

The effect of the duration and temperature of infusion on the heavy metal content of green tea

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Abstract

Tea is one of the most popular drinks in the world, which contains several important essential micro nutrients that are beneficial to human health. On the other hand, the contamination of tea leaves by heavy metals may pose serious problems to human health. In this research, the concentration of toxic metals (Al, As, Pb, Cr, Cd, and Ni) and essential heavy metals (Fe, Zn, Cu and Mn) in Iranian green tea (IGT) leaves and its infusion were determined using atomic absorption spectrometry. Additionally, the effect of the duration (10, 30 and 90 min) and temperature (80 and 100 °C) of tea infusion on the content of heavy metals was studied. There was a significant difference between all samples at different time and temperatures. The concentrations range of heavy metals in IGT leaves was 1332-0.023 mg/kg. The highest and lowest metal content was observed for Al and Cd, respectively. The results showed that the heavy metal contents in IGT infusion varied between 11.7 mg/L for Al to 0.0002 mg/L for Cd. The highest and lowest percentage of metal extracted into IGT infusion was observed for Ni and Fe as 73.6 and 2.4, respectively.

Keywords: Atomic absorption spectrometry, Green tea infusion, Toxic and essential elements.

Introduction

Tea is one of the traditional and healthy drinks most widely consumed in Iran as well as some other countries. The black, green and oolong are the most popular types of tea. The green tea which is prepared from the dried leaves of *Camellia Sinensis* L, began about 5000 years ago in china. It is supposed that green tea has numerous beneficial effects on health include prevention of many diseases such as skin cancers (Wu et al., 2013), Parkinson's disease, cardiovascular diseases (Dogra et al., 2011), coronary artery (Qin and Chen, 2007), regulation of blood sugar and promotion of digestion (Samali et al., 2012). The chemical composition of green tea is similar to fresh tea leaves including flavonols, alkaloids, tannin substances, proteins and amino acids, enzymes, aroma forming substances and vitamins. In addition to above organic compounds, tea is regarded as a rich source of dietary essential elements, including minerals and trace elements (Salahinejad and Aflaki, 2010; Malik et al., 2008). In other hand, plants can take up the heavy metals from the soil and under certain conditions; high levels of them can be accumulated in the leaves and other edible parts of the plant. The presence of heavy metals has become a major problem in today's world due to its direct effect on living organisms. The metal contents in tea plants depend on many factors such as the age of the tea leaves, the soil conditions, rainfall, altitude, and genetic of the plant (Sauerbeck and Hein, 1991; Soon and Bates, 1982; Smith, 1994; Davies, 1992). During of tea infusion, both essential mineral elements and toxic metals are extracted into the beverage. The factors affecting the metal availability in tea leaves influence subsequently the metal concentrations in the infusion. Depending on the concentration, potentially toxic elements can cause damage to human health ranging from

liver and kidney dysfunctions to carcinogenesis. All lead compounds have been classified as human carcinogens (Ahamed and Siddiqui, 2007). Its accumulation in the body may cause several pathological states, including brain damage, kidney failure and serious developmental, learning and behavioral problems in children (Needleman, 2004; Bellinger, 2008; Rowland and Mc Kinstry, 2006). Cadmium is known as carcinogenic, and has been linked to lung and prostate cancer (Waalkes, 2000). Arsenic is strongly associated with lung and skin cancer in humans, and may lead to other internal cancers as well (Devesa et al., 2008; Medeiros et al., 2012). Recent studies show that Al caused some disease such as Alzheimer and Parkinson (Exley and Korchazhkina, 2001). Multiple studies implicated Chromium as a critical cofactor in the action of insulin (Cefalu and Hu, 2004); however, its function as a carcinogenic agent has been demonstrated (Gad, 1989). Nickel ions are essential in the diet for erythrocyte production, but high concentrations can initiate carcinogenesis and function as a tumor promoter (Sunderman Jr, 1989). Copper is essential to human metabolic processes, but accumulation of excess copper is hazardous (Dwivedi and Singh Rajput, 2014). Manganese is a vital element for metabolism and antioxidant systems; nevertheless, excessive intake may lead to manganism (Costa and Aschner, 2014). According to World Health Organization documents (2001) and other researchs (Chasapis et al., 2012), dietary deficiencies in iron and zinc are a concern.

