as epigallocatechin gallate (EGCG), which appears to be most potent, epigallocatechin, epicatechin gallate and epicatechin. A recent meta-analysis shows that the consumption of green tea and coffee appears to reduce esophageal cancer but black tea does not (Zheng et al. 2013). With regard to diabetes, green tea may result in lower fastening glucose levels with no significant HbAlc changes (Maeda-Yamamoto, 2013). There is evidence that insulin resistance may be improved by the antioxidant protective function of the polyphenols (Yan et al. 2012). The consumption of 4 or more cups of tea may provide some protection from depression (Pham et al. 2013).

Tea consumption is associated with heavy metal intake due to their presence in tea materials as contaminants. Hg, As, Cd are considered as contaminants by European commission (2006) and other toxic elements such as Al, Cr and Ni have been found in tea leaves (Han et al., 2006). Heavy metals possess biochemical importance as trace elements but the biotoxic implication of many of them in human biochemistry at excessive dose is of great concern. Pb and Hg affect the development of children and Pb results in a significant decrease in social and average

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developmental quotients (Tang *et al*., 2008). Heavy metals enter human bodies via food, drinking water and air (Lenntech, 2008). Metallic constituents of tea leaves are usually different according to the type (Green or black) and geographical sources (Fernandez-Caceres *et al*. 2001). Depending on the origin of tea leaves, heavy metals accumulation can be derived naturally by soil contamination, use of pesticides and fertilizers (Ebadi *et al*. 2005). Plants can take up heavy metals from soil and under certain soil conditions, high levels can be accumulated in the leaves and other vegetative parts of the plant. For example, a concentration of 23,000 ppm of Al has been reported in tea leaves. That was considered higher than other plants that do not normally exceed 200 ppm (Atta, 1995). Tea bush is known to accumulate trace metals (Basque *et al*. 1990).

Accumulation of heavy metals can have middle term and long term health risk and strict periodic surveillance of these contaminants is therefore advisable (Cabrera *et al*. 1995). The World Health Organization (WHO, 1998) recommends that medicinal plants which are used as raw materials for finished products may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination. Due to the significance of tea quality with reference to heavy metal contamination, each country sets its own allowable limit for certain heavy metals in tea leaves. For instance, in Europe $0.5mgkg^{-1}$ of Pb is allowed in tea leaves while Japan set $20mgkg^{-1}$ and China $2mgkg^{-1}$. Considering the approximately 18-20 billion tea cups consumed daily worldwide (Kirk-Othmer, 1995), the interest of many researchers all over the world is drawn to evaluating the level of heavy metals present in tea from various geographical locations of the world. Sahito *et al*. (2005) reported the contents of 15 essential trace and toxic elements in some green tea samples and their infusion. Karimi *et al*. (2008) worked on the concentrations and health risks of metals in tea samples marketed in Iran, Garba *et al* (2015) assessed heavy metals in selected tea brands marketed in Zaria, Nigeria. Considering the fact that, most of the commercial tea brands sold in southwestern Nigeria are imported from other countries with no information on heavy metal content of the products, it was imperative to evaluate the level of heavy metal contamination in some commercial tea brands with a view to evaluating the potential health risk in adults who consume the products on regular basis.

MATERIALS AND METHODS

Sample collection

Eight (8) commonly consumed tea brands were purchased from selected markets within Ibadan metropolis in Oyo State, Nigeria. The tea brands comprised of three (3) Green tea, and five (5) Black tea.

Sample treatment and heavy metal quantification

One gram (1g) of each tea sample was digested in 20ml of 3:1 nitric and perchloric acids in a micro Kjeldahl flask until the mixture became colorless. After sample digestion, the digest was allowed to cool and thereafter made up to 100ml mark with distilled water in a 100ml capacity volumetric flask. Three replicates were made for each sample. The quantification of heavy metals was made on the basis of calibration standard curves using Buck 260 Atomic Absorption spectrophotometer

Quantification of heavy metals in tea beverage as influenced by temperature

Three samples with the highest heavy metal concentration were used for quantifying the percentage of total metal in tea samples that is leached (bio-available) into tea beverage. Each tea bag of known weight was placed in separate mugs and hot water of three levels of hotness (60^oC, 70^oC and 80^oC) was poured into the mugs. After 10 minutes of tea steeping, the tea bags were removed and tea beverage was thereafter analyzed for heavy metal content.

