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Occurrence, exposure and health risk assessment of heavy metals in green tea samples cultivated in Hangzhou area

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The concentration of heavy metals in 120 green tea samples grown in Hangzhou area were investigated by inductively coupled plasma mass spectrometry (ICP-MS). Except for mercury (Hg) (69.2%), Sb (98.3%), Se (93.3%) and Sn (94.2%), the detection rate of the other 11 elements were all 100.0%. The mean concentration of heavy metals in tea showed the following order: manganese (Mn) > aluminum (Al) > barium (Ba) > copper (Cu) > nickel (Ni) > chromium (Cr) > lead (Pb) > tin (Sn) > lithium (Li) > vanadium (V) > selenium (Se) > arsenic (As),> cadmium (Cd) > antimony (Sb) > mercury (Hg). Mn was the highest element found in the tea samples with a concentration ranging from 202.00 to 2010.00 mg/kg and a mean of 836.00 mg/kg. The lowest element was Hg, with a concentration ranging from ND to 0.017 mg/kg and the mean concentration was 0.0033 mg/kg. And all the 120 samples did not exceed the standard limit value of heavy metal. The pollution level of heavy metals indicated that the single pollution index and comprehensive pollution index of green tea produced in Hangzhou area are far below 0.7, which belong to the clean and safety level. Health risk of inhabitant via consuming green tea was conducted and the HI value was 0.42, less than 1, which indicates that these chemicals pose no risk to human health in Hangzhou area.

Keywords Heavy metals, ICP-MS, Green tea, Risk assessment

Tea, the second most popular drink in the world[1,2] and the most popular in China, is globally known for its health benefits. Indeed, tea is recognized as the best health beverage in the world[1,3]. Over the last decades, the global production of this plant has grown at a rate greater than 1.8%, and global tea consumption has increased by more than 2% per year[4]. Today, 18–20 billion cups of this drink are consumed by about 3 billion people on a daily basis[5–7]. The health benefits of tea are mainly attributed to polyphenols, polysaccharides, and other active ingredients, which endow the beverage with antioxidant properties. However, the presence of toxic elements such as heavy metals and pesticide residues in tea leaves and infusions poses a serious health risk[8–12].

Heavy metals are persistent inorganic pollutants that can bioaccumulate in animals and plants. In tea, the metal content is attributed to the tea processing procedure[2] as well as to the acidic soils in which the tea plant is cultivated[13]. Other sources such as rainfall, atmospheric dust, plant protective agents, and fertilizers also contribute to metal contamination in tea[14]. Although some heavy metals, such as Mn, Cu, Zn, and Fe, play an essential role in metabolism, others, such as Hg, Cd, Pb, and As, are non-essential and can cause adverse health effects, even in trace quantities[15–17]. Dietary intake constitutes the main route of human exposure to heavy metals[18]. Continued exposure to these metals may lead to nervous, bone, and kidney diseases, as well as to cardiovascular and chronic accumulation[19].

After entering the human body, heavy metals such as Pb, Cr, Hg, Cd, As, and Ni will have an adverse effect on physiological function and metabolism. For example, mercury and lead can damage the nervous system, leading to slow reactions and dementia[2]. Meanwhile, cadmium and nickel can cause bone pain necrosis and damaged liver function, respectively. Chromium poisoning of the human body is systemic and may cause dermatitis, eczema, tracheitis, and rhinitis, as well as genetic defects that may lead to metamorphosis and cancer. Mercury, cadmium, arsenic, and lead are also carcinogenic. Unfortunately, their incubation period is long, and thus they can accumulate in the human body to levels that cause irreversible damage, especially since they cannot be easily detected in seemingly healthy individuals[20].

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In this study, we analyze the contents of Al, As, Ba, Cd, Cr, Cu, Hg, Li, Mn, Ni, Pb, Sb, Se, Sn, and V metals in 120 tea samples produced in Hangzhou, using inductively coupled plasma mass spectrometry (ICP-MS). The ratio of the dose of human pollutant intake to the standard reference dose (Standard reference dose, RfD) is used as the evaluation criterion[21], as recommended by the Environmental Protection Agency (USEPA). The health risk is assessed based on the calculated hazard coefficient (hazard Quotients, HQ) and risk index (HI) to ensure the drinking safety of the product and help develop it further.