In this study, the content of the essential and toxic trace elements in IGT leaves and its infusion was determined using atomic absorption spectrometry. Also, effect of the temperature and duration of tea infusion on the extraction of these elements was evaluated.

Materials & methods

All solvents and reagents necessary for experiments or preparation of the standard calibration curve, such as H₂O₂ and HNO₃, were obtained from Merck Company (Germany). All metal ion stock solutions were prepared from their nitrate forms, except for the arsenic solution, which was prepared from As₂O₃.

Sample Collection

Several commonly consumed IGT samples were randomly collected from different markets in Kermanshah (a western province of Iran).

Preparation of Tea Infusions

Exactly 5 gr of each tea sample was added to 200 ml of boiling water and heated to infuse for different times (10, 30 and 90 min) and temperatures (80 and 100°C). After infusion, the solution was filtered and an appropriate amount of distilled water was added in a volumetric flask to make up a 200 mL volume.

Analysis

The Varian atomic absorption instrument, equipped with flame, graphite furnace and hydride vapor, was used to determine selected elements in the tea leaves and their infusions.

Results & Discussion

Lead (Pb)

Average concentration of Pb in the IGT leaves was determined as 0.425 mg/kg (Table 1) which was below the maximum allowable intake limit in Iran (below 1 mg/kg). A study by Tsushida and Takeo (1977) showed that the concentration of Pb in Japanese green tea was in the range from 0.11 to 1.93 mg/kg. Based on a study by Othman et al., (2012) the Pb concentration in nine different imported Chinese green tea samples was found to range from 0.231 to 6.340 mg/kg. The main source of Pb in tea samples can be their growth media such as soil. Tea plant is normally grown in high acidic soils, where Pb is more available for root uptake (Han, 2006). The mean concentration of Pb in IGT infusion was 0.004 mg/L (Table 2) and 27.9 was found as percentage of Pb extracted

from IGT leaves into its infusion (Table 3). Table 4 shows that the Pb concentration rised with increasing infusion time, and decreased when temperature increased. There was also difference between all samples. The tea sample that was infused for 90 min at 80°C has higher Pb concentration (0.0047 mg/L) than other samples, while the lowest concentration of Pb (0.0034 mg/L) was observed for sample infused 10 min at 100°C.

Table 1: The mean concentration (mg/kg) of toxic and essential elements in IGT leaves

Elements										
Sample	Pb	Cd	As	Al	Cr	Ni	Cu	Mn	Fe	Zn
IGT leaves	0.425	0.023	2.32	1332	1.64	8.4	37.4	297.3	351	28.6

Cadmium (Cd)

Cd mean concentration in IGT leaves was observed 0.023 mg/kg (Table 1). The maximum allowable intake limit of Cd in Iran is below 0.1 mg/kg, which is indicated that the Cd concentration in IGT was acceptable. In a study by Zhang and Fang (2007), the Cd concentration in China green tea leaves was found in the range of 0.012 to 0.057 mg/kg. According to their results, the Cd concentration was at the lowest concentration among different trace metals, which was comparable with our results. Table 2 shows that 0.0002 mg/L was the mean concentration of Cd in IGT infusion and the Cd from IGT leaves into its infusion was 26.9% (Table 3). As shown in Table 4, the Cd concentration increased when the infusion time increased, and decreased when temperature increased. The lowest (0.00018 mg/L) and highest (0.00024 mg/L) concentrations of Cd were observed for tea samples that infused for 10 min at 100°C and for 90 min at 80°C, respectively.

Table 2: The mean concentration (mg/L) of toxic and essential elements in IGT infusion

Elements										
Sample	Pb	Cd	As	Al	Cr	Ni	Cu	Mn	Fe	Zn
IGT infusion	0.004	0.0002	0.019	11.7	0.016	0.2	0.2	2.7	0.27	0.31

Arsenic (As)

The mean concentration of As in IGT leaves was 2.32 mg/kg (Table 1) which was higher than the maximum allowable intake limit in Iran (below 1 mg/kg). Yuan et al., (2007) reported that the total amount of As ranged from below the detection limit to 4.81 mg/kg in Chinese tea leaves. According to Shen and Chen studies (2008), no As was found in 15 green tea samples from Taiwan when infusion was prepared from 2.0 g of tea sample with 100 mL of water, while in our studies 0.019 mg/L was determined as As concentration in IGT infusion (Table 2). The percentage of As extracted into IGT infusion was 24.4 (Table 3). As shown in Table 4, the As concentration in IGT infusion depends on the temperature and infusion time and its concentration increased when temperature and infusion time increased. So the highest concentration of As (0.027 mg/L) was observed for tea sample that was infused at 100°C for 90 min.