Brewing duration and heavy metal leachability

Each tea sample was brewed for 5, 10 and 15 minutes to evaluate the effect of brewing time on metal leachability in tea beverage. At the completion of brewing, the tea bags were removed from the various mugs while the extracts were analyzed for heavy metal concentration.

Effects of pH on heavy metal leachability in tea beverage

In order to assess the relationship between water pH and heavy metal solubility in tea infusion, three levels of pH (6, 7 and 8) were established by the addition of drops of 0.1M HCl to 100ml distilled water in a beaker to attain pH 6 while pH 7 and pH 8 were attained by the addition of drops of 0.1M NaOH. The different water samples were subsequently boiled for tea infusion. After infusing the tea for 10 minutes, the bags were removed from the mugs and tea extracts were sent to the laboratory for the determination of Cu, Co, Cr, Cd, Pb and Ni. The experiment was replicated thrice.

Quality control

For every metal analysis, samples were prepared in triplicates. All glass wares used in the course of the study were soaked in 10% HNO_3 for 18 hours and later rinsed with deionized water to prevent contamination from glass ware. The Atomic absorption spectrometer was re-calibrated after analyzing ten samples. Accuracy of the analytical procedure was determined by introducing known amount of heavy metal and re-analyzed. Spiked recovery were 98% for Pb, 97% for Co, 99% for Cu, 98% for Cd, 97.5% for Cr and 98% for Ni. Blank solutions were prepared in order to make room for contamination from analytical reagents used. The detection and quantification limits (LOD and LOQ) were obtained on the basis of the concentration of the analyte that produced signal-to noise ratio of 3x standard deviation of low concentration/slope of the calibration line and 10x standard deviation of low concentration/slope of the calibration line respectively. The detection limits in mg kg^{-1} of the heavy metals were Ni (0.003), Pb (0.05), Co (0.002), Cu (0.003), Cr (0.002) and Cd (0.002.

Determination of Estimated Daily Intake

In order to appraise the health risk associated with heavy metal contamination in the studied commercial tea, estimated daily intake of metals was calculated using the formula:

$$EDI = \frac{C \times CR \times EF}{Bw} \quad (1)$$

Where EDI = estimated daily intake: It is generally the number of milligrams of the contaminant that enters the body for each kilogram of body weight (mg/kg/day).

C = concentration of the contaminant in the exposure pathway being considered (mg/g) of food.

CR= contact rate; Amount of food taken per day (g/day) (tea of 10g per day was considered for each adult in the study)

EF = Exposure Frequency: This number indicates how often the individual is exposed during a year and the number of years (365 days for 10 years was considered in the study)

Bw = Body weight (kg) (Since the study was concentrated on adults, an average weight of 60kg was used)

Determination of Target Hazard Quotient

The health risk associated with heavy metals exposure through the consumption of chocolate was evaluated using the target hazard quotient (THQ) (Liu *et al* 2006). Though THQ does not provide quantitative estimate on the probability of an exposed population experiencing a reverse health effect, it offers indication of the risk level due to contaminant exposure. The THQ index can be defined as the ratio of determined dose of a pollutant to the reference dose (RfD) (µg/kg bw/d).The following were the values used for oral reference dose: Cu (0.040), Ni (0.02) (USEPA, 2010).

$$THQ = \frac{EF \times FD \times DIM}{RfD \times W \times T} \quad (2)$$

Where EF is the exposure frequency (365d/year), FD is the exposure duration (10 years), DIM is the daily metal ingestion (mg/person/day), RfD is the oral reference dose (mg/kg/day), W is the average body weight (60kg), T is the average exposure time for non-carcinogen (365 days/year x number of exposure years). THQ is a highly conservative and relative index (Wang *et al*, 2005). If THQ is less than 1, there is no obvious risk from the substance over a lifetime exposure, while if THQ is higher than 1, the toxicant may produce an adverse effect. The higher the THQ value, the higher the probability of experiencing long term carcinogenic effects. (Song *et al*. 2009)

Statistical analysis

All the data generated from the experiments were subjected to statistical analysis using SPSS version 17. Means were separated using analysis of variance (ANOVA).

