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Assessing the health risks of heavy metals and seasonal minerals fluctuations in *Camellia sinensis* cultivars during their growth seasons

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A R T I C L E I N F O

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A B S T R A C T

The risk assessment of heavy metals in tea is extremely imperative for the health of tea consumers. However, the effects of varietal variations and seasonal fluctuations on heavy metals and minerals in tea plants remain unclear. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to evaluate the contents of aluminum (Al), manganese (Mn), magnesium (Mg), boron (B), calcium (Ca), copper (Cu), cobalt (Co), iron (Fe), sodium (Na), zinc (Zn), arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), and antimony (Sb) in the two categories of young leaves (YL) and mature leaves (ML) of tea (*Camellia sinensis*) cultivars throughout the growing seasons. The results showed significant variations in the contents of the investigated nutrients both among the different cultivars and growing seasons as well. Furthermore, the average concentrations of Al, Mn, Mg, B, Ca, Cu, Co, Fe, Na, Zn, As, Cd, Cr, Ni, and Sb in YL ranged, from 671.58–2209.12, 1260.58–1902.21, 2290.56–2995.36, 91.18–164.68, 821.95–5708.20, 2.55–3.80, 3.96–25.22, 37.95–202.84, 81.79–205.05, 27.10–69.67, 0.028–0.053, 0.065–0.127, 2.40–3.73, 10.57–12.64, 0.11–0.14 mg kg^{-1}, respectively. In ML, the concentrations were 2626.41–7834.60, 3980.82–6473.64, 3335.38–4537.48, 327.33–501.70, 9619.89–13153.68, 4.23–8.18, 17.23–34.20, 329.39–567.19, 145.36–248.69, 40.50–81.42, 0.089–0.169, 0.23–0.27, 5.24–7.89, 18.51–23.97, 0.15–0.19 mg kg^{-1}, respectively. The contents of all analyzed nutrients were found to be higher in ML than in YL. Target hazard quotients (THQ) of As, Cd, Cr, Ni, and Sb, as well as the hazard index (HI), were all less than one, suggesting no risk to human health via tea consumption. This research might provide the groundwork for essential minerals recommendations, as well as a better understanding and management of heavy metal risks in tea.

1. Introduction

Indeed, tea is globally recognized as the second most consumed beverage after water and is enjoyed for its taste, variety, and potential health benefits. It's prepared from the tender buds and leaves of the tea plant (*Camellia sinensis* (L.) O. Kuntze) that have been steeped in boiling water to create a tea infusion. China, India, Sri Lanka, and Kenya are among the Asian and African countries that mainly cultivate tea plants (Li et al., 2015). According to recent statistics, the worldwide area under tea cultivation is 407×104 hm^2, yielding around 6×10^4 metric tons. In

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addition, China accounted for 54% of world tea acreage and 41% of global tea production, respectively (Zhang, Yang, Chen, Li et al., 2018). Tea is a perennial plant that requires pruning at regular intervals throughout the year. The shoots are plucked at regular intervals (6–13 days), removing a large amount of different nutrients from the soil-plant system. Fertilizer applications are necessary to provide these nutrients. The present fertilizer prescription in tea focuses on the application of main nutrients, especially N, P, and K, while minerals are not consistently treated in tea plantations, so their loss from the soil continues in the absence of a planned replacement strategy. Although tea yield increases significantly with increasing N, P, and K up to a certain level (Barbora, 1996). However, other essential nutrients, such as minerals and trace elements, should not be ignored. The daily demand for nutrients increases as the tea plants develop. In general, plants require seventeen (17) important nutrients for growth, with each nutrient performing a distinct role in the plant (Kaur et al., 2023). In short, plants require not only a sufficient amount of nutrients, but they also need them to be present in the right ratios and delivered at the appropriate times.