Aluminum (Al)

Among the investigated metals, Al showed the highest concentration (1332 mg/kg) in IGT leaves (Table 1). Our results indicated that the concentration of Al in IGT samples was higher than maximum allowable intake level in Iran. Al content was 919 mg/kg in green tea which reported by Wróbel et al., (2000). In a study by Fung et al., (2009) the Al concentration was determined as 2633 mg/kg by analyzing of seven green tea samples. The mean Al concentration in IGT infusion was 11.7 mg/L (Table 2), and percentage of Al extracted into IGT infusion was 26.3 (Table 3). Table 4 shows that temperature and infusion time affected Al concentration in IGT infusion. The Al content in IGT infusion increased when the infusion time and temperature increased. The highest Al concentration (13.22 mg/L) was found for tea sample that was infused for 90 min at 100°C.

Table 3: The percentage of toxic and essential elements extracted from IGT leaves into its infusion

Sample	Elements									
	Pb	Cd	As	Al	Cr	Ni	Cu	Mn	Fe	Zn
IGT infusion	27.9	26.9	24.4	26.3	28.8	73.6	17.4	27.3	2.4	32.3

Chromium (Cr)

The mean concentration of Cr in IGT leaves was determined as 1.64 mg/kg (Table 1). Wróbel et al., (2000) reported that the average Cr content in Mexican green tea sample was 0.62 mg/kg. Another study by Sividhya et al., (2011) showed that the Cr content in green tea was lower than the quantification limit. The mean concentration of Cr in IGT infusion was 0.016 mg/L (Table 2). The percentage of Cr extracted into IGT infusion was determined as 28.8 (Table 3). Table 4 shows the influence of temperature and infusion time on Cr content in IGT infusion. The highest Cr concentration (0.018 mg/L) was observed for tea sample which was infused for 90 min at 100°C.

Table 4: The mean concentration (mg/L) of toxic and essential elements in IGT infusion at different temperatures and infusion time

Temperature (°C)	80			100		
Infusion Time (min)	10	30	90	10	30	90
Pb	0.0036	0.0039	0.0047	0.0034	0.0037	0.0044
Cd	0.00019	0.00022	0.00024	0.00018	0.00019	0.00021
As	0.012	0.014	0.019	0.017	0.024	0.027
Al	10.07	11.52	12.3	10.6	12.3	13.22
Cr	0.014	0.014	0.015	0.016	0.017	0.018
Ni	0.182	0.19	0.212	0.197	0.222	0.232
Cu	0.295	0.27	0.25	0.194	0.154	0.142
Mn	2.58	2.71	2.77	2.67	2.77	2.81
Fe	0.23	0.24	0.31	0.23	0.3	0.34
Zn	0.27	0.29	0.29	0.33	0.34	0.33

Nickel (Ni)

As shown in Table 1, the mean concentration of Ni in IGT leaves was found to be 8.4 mg/kg. In a similar study, the mean concentration of Ni in green tea sample which was commonly consumed in South India, was determined as 9.09 mg/kg (Sirvidhya et al., 2011). The concentration of Ni extracted into the IGT infusion was 0.2 mg/L (Table 2). The highest percentage of metal extracted into IGT infusion was observed for Ni as 73.6 (Table 3). As shown in Table 4, the Ni concentration increased when temperature and infusion time increased. So the highest concentration of Ni in IGT infusion was 0.232 mg/L for tea sample that infused for 90 min at 100°C.

Copper (Cu)

The mean concentration of Cu in the IGT leaves was 37.4 mg/kg (Table 1). Qin and Chen (2007) reported that the Cu concentrations of 57 commercial green tea samples purchased from the local market in Beijing, China, varied from 8.22 to 28.71 mg/kg. Table 2 shows that Cu concentration of IGT infusion was 0.2 mg/L and the percentage of Cu extracted into the IGT infusion was observed as 17.4 (Table 3). Table 4 illustrates that, the Cu concentration decreased when temperature and infusion time increased, which is due to the formation of non-soluble complex with polyphenols. The lowest concentration of Cu (0.142 mg/L) was found for tea sample that was infused for 90 min at 100°C.

