International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2013 IJFAS Journal-2013-2-13/383-387
ISSN 2322-4134 ©2013 IJFAS

Contaminations of Heavy Metals in Tea Leaves, Finished Tea Products and Liqour in Gilan Province, Iran

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ABSTRACT: Tea is drunk by most of Iranian people. It is widely cultivated and consumed in the north of Iran. Although tea is rich in many trace inorganic elements, probably some toxic elements also are present in tea leaves due to polluted soil, application of pesticides, industrial activities or fertilizers. As the significant amount of tea consumed (several times in a day) it was necessary to determine the toxic metal contents. To assess the levels of Zinc, Copper, Cadmium and Lead in Tea leaves and finished tea products, 100 samples after harvesting from 12 major Tea farms mostly in 5 cities (Ladhijan, Siahkal, Langerud, Rudsar , Foman) in Gilan province in the north of Iran and 50 samples from 5 Tea Factories in 2012 were randomly collected. Soil samples were collected at each place and time the tea leaves were plucked and soil mixed thoroughly to give a represent able fraction. Analysis of variance (ANOVA) was employed to detect significances among samples from different forms of tea samples (Black tea, tea leaves). Soil results showed that the Cadmium contents in the soil samples collected from all farms in 5 cities in the Gilan province are higher than WHO permissible levels and the mean content of Cadmium in all tea leaves samples is over, while Lead, copper and zinc contents were below the maximum permitted level for making tea has the significant role ($p < 0.003$) in decreasing the cadmium content.

Keywords**:** Tea leaves, finished tea products, Cadmium, contamination

INTRODUCTION

Tea is drunk by most of Iranian people. It is widely cultivated and consumed in the north of Iran. Recent decades, tea could be beneficial for hypertensive patients due to its manganese-rich content and its potassium amount. On the other hand, presence of heavy metal concentration in food and beverages has restricted by several countries according to food laws (Bosque et al. ,1986; IARC. 1999) .Although tea is rich in many trace inorganic elements (Shen and Chen. 2008; Mehmet et al., 2008), probably some toxic elements also are present in tea leaves due to polluted soil, application of pesticides, industrial activities or fertilizers. A few reports are available to confirm the presence of heavy metals such as Cd and Pb in tea(Onianwa. Et al., 1999; Al-Oud. And Pak. 2003; Yemane . et al., 2008).There is a little information available about the safety of tea leaves and finished products with respect to heavy metal (Lead and Cadmium) contamination. With the increasing use of tea and rapid expansion of global market for it, the safety and quality of tea leaves and finished tea products have become a major concern for health authorities, tea manufacturers and the public. The quality of a plant product is determined by the prevailing conditions during growth and good agricultural practices (WHO. 1998) . In fact for tea producers, an integral part of quality control consists of: Method of collection, temperature of processing, exposure of light, availability of water, nutrients, drying, packaging, transportation of raw tea leaves and storage can mostly affect the quality. Due to significant amount of tea consuming (several times in a day) there is need for an adequate quality assurance system. This assurance is also required during cultivation, harvesting, primary processing, handling, storage, packaging and distribution (Joint FAO/WHO 2005). Tea plantations are found in northern Iran in Gilan and

Mazandaran provinces where mist and high humidity ensure that the leaves grow slowly and remain tender. Most of the tea consumed in Iran is grown in these northern regions.

Unfortunately, Cu, Zn, Cd and Pb content of consumed tea in Iran were not been Studied in Abidjan (Gilan province). Only, one study determined Cadmium and Lead Contents in some Black Tea brands and Tea Liquor from Iran in 2010 (Zazouli. et al., 2010). Therefore, the objectives of this study were to determine and monitor Zn, Cu,Cd and Pb contents of in Tea leaves (*Camellia sinensis* L) and finished tea products in Gilan , as the significant amount of tea consumed (several times in a day) it was necessary to determine the toxic metal contents and to compare the release of Cd and Pb from them by infusion. Finally, the results are compared with other related reports in this respect.