RESULTS

Total concentration of heavy metals in tea samples

Result (Figure 1) shows that total Cu in the studied commercial tea brands ranged between 11.02 and 24.12mgkg^{-1} with a mean value of 17.77mgkg^{-1}. HB black tea had the lowest value while KKL green tea had the highest level of total Cu. Cr concentration in the examined tea brands ranged between 5.50 and 23.00mgkg-1 with a mean value of 15.80mgkg-1.Among the heavy metals investigated, Ni had the lowest concentration in the tea samples. The concentration of total Ni ranged between 3.50 and 9.00mgkg-1 with an average value of 6.59mgkg-1. STG black tea had the highest Ni value while HB tea had the lowest Ni concentration.

Bioavailability of heavy metals in aqueous tea extract

Results from tea infusion shows that 16.25 to 40.11% of total Cu in tea samples got leached into aqueous tea extract (Table 1). The highest concentration of Cu leached into tea extract was from green tea. Among the three commercial tea brands considered for the determination of heavy metal leachability in tea infusion, the green tea (HM green tea) with total Cu (18.00mgkg-1) released concentration of 7.22mg Cu per kilogram of tea sample while STG black tea with total Cu (18.50mgkg-1) and LP black tea with total Cu (18.21mgkg-1) released 6.98 and 2.96mg Cu per kilogram of tea sample respectively. The study on the impact of temperature on heavy metal leachability in tea infusion showed direct proportionality between hotness of water and leachable metals in aqueous tea extract.

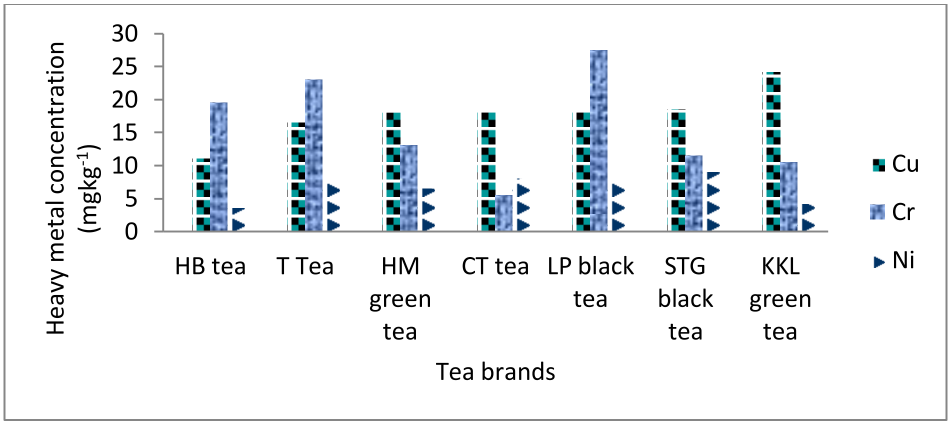

Figure 1: Total content of heavy metals in commercial tea brands.

Result (Table 1) showed that 40.11% of the total Cu in HM green tea was leached into tea drink when tea sample was infused in hot water of 80^{o}C. On the other hand, 32.39% and 25.00% of total Cu was leached into aqueous tea extract when the green tea was infused in hot water of 70^{o}C and 60^{o}C respectively. Similar observation was made in STG black tea in which 37.73, 22.00 and 14.00% of total Cu in tea sample was leached into tea beverage after brewing the tea with hot water of 80, 70 and 60^{o}C respectively. LP black tea had 16.25, 14.99 and 13.73% of total Cu in tea sample leached into tea beverage when prepared with hot water of 80, 70 and 60^{o}C respectively.