Materials and methods

Chemicals and equipment

The standard stock solution of mixed Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Li, Mg, Mn, Ni, Pb, Sb, Sn, Sr, Ti, Tl, V, and Zn metals (100 µg/mL) was purchased from Bruker and the National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials. Meanwhile, the standard stock solutions of Hg and Se (100 µg/mL) were bought from TM Standard and NCS Testing Technology Co., Ltd, respectively. The standard working solutions of the metal mixture and Se (0.0, 1.0, 2.0, 4.0, 8.0, 20.0, and 40.0 µg/L) were prepared by step dilution of standard stock solutions with 1% HNO_3, while those of Hg (0.0, 0.2, 0.4, 1.0, 1.5, and 2.0 µg/L) were prepared with 1% HCl. Trace metal grade HNO_3 and Suprapur HCl were obtained from Thermo Fisher and Merck, respectively. High-purity deionized water (18.2 MΩ cm) was used in all preparations. To eliminate the blank of the trace analytes and other contaminants, all containers were dipped in 20% HNO_3 over 24 h and rinsed with high-purity deionized water prior to use. Sample digestion and metal analyses were achieved using a microwave digestion system bought from CEM, USA, and an 8900 ICP-MS system obtained from Agilent, USA.

Sample collection

A total of 120 green tea samples were collected from supermarkets (67 of 120), local markets (43 of 120), and planting link (10 of 120) in Hangzhou. They were planted in Xihu District (33 of 120), Yuhang District (26 of 120), Fuyang District (18 of 120), Linan District (13 of 120), Chunan County (12 of 120), Jiande city (9 of 120), and Tonglu County (9 of 120) of Hangzhou area. The experiment complied with relevant institutional, national, and international guidelines and legislation.

Pretreatment of samples

Samples of tea leaves (0.3 g, accurate to 0.0001 g) were placed in a microwave digestion tube and mixed with 5 mL HNO_3. Two hours later, the capped tubes were moved to the microwave digestion instrument and heated as per the following program: step 1: heating time: 15 min, holding temperature 140 °C, holding time 2 min; step 2: heating time: 6 min, holding temperature 180 °C, holding time 30 min). Afterward, the samples were cooled to room temperature, and the solution was eluted with ultrapure water to 25.00 mL. The obtained solutions were analyzed on the quadrupole inductively coupled plasma-mass spectrometer (ICP-MS), and the concentrations of different metals in each solution were determined based on the calibration curve constructed using standard multi-element solutions of different concentrations. The element indium (In) (3 µg/L) was used as an internal standard. All instrument operating parameters are presented in Table 1.

Statistical analysis

As recommended by the World Health Organization[22], all non-detected (ND) values were assumed to be anywhere between zero and the limit of detection (LOD), with zero being the lower bound (LB) and LOD being the upper bound (UB). middle bound (MB) is defined as LOD/2. Herein, the ND level of a heavy metal was considered to be 0 when more than 80% of results were less than the LOD, and it was considered to be LOD when more than 60% but less than 80% of results were less than the LOD.

The correlation between seven heavy metal elements was analyzed using the SPSS19.0 (https://spss.en.softonic.com/) statistical analysis software.