MATERIALS AND METHODS

Sampling method

To assess the levels of Zinc, Copper, Cadmium and Lead in Tea leaves and finished tea products, 100 samples after harvesting from 12 major Tea farms mostly in 5 cities (Ladhijan, Siahkal, Langerud, Rudsar, Foman) in Gilan province in the north of Iran and 50 samples from 7 Tea Factories (Zaboli, Fajr,Qaem,Roshan Bahar, Fatehkar, Sargole Sarcheshmeh, Golkhal Sarcheshme) in 2012 were randomly collected. Soil samples were collected at each place and time the tea leaves were plucked and soil mixed thoroughly to give a represent able fraction.

Tea samples

All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid at least overnight, and rinsed abundantly in deionized water and dried in such a manner to ensure that any contamination does not occur. Five-point calibration curves (five standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \geq 0.996$ before the start of the sample analysis. To assess the levels of Zinc, Copper, Cadmium and Lead in Tea leaves and finished tea products, 100 samples after harvesting and 50 samples from Tea Factory were randomly collected from 12major Tea farms in 2012 from Gilan province were analyzed. Approximately 0.5 g of each sample (Tea leaves and finished tea products), accurately weighed and digested in accordance with U.S. Analar grade nitric acid, hydrogen peroxide (about 30%) and concentrated Hydrochloric acid were used for the digestion. As Tea leaves contain a number of organic substances of different stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution, which can disturb spectrochemical measurements. Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 (Merck) for mineralization of tea leaves to the complete digestion of samples (Praveen Sorajam. 2011) following Environmental Protection Agency (EPA) Method 3052 was done. The digested samples were diluted with 0.1% HNO3 and brought up to 50 mL and analyzed by a graphite furnace atomic absorption spectrophotometry, (GFAAS). The measurements were performed using a PerkinElmer PinAAcle 900T atomic absorption (AA) spectrophotometer and using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (AOAC 1998).

Soil samples

Soil samples were collected at each place and time the tea leaves were plucked in Gilan province and soil mixed thoroughly to give a represent able fraction**.** The soil samples were air dried ground and sieved through 200μm mesh size. The collected soil sub-samples were thoroughly mixed, pooled together to make a composite of each soil sample. The method developed by the United States Environmental Protection Agency for (total sobbed) heavy metals in soil, sediments and sludge (USEPA SW-846, method 3050) (US EPA. 1996; US EPA. 2002) , was used in the preparation of the soil samples for the determination of total metal content in this study**.** One gram (1g) of the soil sample was weighed for acid digestion. Analar grade nitric acid, hydrogen peroxide (about 30%) and concentrated Hydrochloric acid were used for the digestion. The digest was filtered through whatman filter paper. Each filtrate was collected in 100ml volumetric flask and deionized water was used to rinse the filter paper into volumetric flask. Each filterate was later made up to 100ml with deionized water. Standards were prepared with serial dilution techniques within the range of each metal determined. The instrument was first calibrated with stock solutions of the prepared standards before analyzed using flame atomic absorption spectrophotometer**.**

Tea infusion samples

Tea infusion was conducted with 15 minutes .The first step, about 10 g dried and milled sample was divided to 5 g. To each 5.0 g sample of tea was dried as above, the Iranian manner of tea infusion was performed by addition of 50 mL of boiling deionized water and the mixture kept at 80 °C on a water bath for 15 min. The mixture was held for 5 min at room temperature and then filtered. At the end of the infusion period, the tea extract diluted to 100 mL with deionized water (Zazouli. et al., 2010).

Statistical Method:

Analysis of variance (ANOVA) was employed to detect significances among samples from different forms of tea samples (Black tea, tea leaves).

RESULTS

Results were determined as mean ± SD of dry weight from three replicates in each test. The results of Zinc, Copper, Cadmium and Lead contents in Tea leaves and soil from 12 farms from 5 cities and finished tea products in 2012 in Gilan province, from 5 Tea Factories and their liquor forms (tea made) summarized in table 1. Results revealed that the mean content of Cadmium in all tea leaves samples from Lahidjan is over, while Lead, copper and zinc contents were below the maximum permitted level for tea. The results of the current research showed the Pb level in all tea samples except one finished tea product from Roshan Bahar was less than (10 mg/ kg) permissible limit of lead in food samples as per World health Organization and Federal Drug Administration for edible plant parts and international standards in United Kingdom, India and Kenya (WHO 1998; Sushama et al., 2012; Wang. et. al., 1993; Karak, and Bhagat. 2010; Lagerwerff. et al. 1979; Waalkes. 2000).