Table 1: Water temperature and heavy metal leachability in tea beverage

Infusion Temp (°C)	Percentages of total Cu leached in tea drink			Percentages of total Ni leached in tea drink		
	HM G tea	STG Btea	LP Btea	HM Gtea	STG Btea	LP Btea
80	40.11^{a}	37.73^{a}	16.22^{a}	69.23^{a}	16.22^{a}	15.20^{a}
70	32.39^{b}	22.00^{b}	14.99^{a}	51.23^{b}	9.67^{b}	12.13^{a}
60	25.00^{c}	14.00^{c}	13.73^{a}	47.69^{b}	3.22^{c}	6.13^{b}

Key: different alphabets on same column are significantly different (P<0.05)
HM:Halmak; STG:Strong; LP: Lipton
G tea= Green tea; B tea= Black tea

Bioavailability of Ni in infused tea was considerably high in the green tea compared with black tea. In HM green tea, 69.23% of total Ni in tea sample was leached into aqueous tea extract prepared with hot water of 80^{o}C while 51.23 and 47.69% of total Ni was leached into aqueous tea brewed with hot water of 70 and 60^{o}C respectively. In the case of STG black tea, 16.22, 9.67 and 3.22% of total Ni was leached into tea extract when sample was brewed in hot water of 80, 70 and 60^{o}C respectively. Similarly, 15.20, 12.13 and 6.13% of total Ni was extracted into tea beverage when LP black tea was brewed with hot water of 80, 70 and 60^{o}C respectively.

Infusion duration and metal bioavailability

The determination of the relationship between duration of tea brewing and heavy metal bioavailability in tea beverage showed positive correlation between brewing time and leachability of heavy metals. Result (Table 2) shows that 21.17% of total Cu was leached into tea beverage when HM green tea bag steeped in hot water for 5 minutes. Steeping tea bag for 10 minutes resulted in 21.61 % of total Cu leached into aqueous tea while at a steeping time of 15 minutes, 39% of the total Cu in tea sample had become bioavailable in tea drink. A similar trend of Cu leach in tea drink was observed in STG black tea in which 14.16, 28.32 and 37% of total Cu in tea material was extracted into tea drink when subjected to steeping period of 5, 10 and 15 minutes respectively. In the case of LP black tea, 10.76, 14.99 and 16.20% of total Cu was leached into tea beverage after steeping period of 5, 10 and 15 minutes respectively. Bioavailability of Cu in aqueous tea followed a decreasing order of HM green tea>STG black >LP black tea.

Table 2: Effect of steeping duration on heavy metal leachability in tea beverage

Steeping time (mins)	Percentages of total Cu leached in tea drink			Percentages of total Ni leached in tea drink		
	HM G tea	STG Btea	LP Btea	HM G tea	STG Btea	LP Btea
5	21.17[b]	14.16[c]	10.76[bc]	55.54b[c]	0	6.67c
10	21.61[b]	28.32[b]	14.99[ab]	60.31[ab]	10.67[b]	11.60[b]
15	39.00[a]	37.29[a]	16.20[a]	64.15[a]	16.00[a]	14.13[a]

Key: different alphabets on same column are significantly different ($P<0.05$)
HM: Halmak; STG: Strong; LP: Lipton
G tea= Green tea; B tea= Black tea

Bioavailability of Ni in brewed tea was observed to increase as the steeping time increased. In HM green tea, 55.54, 60.31 and 64.15% of total Ni in tea material was leached into aqueous tea extract when steeped for 5, 10 and 15 minutes respectively (Table 2). In the case of LP black tea 6.67, 11.60 and 14.13% of total Ni in tea sample was bioavailable in tea drink after steeping it for 5, 10 and 15 minutes respectively. On the other hand, no detectable Ni was recorded in aqueous tea extract when STG black tea spent 5 minutes in the hot water. However, at 10 and 15 minutes steeping time, 10.67 and 16.00% of total Ni in tea sample was leached into tea drink respectively.

pH of water and heavy metal leachability in tea beverage

Experiment to estimate the effect of pH of water and heavy metal leachability in tea infusion shows that, initial pH of water before boiling does not have any significant effect on heavy metal leachability from tea material into tea drink. In an attempt to evaluate this, pH of water was adjusted to 6 and 7 by the addition of drops of 0.1M HCl to water while pH 8 was attained by addition of drops of 0.1M NaOH before boiling. In HM green tea, 34.44, 33.94 and 29.33 % of total Cu in tea sample was leached into tea drink when water with initial pH of 6, 7 and 8 was respectively used in tea drink preparation (Table 3). In STG black tea, 15.67, 12.59 and 14.16% of total Cu was extracted into aqueous tea when water of pH 6, 7 and 8 was respectively used. Similarly, 14.88, 16.14 and 15.76% of total Cu was bioavailable in the drink when water with pH 6, 7 and 8 was respectively used.