Fe, zinc Zn, Mn, Cu, B, and Ni are now recognized minerals; they are normally found in quantities of less than 0.01% of dry tissue weight (Grusak, 2001). Other minerals, such as Co and Na are necessary or beneficial for specific plant species, but their broad significance has yet to be conclusively proven in the broader context of plant growth and development. Many more elements can be detected in plants, but they are assumed to enter plants in an indiscriminate manner. The majority of these non-essential elements have no known value to the plant, and several, such as As, Sb, Cd, or Cr, are actually harmful to plant growth (Grusak, 2001). Furthermore, the tea plant is known to be a hyperaccumulator of Al, accumulating large amounts of Al in its leaves ranging from 8700 to 23000 mg kg^{-1}, and even up to 30, 000 mg kg^{-1} (Mukhopadyay et al., 2012). A suitable Al concentration plays an important function in promoting the growth of tea plants (Li et al., 2017). Mn is a key component in plant functions such as secondary metabolism, lipid formation, photosynthesis, and oxidative stress (Williams and Pittman, 2010). The tea plant has the ability to collect significant levels of Mn (more than 1000 mg kg^{-1}) in its leaves (Zaman et al., 2023). Several studies have found that the Mn content of tea leaves is extraordinarily high (Peng et al., 2018; Wen et al., 2018), demonstrating that the tea plant is Mn hyperaccumulator. Mn fertilization boosts fresh tea production in Mn-deficient areas, whereas it diminishes tea production in Mn-abundant areas (Ishibashi et al., 2004). Besides, Mg, the second most prevalent intracellular cation, has been shown to play an important role in plants due to its involvement in chlorophyll synthesis and catalytic activity (Gerendás and Führs, 2013). Ca is the third most abundant essential plant nutrient, following N and K, and it has a similar availability to P in plant tissue. Growers have access to a wide range of commercial minerals supplements. These range from single-element products to multi-mineral combinations. These multi-minerals combinations have the potential to treat minerals deficiencies, particularly when several micronutrient shortages exist (Kumar, 2017).

Heavy metals such as As, Cd, Cr, Ni, and Sb, have been found in tea leaves, brewed tea, and tea infusions, according to several studies (Li et al., 2015; de Oliveira et al., 2018; J. Sun et al., 2019). According to these investigations, tea contains hazardous heavy metals, and long-term exposure may promote their accumulation in the human body. However, to our knowledge, a comparative investigation on the natural variations, seasonal dynamics of hyperaccumulators, minerals and health risk evaluation of the aforementioned heavy metals throughout the growing seasons of diverse tea cultivars is lacking. Consequently, the aims of this work were to 1) measure the concentrations of Al, Mn, Mg, B, Ca, Cu, Co, Fe, Na, Zn, As, Cd, Cr, Ni, and Sb in tea leaves (young and mature) during different growth seasons of the year in diverse tea cultivars using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES); and 2) determine the natural variations and seasonal dynamics of these nutrients in tea varieties; 3) using the target hazard quotient (THQ) and hazard index (HI) approaches, assess the human health risk of heavy metals (As, Cd, Cr, Ni, and Sb) in tea leaves through tea intake. This research can assist as a valuable resource and groundwork for understanding the natural variations and seasonal minerals fluctuations in tea germplasm, as well as mitigating the potential risks of heavy metals in tea.

2. Materials and methods

2.1. Plant materials

The plant material for the investigation was collected from Huazhong Agricultural University's tea garden in Wuhan (30°32.87´N, 114°25.01´E), China. A total of 87 elite tea cultivars aged five years were used and they were either suitable for black tea, or green tea, or oolong tea. The detailed information of the tea cultivars, breeding method, genetic background, origin and suitability for tea categories, were according to our previous study (Zaman et al., 2022b). The tea garden has been maintained as an integrated yearly fertilization strategy during the growing season, with basal fertilizer supplied once in autumn (between mid-October and mid-November) and topdressing fertilizer used twice during the growth season (before sprouting early in March and immediately after spring tea harvest. According to an earlier study (J. Zhang et al., 2020), nitrogen, phosphorus (P_2O_5), and potassium (K_2O with SO_4^{2-} type) fertilizers were used as base supplements. Weeds, insects, and diseases were controlled using chemical or manual methods to ensure vigorous growth and a predictable yield. The experimental area has a warm and subtropical climate, with an average annual temperature of 15.8 °C–17.5 °C, an average annual rainfall of 1269 mm, and an average annual frost-free time of 270 days during the last three decades. Watering was accomplished through the use of gun sprinkler irrigation. Before basal fertilizer application, the soil pH was 4.5, organic matter was 42.65 g kg^{-1}, alkali-hydrolyzable N was 164.83 mg kg^{-1}, available P was 68.06 mg kg^{-1}, and available K was 210.36 mg kg^{-1}, showing highly fertile soil for tea cultivation.