The permissible limit of copper is 150 µg/g under Prevention of Food Adulteration Act, 1954 (PFA), United kigdam, India and Kenya (Karak, and Bhagat. 2010). Wang et al. (1993), who found that the Cu content in Chinese tea samples was varied between 9.6 and 20. 9 mg/ kg , while in this research the copper contents of black tea leaves in Ladhijan was varied between 21.573 and 87.7075 mg/kg DW and the mean level was 37.2888 ± 2.63807 mg/kg DW which is much higher than Chinese tea samples.

Table 1. Experimental Conditions for the Detection of Heavy Metals in All Tea samples, Tea made, and the soils

sample	Mean Cd (mg/kgDW) ± SD	Mean Pb (mg/kgDW) ± SD	Mean Cu (mg/kgDW) ± SD	Mean Zn (mg/kgDW) ± SD
Black tea	1.74929 ± 0.10392	8.54182 ± 0.61823	37.2888 ± 2.63807	40.47945 ± 2.85983
Tea leaves	2.220815 ± 0.15299	6.918365 ± 0.35998	40.06409 ± 2.04376	58.2784 ± 4.24711
Liquor of Black tea	0.01638 ± 0.00164	1.40968 ± 0.057	3.1132 ± 0.25232	2.85424 ± 0.24574
Soil	26.6864 ± 1.49658	1.56738 ± 0.08622	52.1398 ±1.1830	65.1309±7.5625

Soil results showed that the Cadmium contents in the soil samples collected from all farms in 5 cities in the Gilan province are higher than WHO permissible levels (figure 1),while the results of the current study also revealed that the Pb level in all tea soil samples was less than 50 mg /kg. The mean contents of zinc and copper in all tea and soil samples were lower than permissible levels.

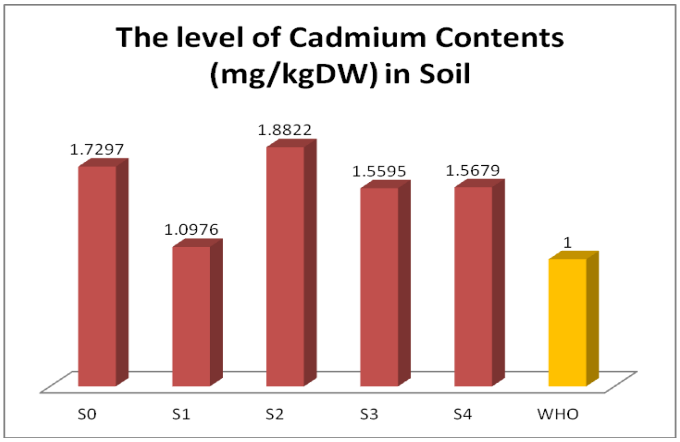

Figure 1. Concentrations of Cadmium in tea farm soil samples from 5 cities in Gilan

Average concentrations of cadmium in Tea leaves samples were lower than black tea and the remained heavy metals contents in liquor decreased dramatically (figures 2) as the percentage of tea liquor samples were exceeded from permissible levels was 0% and the results show that making tea has the significant role ($p < 0.003$) in decreasing the cadmium content (figure 3).