Table 3: Effect of water pH on heavy metal leachability in tea beverage

Water pH	Percentages of total Cu leached in tea drink			Percentages of total Ni leached in tea drink		
	HM G tea	STG B tea	LP B tea	HM G tea	STG B tea	LP B tea
6	34.44[a]	15.67[a]	14.88[a]	59.85[a]	8.67[a]	13.60[a]
7	33.94[a]	13.59[a]	16.14[a]	60.00[a]	7.22[a]	13.06[a]
8	32.33[a]	14.16[a]	15.76[a]	57.54[a]	6.78[a]	13.33[a]

Key: different alphabets on same column are significantly different ($P<0.05$)
HM: Halmak; STG: Strong; LP: Lipton
G tea= Green tea; B tea= Black tea

Estimated daily intake of heavy metals

Since the study was targeted at adults who are regular consumers of tea drinks, it is important to assess the daily intake of metals from tea and compare the values with the oral estimated daily intake set by international bodies which deal with health safety. The calculated estimated daily intake of Cu ranged between 1.80 and 4.38µgkg^{-1}bwd^{-1} with a mean value of 3.48 µgkg^{-1}bwd^{-1}(Figure 2).

Estimated daily intake obtained for Ni in the studied tea samples ranged between 0.69 and 2.74 µgkg^{-1}bwd^{-1} with a mean value of 1.44 µgkg^{-1}bwd^{-1} (Figure 2). HM green tea had the highest EDI value.

Target hazard quotient (THQ)

The calculated target hazard quotient for Cu ranged between 0.012 and 0.030 with a mean value of 0.024 (Figure 2) while that of Ni ranged between 0.010 and 0.038 with a mean value of 0.020.

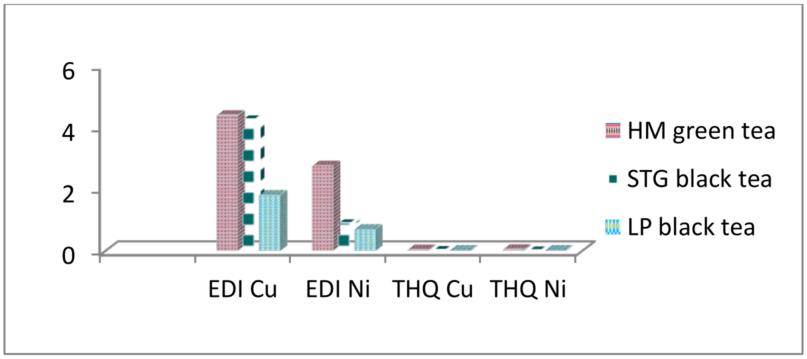

Figure 2: Estimated daily intake and target hazard quotients of heavy metals

DISCUSSION

The variation in Cu concentration among the tea brands is attributable to differences in tea types, manufacturing equipment and geographical origin. The obtained mean value of Cu concentration in the study was higher than 13.20mgkg^{-1}and 16.18mgkg^{-1}reported by Naithani and Kakkar, (2005) and Soliman, (2016) respectively while it is lower than the values 27.70 mgkg^{-1}, 24.80mgkg^{-1} and 18.10mgkg^{-1} reported by Matsuura *et al* (2001), Narin *et al*. (2004) and Ashraf and Mian (2008) respectively in commercial tea. Copper is known to be one of the metals commonly found in tea. It is central to polyphenol oxidase enzyme (Soliman, 2016). According to Seenivasan *et al*. (2008), it is one of the major elements next to Al and Zn in tea. Although, Cu is an essential enzymatic element for normal plant growth and development, it can become phytotoxic at excessive level. Accumulation of Cu in the body can result in hyperactivity in autistic children. It can cause stuttering, insomnia and hypertension (Hussain *et al*. 2006).