2.2. Sampling and measurements of (minerals and hyperaccumulating nutrients) and heavy metal levels

One bud with two expanding/young leaves (YL) (hundred buds/cultivar) and mature canopy leaves (ML) (hundred leaves/cultivar) were randomly sampled to elucidate their natural and nutritional importance. Photosynthesis is influenced by the amount of leaf area in the canopy, which in turn affects tea production. The duration that leaves last is determined by climate variations and varies by variety. The proportion of canopy leaves varies greatly amongst the tea germplasm. Tea plants at the end of each tea row were not taken into account to avoid border impacts. In 2019, from March to October, four rounds of YL and ML were sampled two rounds in spring (22nd March and 05th May), one in summer (19th July) and one in autumn season (05th October) replicated four times for the evaluation natural and seasonal dynamic variations of minerals, hyperaccumulating and certain heavy metals. The samples were instantly treated at 120 °C for 2–3 min, followed by 80 °C to the constant weight, and then milled to a fine powder for nutritional analysis (Naozuka et al., 2011; L. L. Sun et al., 2019). The 0.05 g of fine powdered tea leaf sample was digested with concentrated H_2SO_4, and the concentrations of all the investigated minerals were determined by an Inductively Coupled Plasma optical emission spectrometer (ICP-OES) (Zaman et al., 2022a). The optimized ICP-OES instrumental operating conditions are shown in Table 1.

2.3. Health risk evaluation

The estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) were used to evaluate the health hazards to tea users

Table 1
Optimized ICP-OES instrumental operating conditions.

Instrument	Agilent 5110
R. f. frequency	49.9 Hz
Operating power	1.2 KW
Plasma gas flow rate	12 L/min
Auxiliary gas flow rate	1.00 L/min
Torch type	Easy – fit torch one piece 5100 DV ICP-OES
Nebulizer type	Sea-spray nebulizer (glass) U – series1/pk
Nebulizer pressure	150 Kpa
Sample flow rate	15 ml/min
Pump rate	21 rpm
Integration time	5s
Peak search window width	0.08 nm
Viewing height	8 nm
Background modification	Default
Grating order	Default
Filter position	Default
Snout purge	Low

as a result of prolonged intake of As, Cd, Cr, Ni, and Sb from tea consumption (Zhang et al., 2018b). The following are the formulas for calculating EDI, THQ, and HI:

$$EDI_i = (C_i \times IR \times TR_i)/\ (BW \times 1000) \qquad (1)$$

$$THQ_i = EDI_i/RfD_i \qquad (2)$$

$$HI = THQ_1 + THQ_2 + \cdots + THQ_n \qquad (3)$$

where C_i is metal i concentration in tea leaves (mg kg^{-1}), IR is the consumption rate of tea leaves (11.4 g $person^{-1}$ d^{-1}) (Peng et al., 2018), TR_i is the transferal rate of metal i from tea leaves into tea infusion (Zhang et al., 2018b), BW is bodyweight, specifically 70 kg for adults (de Oliveira et al., 2018), RfD_i (mg kg^{-1} bw d^{-1}) is the US Environmental Protection Agency's (USEPA) oral reference dosage for metal i, THQi is the target hazard quotient of metal i, whereas HI denotes the overall health risk exposure to hazardous metals (n = 5). The RfD values for As, Cd, Cr, Ni and Sb were 3.0×10^{-4}, 5.0×10^{-4}, 1.5, 0.02 and 4.0×10^{-4} mg kg^{-1} bw d^{-1}, respectively (US EPA, 2019; Cai et al., 2019).

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was used to calculate the significance of variations in nutritional concentrations in the two categories of tea leaves, cultivars and growing seasons using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA, 2004). The least significant difference (LSD) test was used to compare the differences in mean values of each parameter. Microsoft Excel 2016 was used to summarize and compute descriptive statistics (maximum value (Max), minimum value (Min), mean, standard deviation (SD), and coefficient of variation (C.V). The heatmap was illustrated using online software, www.heatmaper.ca. Significances were indicated as follows, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Figures were drawn with the Origin Pro 7.0 software.