Manganese (Mn)

The average concentration of Mn in IGT leaves was observed as 297.3 mg/kg (Table 1). Higher Mn concentration (508 mg/kg) in South India green tea samples was reported by Sirvidhya et al., (2011). The mean concentration of Mn in IGT infusion was determined as 2.7 mg/L (Table 2) and the amount of Mn extracted into the IGT infusion was 27.3% (Table 3). As shown in Table 4, the Mn amount which was extracted into the IGT infusion depends on the temperature and infusion time. When the infusion time and temperature increased, the Mn extracted into the infusion increased. So the highest Mn concentration was observed for tea sample which was infused for 90 min at 100°C as 2.81 mg/L.

Iron (Fe)

The mean concentration of Fe in IGT leaves was found to be 351 mg/kg as shown in Table 1. Analyzing of 30 tea samples including green, black, semi-fermented and white teas imported to the Czech Republic, showed that the Fe concentrations in tea leaves varied between 103–523 mg/kg (Street et al., 2006) which was comparable with our results. Fe concentration in IGT infusion was determined as 0.27 mg/L (Table 2). The lowest percentage of metal extracted into the IGT infusion was 2.4 for Fe (Table 3). Table 4 shows that, Fe contents extracted into the IGT infusion depend on temperature and infusion time so that, increased temperature and infusion time cause to the increased Fe concentration. Then the highest Fe concentration (0.34 mg/L) was observed for tea sample which infused for 90 min at 100°C.

Zinc (Zn)

Zn concentration in IGT leaves was observed as 28.6 mg/kg (Table 1). In a comparable study with analyzing of several green tea samples, which consumed in South India, the Zn concentration was determined as 26.39 mg/kg (Sirvidhya et al., 2011). The mean concentration for Zn extracted into the IGT infusion was found to be 0.31 mg/L (Table 2). The percentage of Zn extracted into the IGT infusion was 32.3 as shown in Table 3. Table 4 illustrates that, increasing of temperature, increased the Zn concentration in IGT infusion. The lowest (0.27 mg/L) and highest (0.34 mg/L) Zn concentrations were observed for tea samples that were infused for 10 min at 80°C and for 30 min at 100°C, respectively.

Conclusion

The mean concentration of heavy metals determined in IGT leaves arranged in the following order: Al> Fe> Mn> Cu> Zn> Ni> As> Cr> Pb> Cd. We found that the Al and As concentrations in IGT leaves were higher than the maximum allowable intake level in Iran. The relative concentrations in IGT infusion were observed as Al> Mn> Zn> Fe> Cu, Ni> As> Cr> Pb> Cd. The highest and lowest percentages of metal extracted into IGT infusion were found for Ni and Fe, respectively. The results indicated that the concentrations of all elements (except Pb, Cd and Cu) in IGT infusion increased with enhanced temperature. With the exception of Cu, concentrations in IGT infusion increased with raised infusion time.

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References

Ahamed, M. & Siddiqui, M.K. (2007). Low level lead exposure and oxidative stress: current opinions. *Clinica Chimica Acta*, 383, 57-64. <http://www.sciencedirect.com/science/article/pii/S0009898107002744>