The investigation of metal leachability in tea infusion showed a significant release of Cu from green tea sample into aqueous tea extract compared with the various black tea samples. Result of heavy metal analysis in tea samples showed that the green tea sample had lower concentration of total Cu compared with the black tea samples. However, after tea infusion, the percentage of total Cu released into aqueous tea extract was highest in the green tea. The observed higher leachability of Cu in green tea can be explained on the basis of the formation of chelate between oxidative products of tannin and Cu during fermentation of black tea. Owing to the fact that green tea processing does not undergo fermentation/oxidation, the oxidative products which would have formed complex with metals as obtained in black tea are lacking. Hence, Cu in green tea is less bound compared with black tea. As a result, the metal was easily leached into aqueous tea extract. Schwalfenberg *et al*. (2013) reported higher leaching of Pb from green tea than counterpart black tea in aqueous tea extract. In the course of the study, result showed that, Cd and Pb were not detected in aqueous extract after tea infusion though present in commercial tea samples. It then suggests that, the two metals were tightly bound to tea materials resulting in low solubility in hot water. The observation is in agreement with the report of Gebretsadik and Chandravanshi (2010) who found Cd and Pb below detection limit in Ethiopian commercial black tea. Due to the fact that polyphenols poorlyabsorb chemical species (Manach *et al*., 2004), Pb is firmly bound to them and may not be available in tea infusion (Arpadjan *et al*., 2008).

The concentration range of Cr in the commercial tea was higher than the values reported by Hussain *et al*. (2006). In their report, the concentration range of Cr was between 0.25 and 14.00mgkg^{-1}. Mandiwana *et al*. (2011) reported Cr concentration of 0.7mgkg^{-1} in Chinese tea. Falahi and Hedaiati (2013) reported mean concentration of 8.2mgkg^{-1} Cr in commercial tea while Li *et al*., (2013) found higher total Cr concentration in black tea compared with those in green tea. Cr (III) plays important role in the body while other forms of Cr are toxic with no physiological function in human body. Cr (III) is present in the pancreas which produces insulin (Hussain *et al*. (2006). One usable form of Cr is the Glucose Tolerance factor (GTF) (Gala, 1984). GTF is essential for the efficient use of insulin but its deficiency in the body decreases the efficiency of insulin and increase sugar and cholesterol in the blood (Rajurkar and Perdeshi, 1997). Though Cr (III) was detected in all the tea samples, infusion of the various tea brands did not produce detectable Cr in aqueous extracts. The absence of Cr (III) in aqueous extract was due to complex formation with phytochemicals within tea material (Soomro *et al*. 2008). According to Brzezicha-Cirocka *et al*. (2015), tannic acid as well as tannins reacts with elements in tea leaves. Precipitation of chelates significantly decreases the concentration of metals in brewed tea extract. Dambiec *et al*. (2013) suggested that teas characterized with lower tannin levels reveal better percentage of leaching of particular elements to tea infusion. On the other hand, Seenivasan *et al* (2008) reported low Cr concentration (0.04-0.42 $mgkg^{-1}$) in black tea infusions brewed for 6 min while a total concentration of Cr (2.11$mgkg^{-1}$) was detected in tea infusion obtained from leaves containing a total concentration of 5.63$mgkg^{-1}$ (Chen *et al*., 2014).

The variation in the concentration of Ni among all the investigated tea samples was an indication that teas materials from different geographical origin do not have same level of metal content. The report of Hussain *et al*. (2006) showed that Ni in commercial tea in Pakistan ranged between 0 and 2.5$mgkg^{-1}$. Scancar et *al*., (2013) reported a total concentration of 6.19 to 14.4$mgkg^{-1}$ Ni in tea leaves. The reported values were lower than what was obtained in the present study. The mean value obtained for total Ni in the study is comparable with the value (5.10$mgkg^{-1}$) reported by Al-Othman *et al*. (2012) in commercial tea. In all the tea samples, the percent Ni leached into aqueous tea extract increased with temperature of water. The percentage of total Ni leached from green tea into aqueous tea was significantly ($p< 0.05$) higher than those from black tea (Table 1). On the contrary, Shen and Chen (2008) found that the percentage of heavy metal released into hot water infusion was higher in black than green tea. Ni is required in minute quantity in the body and it is mostly present in the pancreas where it plays vital role in the production of insulin. Its deficiency can result in the disorder of the liver (Nath, 1986). However, increased concentration of Ni in the kidney can lead to kidney damage. The study on evaluation of brewing time and heavy metal bioavailability in aqueous tea extract showed direct proportionality between brewing time and heavy metal concentration in tea drink. The observed increase in metal concentration in brewed tea is in consonance with the report of Schwalfenberg *et al*. (2013) where concentration of Pb, Al, As and Cd leached into aqueous tea extract increased with steeping time. According to the report, steeping tea for longer periods of time over 3 minutes increases the levels of heavy metal contaminants by 10 to 50%. In addition, Pb (0.802-1.367 mg/kg) and Cd (0.135-0.343mg/kg) have been found in black tea infused for 5, 15 and 60 min (Shekoohiyan *et al*., 2012). The transfer of metals from tea to brew depends on infusion time (Yuan *et al*., 2007; Shekoohiyan *et al*., 2012; Schwalfenberg *et al*., 2013; Al-Masri *et al*., 2004). The experiment designed to evaluate the effect of pH on heavy metal leachability in tea infusion showed that the initial pH of water before boiling has no significant effect on the amount of heavy metal leached from tea material into aqueous tea extract. This might be due to adjustment that occurs in pH of water as temperature increases from 20°C to 100°C in the process of heat application. A study carried out by Edward, (1962) established that whatever the pH of the particular sample of water at 20°C, it rises gradually until at 100°C.