3. Results

3.1. Concentrations of hyperaccumulators (Al, Mn and Mg) and essential nutrients in the two categories of tea leaves

The average concentration of Al, Mn and Mg in the YL and ML of 87 tea cultivars throughout the growing seasons is presented in Fig. 1. The results revealed that the average concentration of Al in the YL increased from 671.58 mg kg to 1 in the 1st spring tea to 850.38 mg kg-1 in the 2nd spring tea, it decreased to 747.289 mg kg-1 in the summer tea, and then increased to 2209.12 mg kg-1 in autumn tea (Fig. 1). Correspondingly, the average concentration of Al in the ML decreased from 7834.60 mg kg to 1 in the 1st spring tea to 5621.30 mg kg-1 in the 2nd spring tea. Subsequently, it decreased to 4478.13 mg kg-1 in summer tea and then further decreased to 2626.41 mg kg-1 in autumn tea. The natural variations of Al concentrations among the investigated cultivars were indicated by coefficient variation (CV) Table S1. The results demonstrated that the CVs for Al concentration in the YL were 30.63% (summer tea) > 24.08% (the 1st spring tea) > 20.83% (the 2nd spring tea) > 7.09% (autumn tea) while the CVs for ML were 65.34% (the 2nd spring tea) > 59.60% (summer tea) > 22.68% (the 1st spring tea) > 9.21% (autumn tea).

Furthermore, in terms of average Mn concentrations in the YL, Mn increased from 1368.81 mg kg to 1 in the 1st spring tea to 1389.23 mg kg-1 in the 2nd spring tea, to 1902.21 mg kg-1 in the summer tea and then significantly decreased to 1260.58 mg kg-1 in autumn tea. Whereas in ML the Mn concentration constantly decreased from 6473.64 mg kg to 1 in the 1st spring tea to 5547.68 mg kg-1 in the 2nd spring tea, further decreased to 4926.24 mg kg-1 in summer tea and finally decreased to 3980.82 mg kg-1 in autumn tea (Fig. 1). Besides, the average concentration of Mg ranged from 2290.56 mg kg-1 to 2995.36 mg kg-1 in the YL, while in the ML it varied from 3335.88 mg kg-1 to 4537.48 mg kg-1 (Fig. 1).

The average concentrations of B, Ca, Co, Cu, Fe, Na, and Zn mg kg^{-1} are presented in Fig. 2. The results showed that the levels of the aforementioned nutrients were higher in ML than in YL (Fig. 2). The average content of B ranged from 91.18 mg kg-1 to 164.68 mg kg-1 in the YL and in the ML, it varied from 327.33 mg kg-1 to 501.70 mg kg-1 (Fig. 2a). Likewise, the average content of Ca ranged from 821.95 to 5708.20 mg kg-1 in the YL, whereas in the ML it varied from 9619.89 to 13153.68 mg kg-1 (Fig. 2b). Similarly, in terms of Co, the average Co content in YL ranged from 2.55 mg kg-1 to 3.80 mg kg-1 whereas it ranged from 4.23 mg kg-1 to 8.18 mg kg-1 in the ML (Fig. 2c). Furthermore, the average Cu content ranged from 1.18 to 8.95 mg kg-1 and from 17.23 to 34.20