Evaluation method of heavy metal pollution level

The comprehensive pollution index, also known as the Numero integrative pollution index, is proposed by American scholar named Numero in his book《Scientific Analysis of River Pollution》. It is one of the most commonly used methods to calculate the comprehensive pollution index at home and abroad. The comprehensive pollution level of heavy metals in tea was evaluated by calculating the comprehensive pollution index (P), as per Eqs. (1) and (2)[23]:

Mode	Collision	Mode	Collision
RF power (kW)	1.55	Sampling depth (mm)	10.0
Auxiliary flow (L/min)	0.80	First extraction lens (V)	−2.0
Nebulizer flow (L/min)	0.95	Second extraction lens (V)	−165.0
CeO^+/Ce^+ (%)	1.2	Ba^{++}/Ba^+ (%)	1.1
Collision gas	He	He flow (mL/min)	4.0

Table 1. Operating parameters of the ICP-MS system. Monitored isotopes ^{7}Li, ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{75}As, ^{78}Se, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{202}Hg, $^{206,207,208}Pb$

Heavy metals	Standard limit	Reference standard
As	2.0	NY659-2003
Cd	1.0	NY659-2003
Cr	5.0	NY659-2003
Hg	0.3	NY659-2003
Pb	5.0	GB2762-2022

Table 2. Standard limit values (mg/kg) of heavy metals in tea.

Grade	*P*	Class of pollution	Pollution level
1	$P \leq 0.7$	Safe	Cleaning
2	$0.7 < P \leq 1$	Warn	Nearly cleaning
3	$1 < P \leq 2$	Mild pollution	Began to be contaminated
4	$2 < P \leq 3$	Middle level pollution	Moderately polluted
5	$3 < P$	Heavy pollution	Heavily polluted

Table 3. Comprehensive pollution index classification standard[23]. Risk assessment estimation. To determine the degree of risk posed by the detected heavy metals on human health, an exposure evaluation was conducted based on the results of heavy metal monitoring in tea. The Estimated Daily Intake (EDI; mg/kg.bw.d) factor of each metal was calculated according to Eq. (3)[21,24,25]:

$$P_i = \frac{Ci}{Si} \tag{1}$$

$$P = \frac{\sqrt{\left[\left(\frac{Ci}{Si}\right)\max^2 + \left(\frac{Ci}{Si}\right)\mathrm{ave}^2\right]}}{2} \tag{2}$$

where P_i is the single factor pollution index of heavy metal i in tea, C_i is the measured content of heavy metal i in tea (mg/kg), S_i is the evaluation limit standard of heavy metal i, max is the maximum value, and ave is the average value. Table 2 summarizes the standard limit values of specific metals, and Table 3 shows comprehensive pollution index classification criteria.

$$EDI = \frac{C\,x\,EF\,x\,ED\,x\,FIR}{bw\,xTAx1000} \tag{3}$$

Where C is the metal content (mg/kg), E_D is the exposure duration, E_F is the exposure frequency (365 days/year), F_{IR} is the tea ingestion rate (g/person/day), T_A is the average exposure time ($E_F \times E_D$), and bw is the consumer body weight in kg. According to "world health statistics 2023: monitoring health for the SDGs, sustainable development goals", life expectancy in China is 77.4 years (E_D)[26], and based on the fifth China total diet study performed by Wu et al.[27], the citizens of Zhejiang Province consume 0.73 g of green tea per day. As for the mean weight of the Hangzhou citizens aged between 20 and 59 years (23,868 citizens), it is 64.36 kg, as per the Hangzhou Citizen Physical Fitness Test Data Analysis Report published in 2019[28]. The calculated EDI values were compared with the established RfD values of the corresponding metals to assess the risk.

The hazard quotient (HQ) was used to assess long-term exposure to heavy metals, and it was calculated using the following formula[29]:

$$HQ = \frac{EDI}{RfD} \tag{4}$$

According to the EPA Integrated Risk Information Database (IRIS) and International The RfDs of Cu, As, V, Al, Mn, Sn, Hg, Cd, Ni, Cr, Ba, Li, Se and Sb are 0.04, 0.0003, 0.007, 1, 0.024, 0.6, 0.0003, 0.0018, 0.02, 1.5, 0.2, 0.002, 0.005 and 0.0004 mg/(kg day) respectively[30–39]; Tolerable daily intake (TDI) for Pb is 3.6 μg/(kg day)[40,41]. HQ values greater than 1 signify that the exposed consumers are unlikely to experience obvious toxic effects.