Estimated daily intake is the estimate of the amount of a substance in food or drinking water, expressed on a body mass basis which can be ingested daily over a lifetime without appreciable health risk. Under regular consumption of tea, it is necessary to evaluate the daily intake of heavy metals and compare the values with safe limit set by international organizations for health safety. The estimated daily intake values were obtained with reference to adults on average weight of 60kg who consume one tea bag (10g) per day. The estimated daily intake of Cu obtained in the study (Figure 2) is higher than the value reported for Cu by Soliman (2016) in a study on commercial tea in Egypt. However, the obtained values of estimated daily intake for Cu in the studied tea samples are far below 500 $\mu gkg^{-1}bwd^{-1}$which is the maximal tolerable daily intake set by the Joint Expert Committee on food Additives (JECFA). The values are also lower than 160$\mu gkg^{-1}bwd^{-1}$ which is the safe upper level recommended by EVM (2003).

Result (Figure 2) indicates that estimated daily intake of nickel in HM green tea is more than 50% of tolerable daily intake of Ni (5 $\mu gkg^{-1}bwd^{-1}$) set by the World Health Organization. By implication, the consumption of 1 tea bag (10g) of HM green tea provides 54.8% of total tolerable daily intake for Ni. It implied that, an adult who consumes this particular tea brand (HM green tea) on daily basis may end up exceeding the tolerable daily intake of Ni (5 $\mu gkg^{-1}bwd^{-1}$) if other food sources taken on daily basis by the individual have considerable amount of Ni. It therefore suggests that caution has to be taken with respect to steeping time whenever green tea is brewed in order to limit the concentration of heavy metals that get leached into tea drinks. The high value of calculated EDI for Ni compared with Cu is due to its higher leachability in hot water as revealed by the study. Table 1 shows that, 69.23% of total Ni was leached into tea drink while 40.11% of total Cu was leached into the drink in 80°C hot water. According to the report of Salahinejad and Aflaki, (2010) Ni had the highest rates of solubility in tea infusion compared with Cr, Pb and Cd with lower rates of solubility. The low value of THQ obtained for Cu and Ni in the study suggests that consumption of 1 tea bag (10g) on a daily basis by an adult who weighs 60kg unlikely to pose health threat on regular consumer of the products since the calculated THQ values are less than unity. However, indiscriminate consumption should be discouraged considering the impact of excessive Ni on the liver and kidney.

CONCLUSION

The study shows that commercial tea brands from different geographical regions contain variable levels of heavy metals. Leachability of heavy metals from tea materials into aqueous tea extract is higher in green tea compared with black tea. The study revealed that the longer a tea material stays in hot water, the more the concentration of heavy metals that is extracted from tea sample into tea drink. Due to high leachability of nickel from green tea into tea beverage, steeping of tea material in hot water should not exceed 5 minutes. The high extractability of nickel in green tea infusion is a signal that, indiscriminate consumption of tea could significantly raise dietary intake of nickel. The low values of the estimated daily intake and target hazard quotient obtained from the study implies that, daily consumption of one bag (10g) of any of the investigated commercial tea cannot pose health threat from heavy metal contamination.

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