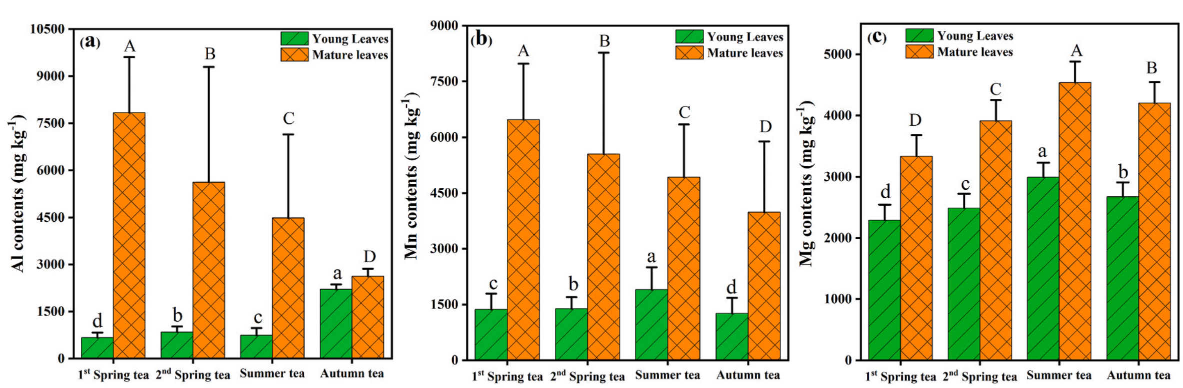

Fig. 1. Concentrations of hyperaccumulators (Al, Mn and Mg) in young leaves (YL) and mature leaves (ML) (mg kg^{-1}) over the four growing seasons, the 1st spring, the 2nd spring, summer and autumn. The different lowercases and uppercases indicated that nutrient concentrations in YL and ML significantly differed between each season, respectively ($p < 0.05$).