The risk assessment of cumulative exposure to the detected heavy metals was performed by calculating the hazard index (HI), which is equivalent to the sum of HQs of the heavy metals that a consumer is exposed to:

$$HI = \sum HQ \tag{5}$$

HI values greater than 1 indicate that green tea consumption poses a risk to human health.

Results and discussion
Method validation
To validate the analytical method applied in this study, the precision, accuracy, linearity, LOD, and uncertainty values were calculated for each element according to the according to the European Commission's SANCO/3103/2000 guidelines[42,43]. For all analytes, the correlation coefficients (R) of the calibration curves were greater than 0.9995–0.9999, and the LOD values were between 0.0003 and 0.03 mg/kg. To test the feasibility of the method, heavy metals were added to the matrices. The accuracy of the method was validated using standard material for biocomposition analysis of green tea [GBW10052(GSS-30)], and the recoveries were between 86.4% and 97.5%.

Sample analysis
Previous studies indicate that when the levels of heavy metals in tea are very high, the absorption of these elements into the human body may cause poisoning, resulting in several health problems. Table 4 summarizes the concentrations of heavy metals in the tea samples analyzed herein. Of the 15 analytes, 11 have a detection rate of 100%. The detection rates of Hg, Sb, Se, and Sn are 69.2%, 98.3%, 93.3%, and 94.2%, respectively. The mean concentrations of heavy metals in tea decrease in the following order: Mn > Al > Ba > Cu > Ni > Cr > Pb > Sn > Li > V > Se > As > Cd > Sb > Hg, which agrees well with the data reported by Fatemeh Pourramezani[44].

The mean concentration of heavy metals in green tea plant soil in west lake scenic area was in the following order: Zn > Cr > Ni > Pb > Cu > Co > As > Cd > Hg[45], except Cu, the mean concentration trend of heavy metals in green tea plant soil and in green tea were similar. According to Peng et al.[46], the mean concentration of Cu in Indian and Sri Lankan black tea is higher than those of Pb and Cd, and the concentrations of heavy metals decrease in the following order: Cu > Pb > As > Cd > Hg, which is consistent with our results. Why the mean concentration of Cu in tea plant soil was lower than Cr, Ni and Pb, but in tea it was higher than Cr, Ni and Pb. This maybe for the reason that the heavy metal enrichment capacity of soil-tea tree follows the following order: Cu > Cd > Zn > Ni > Hg > As > Cr > Pb[47], the enrichment capacity of Cu is the highest, meanwhile, green tea contains Cu itself.

The elements with the highest and lowest concentrations detected in tea are Mn and Hg, and their concentrations range from 202.00 to 2010.00 mg/kg (mean of 836.00 mg/kg) and from ND (not detected) to 0.017 mg/kg (mean of 0.0033 mg/kg), respectively. Notably, none of the 120 samples analyzed herein show heavy metal levels exceeding the standard limit value (Table 2). Jia et al.[48] studied the heavy metal pollution in different types of soil in Hangzhou city, the results revealed that the mean concentration of Mn was the highest in all types of soil studied.

The correlation between the concentrations of seven heavy metals in green tea was assessed. Probability (p) results were in the bracket in Table 5, where $p < 0.05$ indicates correlation reached a significant level, and $p < 0.01$ indicates that correlation reached a highly significant level. The results demonstrate that Pb concentration is positively correlated with the concentrations of Cd, Cr, Mn, and As. Meanwhile, Cd has positive correlations with Hg and As; Cr with Mn and As; Hg with Mn and As; and Mn with As. The correlation analysis shows that with the exception of Cu, the heavy metal concentrations are correlated, and thus there may be synergy between these elements.

Heavy metal pollution level
The Numero integrative pollution index was adopted to evaluate the level of heavy metal pollution in tea samples. Considering that the single and comprehensive pollution index values listed in Table 6 are far below 0.7, the green tea produced in Hangzhou area is clean and safe to drink.