- Bellinger, D.C. (2008). Lead neurotoxicity and socioeconomic status: Conceptual and analytical issues. *NeuroToxicology*, 29, 828-832. <http://www.sciencedirect.com/science/article/pii/S0161813X08000569>
- Cefalu, W.T. & Hu, F.B. (2004). Role of chromium in human health and in diabetes. *Diabetes Care*, 27, 2741-2751. <http://care.diabetesjournals.org/content/27/11/2741.short>
- Chasapis, C.T., Loutsidou, A.C., Spiliopoulou, C.A. & Stefanidou, M.E. (2012). Zinc and human health: an update. *Archives of Toxicology*, 86, 521-534. <http://link.springer.com/article/10.1007/s00204-011-0775-1>
- Costa, L. & Aschner, M. (2014). *Manganese in Health and Disease*. Cambridge, UK: RSC Publishing. <http://pubs.rsc.org/en/content/ebook/978-1-84973-943-6#!divbookcontent>
- Davies, B.E. (1992). Inter-relationship between soil properties and the uptake of cadmium, copper, lead and zinc from contaminated soils by radish (*Raphanus sativus* L.). *Water, Air, & Soil Pollution*, 63, 331-342. <http://link.springer.com/article/10.1007/BF00475500>
- Devesa, V., Vélez, D. & Montoro, R. (2008). Effect of thermal treatments on arsenic species contents in food. *Food and Chemical Toxicology*, 46, 1-8. <http://www.sciencedirect.com/science/article/pii/S0278691507003109>
- Dogra, D., Ahuja, S., Krishnan, S., Kohli, S., Ramteke, A., Atale, N. & Rani, V. (2011). Phytochemical screening and antioxidative activity of aqueous extract of Indian *Camellia sinensis*. *Journal of Pharmacy Research*, 4, 1833-1835. EBSCOHOST: <http://connection.ebscohost.com/c/articles/74457565/phytochemical-screening-antioxidative-activity-aqueous-extract-indian-camellia-sinensis>
- Dwivedi, A.K. & Singh Rajput, D.P. (2014). Studies on adsorptive removal of heavy metal (cu, cd) from aqueous solution by tea waste adsorbent. *Journal of Industrial Pollution Control*, 30, 85-90. <http://www.icontrolpollution.com/articles/studies-on-adsorptive-removal-of-heavy-metal-cu-cdfrom-aqueous-solution-by-tea-waste-adsorbent-.php?aid=45313>
- Fung, K.F., Carr, H.P., Poon, B.H.T. & Wong, M.H. (2009). A comparison of aluminum levels in tea products from Hong Kong markets and in varieties of tea plants from Hong Kong and India. *Chemosphere*, 75, 955-962. [http://linkinghub.elsevier.com/retrieve/pii/S0045-6535\(09\)00007-1](http://linkinghub.elsevier.com/retrieve/pii/S0045-6535(09)00007-1)
- Exley, C. & Korchazhkina, O.V. (2001). Promotion of formation of amyloid fibrils by aluminium adenosine triphosphate (AlATP). *Journal of Inorganic Biochemistry*, 84, 215-224. <http://www.sciencedirect.com/science/article/pii/S0162013401001714>
- Gad, S.C. (1989). Acute and chronic systemic chromium toxicity. *Science of the Total Environment*, 86, 149-157. <http://www.sciencedirect.com/science/article/pii/0048969789902015>
- Han, W.Y., Liang, Y.R., Yang, Y.J., Shi, Y.Z., Ma, L.F. & Ruan, J.Y. (2006) Effect of processing on the Pb and Cu pollution of tea in Chinese. *Journal of Tea Science*, 26, 95-101. http://en.cnki.com.cn/Article_en/CJFDTOTAL-CYKK200602003.htm
- Malik, J., Szakova, J., Drabek, O., Balik, J. & Kokoska, L. (2008). Determination of certain micro and macroelements in plant stimulants and their infusions. *Food Chemistry*, 111, 520-525. <http://www.sciencedirect.com/science/article/pii/S0308814608004275>
- Medeiros, R.J., Santos, L.M.G., Freire, A.S., Ricardo, E., Santelli, R.E., Braga, A.M.C.B., Krauss, T.M. & Jacob, S.C. (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. *Food Control*, 23, 535-541. <http://www.sciencedirect.com/science/article/pii/S0956713511003483>
- Needleman, H. (2004). Lead poisoning. *Annual Review of Medicine*, 55, 209-222. <http://www.annualreviews.org/doi/abs/10.1146/annurev.med.55.091902.103653>