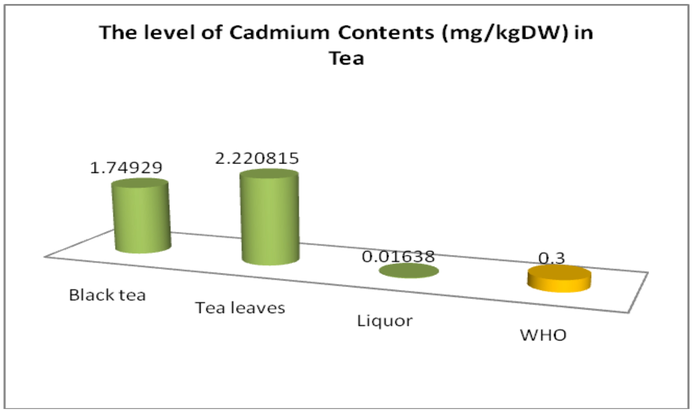

Figure 2. Mean Content of Cadmium in all studied Tea Samples

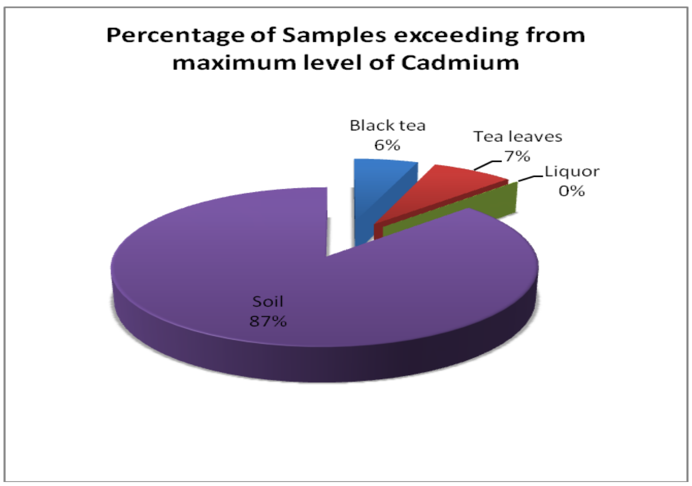

Figure 3. Comparison the contamination of Cadmium in all tea and soil samples

CONCLUSION

By a comparison between acceptable global standards and the level of Cadmium on investigated tea samples, our results showed that the quantities of Cadmium in all of samples were higher than acceptable intake recommended by global standards. JECFA has set PTWI for the Cadmium at 7 μg/kg of body weight (WHO, 2004). The total dietary exposure levels of Lead and Cadmium determined in this study were compared with the provisional tolerable weekly intakes (PTWLS) by the JECFA and showed lower than it. Due to the lack of information specifying the consumption of tea in Iranian diet, the risk assessment of drinking tea cannot be precisely estimated. Considerable attention should be done especially for people who may intake this drunk for a long time. People who drink a lot of tea from regions containing the higher levels of cadmium could be significantly exposed. Cadmium (Cd) has been designated as a human carcinogen (Tanmoy Karak, and Bhagat. 2010 ; Waalkes. 2000) and is clearly a potent, multi-tissue animal carcinogen (Tanmoy Karak, and Bhagat. 2010; Satarug. Et al., 2002). Phosphate fertilizer application is a significant contributor of trace elements, especially for Cd accumulation in cropped soils(Tanmoy Karak, and Bhagat. 2010 ; Cupit. Et al., 2002). Repeated application of phosphatic fertilizers may lead to a gradual buildup of Cd in agricultural soils over time(Tanmoy Karak, and Bhagat. 2010 ; Kabata-Pendias, A. and Pendias 2001). According to Meeüs, Eduljee, and Hutton(Tanmoy Karak, and Bhagat. 2010 ; Meeüs. Et al., 2002) Cd from phosphate fertilizers constituted more than 50% of the total input in avily polluted or heavily industrialized.

To avoid entrance of metals into the food Chain, municipal or industrial waste should not be drained into rivers and farm lands without prior treatment. A port from treating the discharge that enters into the farms, it is also imperative to utilize alternative measures of cleaning up the already contaminated substrates. Probably the most important conclusion that could be drawn up from the findings of this study is that science Tea leaves (*Camellia sinensis* L) tends to absorb and accumulate Cd in the leaves part. shown to accumulate relatively high concentration of heavy metals in compare to other crops , it is recommended that these type of planets should not be cultivated in the fields which irrigated by urban and industrial waste water or water contaminated by heavy metals. As heavy metal toxicity through contamination of preparation continues to be recognized risk, voluntary programs to provide community education regarding the potential risk of herbal preparations should be supported by the availability free heavy metal testing services**.**

ACKNOWLEDGEMENT

Financial Supports from Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) is gratefully acknowledged.

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