Heavy metals	Detection rate (%)	Concentration (mg/kg)	Mean concentration (mg/kg)	Median (mg/kg)
Al	100.0	126.00-975.00	276.50 ± 148.50	227.00
As	100.0	0.013–0.17	0.054 ± 0.030	0.047
Ba	100.0	4.01–103.00	21.40 ± 15.92	16.75
Cd	100.0	0.0093-0.17	0.040 ± 0.024	0.034
Cr	100.0	0.044–3.52	0.62 ± 0.55	0.42
Cu	100.0	7.48-31.00	13.80 ± 3.68	13.25
Hg	69.2	ND-0.017	0.0033 ± 0.0034	0.0028
Li	100.0	0.025–1.02	0.18 ± 0.15	0.15
Mn	100.0	202.00-2010.00	836.00 ± 274.76	814.50
Ni	100.0	2.03–29.90	10.70 ± 4.37	10.35
Pb	100.0	0.051–1.71	0.45 ± 0.30	0.37
Sb	98.3	ND-0.22	0.022 ± 0.022	0.018
Se	93.3	ND-0.18	0.076 ± 0.040	0.076
Sn	94.2	ND-12.80	0.18 ± 1.22	0.022
V	100.0	0.016–0.71	0.11 ± 0.11	0.083

Table 4. Concentrations (mg/kg) of heavy metals in tea samples.

Heavy metals	Pb	Cd	Cr	Cu	Hg	Mn	As
Pb	1.0	0.21(0.022)	0.31(< 0.001)	0.066(0.47)	0.099(0.29)	0.18(0.044)	0.65(< 0.001)
Cd		1.0	0.1(0.28)	0.035(0.70)	0.2(0.028)	0.16(0.073)	0.28(0.002)
Cr			1.0	−0.15(0.11)	0.14(0.12)	0.26(0.005)	0.33(< 0.001)
Cu				1.0	0.085(0.35)	0.007(0.94)	0.16(0.066)
Hg					1.0	0.25(0.005)	0.19(0.036)
Mn						1.0	0.21(0.023)
As							1.0

Table 5. Correlation of Pb, Cd, Cr, Cu, hg, Mn, and as concentrations in green tea.

Heavy metals	Single	Comprehensive
As	0.027	0.094
Cd	0.040	
Cr	0.12	
Hg	0.011	
Pb	0.090	

Table 6. Evaluation of heavy metal pollution in tea. Health risk posed by green tea consumption.

Heavy metals	RfD (μg/kg.bw.d)	EDI (μg/kg.bw.d)	HQ	HI
Al	1000	3.13	0.0031	0.42
As	0.3	0.00061	0.0020	
Ba	200	0.24	0.0012	
Cd	1.8	0.00045	0.00025	
Cr	1500	0.0070	4.7×10^{-6}	
Cu	40	0.16	0.0039	
Hg	0.3	0.000037	0.00012	
Li	2	0.0020	0.0010	
Mn	24	9.48	0.40	
Ni	20	0.12	0.0061	
Pb	3.6	0.0051	0.0014	
Sb	0.4	0.00025	0.00062	
Se	5	0.00086	0.00017	
Sn	600	0.0020	3.4×10^{-6}	
V	7	0.0012	0.00018	

Table 7. Estimated EDI, HQ, and THQ values of individual metals.

The calculated EDI, HQ, and HI values of the metals ingested into the human body through tea consumption are presented in Table 7. Among the investigated metals, Mn and Sn have the highest (0.40) and lowest (3.4×10^{-6}) HQ values, respectively. Considering that the HI value of the heavy metals (0.42) is well below 1, it is not likely that green tea consumption may pose a risk to human health.

Conclusions
The concentrations of heavy metals in green tea samples produced in Hangzhou were determined by ICP-MS. All the 120 samples did not exceed the standard limit value of heavy metal. The pollution level of heavy metals indicated that the single pollution index and comprehensive pollution index of green tea produced in Hangzhou area are far below 0.7, which belong to the clean and safety level. Health risk of inhabitant via consuming green tea was conducted and the HI value was 0.42, less than 1 indicate that green tea consumption are unlikely pose risk to human health.

Data availability
The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from

the corresponding author upon reasonable request. All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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Competing interests
The authors declare no competing interests.

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