- Othman, A., Al-Ansi, S. & Al-Tufail, M. (2012). Determination of Lead in Saudi Arabian imported green tea by ICP-MS. *E-Journal of Chemistry*, 9, 79-82. <http://www.hindawi.com/journals/jchem/2012/132683/abs/> Accessed on [2011-07-13]
- Qin, F. & Chen, W. (2007). Lead and copper levels in tea samples marketed in Beijing, China. *Bulletin of Environmental Contamination and Toxicology*, 79, 249-250. <http://www.springerlink.com/index/X83472T82505G52L.pdf>
- Rowland, A.S. & Mc Kinstry, R.C. (2006). Lead toxicity, white matter lesions, and aging. *Neurology*, 10, 1464-1465. <http://www.neurology.org/content/66/10/1464.short>
- Samali, A., Kirim, R.A., Mustapha, K.B. (2012). Qualitative and quantitative evaluation of some herbal teas commonly consumed in Nigeria. *African Journal of Pharmacy and Pharmacology*, 6, 384-388. <http://www.academicjournals.org/journal/AJPP/article-abstract/4F45AF234396>
- Salahinejad, M. & Aflaki, F. (2010). Toxic and essential mineral elements content of black tea leaves and their Tea infusions consumed in Iran. *Biological Trace Element Research*, 134, 109–117. <http://link.springer.com/article/10.1007/s12011-009-8449-z>
- Sauerbeck, D.R. & Hein, A. (1991). The nickel uptake from different soils and its prediction by chemical extraction. *Water, Air, & Soil Pollution*, 57-58, 861-871. <http://link.springer.com/article/10.1007/BF00282949>
- Shen, F. & Chen, H. (2008). Element composition of tea leaves and tea infusions and its impact on health. *Bulletin of Environmental Contamination and Toxicology*, 80, 300–304. <http://dx.doi.org/10.1007/s00128-008-9367-z>
- Sirvidhya, B., Subramanian, R. & Raj, V. (2011). Determination of Lead, Manganese, Copper, Zinc, Cadmium, Nickel and Chromium in tea leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3, 257-258. <http://www.ijppsjournal.com/Vol3Issue4/2610.pdf>
- Smith, S.R. (1994). Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. I. Nickel, copper and zinc uptake and toxicity to regress. *Environmental Pollution*, 85, 321-327. <http://www.sciencedirect.com/science/article/pii/0269749194900035>
- Soon, Y.K. & Bates, T.E. (1982). Chemical pools of cadmium, nickel and zinc in polluted soils and some preliminary indications of their availability to plants. *Journal of Soil Science*, 33, 477-488. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2389.1982.tb01782.x/full>
- Sunderman, F.W., Jr. (1989). Mechanisms of nickel carcinogenesis. *Scandinavian Journal of Work, Environment & Health*, 15, 1-12. <http://www.jstor.org/stable/40965615>; http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/; <http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2740280306/full>
- Street, R., Száková, J., Drábek, O. & Mládková, L. (2006). The status of micronutrients (Cu, Fe, Mn, Zn) in tea and tea infusions in selected samples imported to the Czech Republic. *Czech Journal of Food Sciences*, 24, 62–71. <http://81.0.228.28/publicFiles/50276.pdf>
- Tsushida, T. & Takeo, T. (1977). Zinc, copper, lead and cadmium contents in green tea. *Journal of the Science of Food and Agriculture*, 28, 255–258.
- Waalkes, M.P. (2000). Cadmium carcinogenesis in review. *J Inorg Biochem*, 79, 241–244.
- World Health Organization (2001). *Iron deficiency anaemia: assessment, prevention and control*. Geneva : World Health Organization.
- Wróbel, K., Wróbel, K. & Urbina, E.M.C. (2000). Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdariffa*, and *Ilex paraguariensis* (mate) by ETA-AAS. *Biological Trace Element Research*, 78, 271–280. <http://dx.doi.org/10.1385/BTER:78:1-3:271>

- Wu, Y., Zhang, D. & Kang, S. (2013). Black tea, green tea and risk of breast cancer: an update. *SpringerPlus*, 2, 1-5. <http://springerplus.springeropen.com/articles/10.1186/2193-1801-2-240>
- Yuan, C., Gao, E., He, B. & Jiang, G. (2007). Arsenic species and leaching characters in tea (*Camellia sinensis*). *Food and Chemical Toxicology*, 45, 2381–2389. [http://linkinghub.elsevier.com/retrieve/pii/S0278-6915\(07\)00202-5](http://linkinghub.elsevier.com/retrieve/pii/S0278-6915(07)00202-5)
- Zhang, M. & Fang, L. (2007). Tea Plantation-induced activation of soil heavy metals. *Communications in Soil Science and Plant Analysis*, 38, 1-12. <http://www.tandfonline.com/doi/abs/10.1080/00103620701378417>