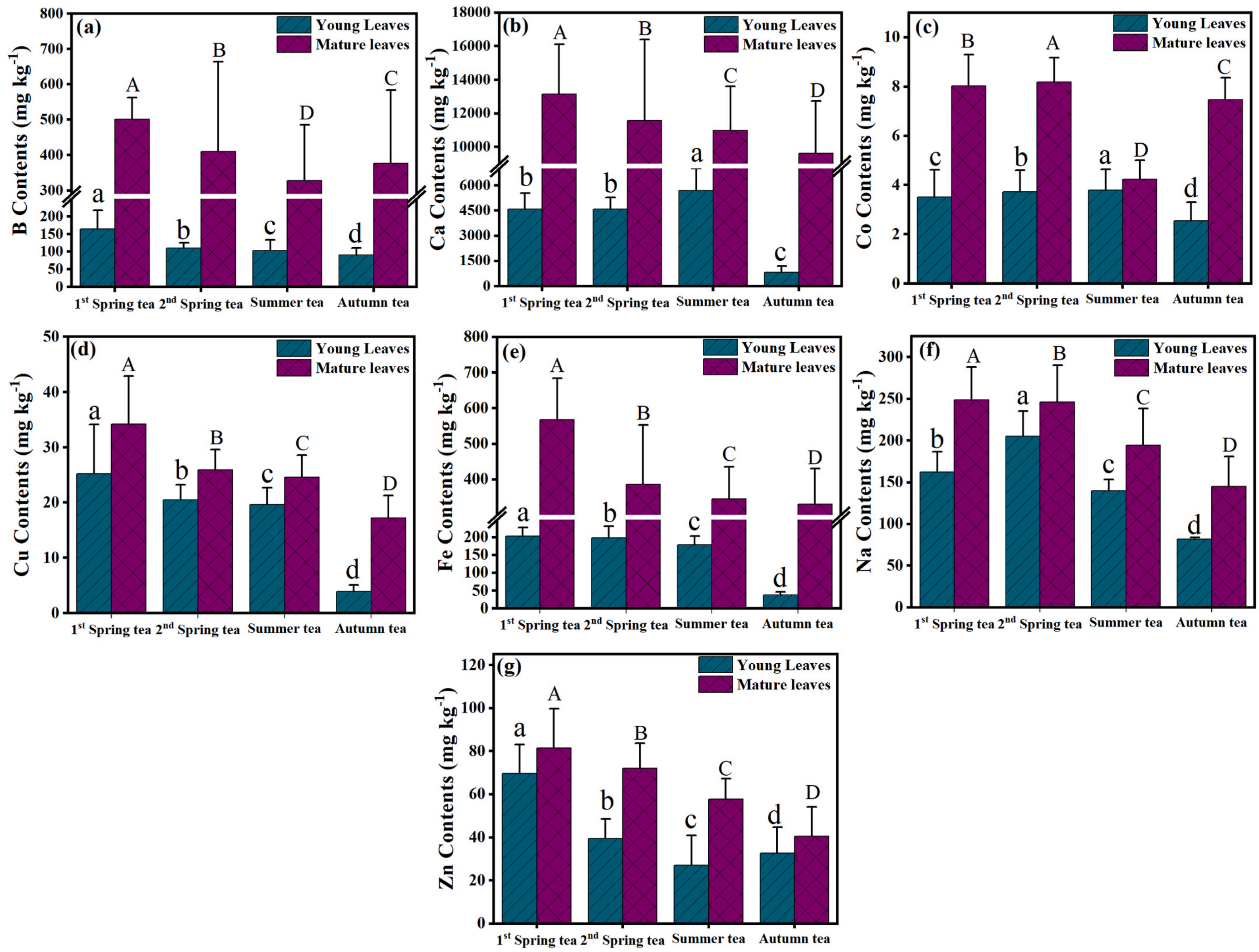

Fig. 2. Concentrations of B, Ca, Co, Cu, Fe, Na, and Zn in young tea leaves (YL) and mature leaves (ML) (mg kg^{-1}) over the four growing seasons, the 1st spring, the 2nd spring, summer and autumn. The different lowercases and uppercases indicated that nutrient concentrations in YL and ML significantly differed between each season, respectively ($p < 0.05$).

mg kg-1 in YL and ML, respectively (Fig. 2d). The average Fe content varied from 37.95 to 202.84 mg kg-1 and from 329.39 to 567.19 mg kg-1 in YL and ML, respectively (Fig. 2e). Similarly, the average Na content varied from 81.79 to 205.05 mg kg-1 in YL, whereas it ranged from 145.36 to 248.69 mg kg-1 in ML (Fig. 2f). For the average Zn content in YL, it varied from 27.10 to 69.67 mg kg-1 while in ML, it ranged from 40.50 to 81.42 mg kg-1 (Fig. 2g). The natural variations and seasonal changes of all these nutrients in the investigated tea plants were specified by coefficient variation (CV) Table S2, which demonstrated that each nutrient in a particular leaf category had a different coefficient of variation for each growing season, reflecting the extensive and limited fluctuations among all the growing seasons.

3.2. Heavy metal content in the two categories of tea leaves

Fig. 3 depicts the mean concentrations of As, Cd, Cr, Ni and Sb in YL and ML of 87 investigated tea cultivars over the four growing seasons. The results showed that all tea samples contained lower levels of As, Cd, Cr, Ni and Sb than the World Health Organization's (WHO) and Chinese national food safety regulations for maximum levels of contaminants in foods (GB 2762–2017 and NY 659–2003) (de Oliveira et al., 2018; Zhang, Yang, Chen, Li, et al., 2018). Furthermore, Cd contents in all YL samples were lower than European Union (EU) limits of 1 mg kg-1 (Zhang et al., 2018b). Comparatively, the heavy metal As, Cd, Cr, Ni and Sb contents were found higher in ML than in YL (Fig. 3). The mean content of As ranged from 0.028 to 0.053 mg kg-1 in YL, whereas it ranged from 0.089 to 0.169 mg kg-1 in ML. The coefficient of variation (CV) was used to denote the natural variations in As concentrations among the examined cultivars Table S3. Similarly, the average Cd content ranged from 0.065 to 0.127 mg kg-1 in YL whereas in ML it varied between 0.227 and 0.272 mg kg-1 (Fig. 3b). Based on the coefficient of variation (CV) Table S3 for Cd in YL and ML, the growing seasons can be ranked as the 1st spring tea > the 2nd spring tea > summer tea > autumn tea. Furthermore, the average Cr content decreased from 3.73 mg kg to 1 in the 1st spring tea to 3.43 mg kg-1 in the 2nd spring tea, further decreased to 2.92 mg kg-1 in summer tea and finally decreased to 2.40 mg kg-1 in autumn tea in YL while in ML it varied between 5.24 and 7.89 mg kg-1 (autumn-the 1st spring) also a maximum Cr content of 4.63 mg kg-1was observed in the 1st spring tea and a minimum Cr content of 1.64 mg kg-1 in autumn tea in YL, whereas in ML a maximum Cr content of 9.83 mg kg-1 and a minimum Cr content of 3.38 mg kg-1 (Fig. 3c). Additionally, the average Ni content fluctuated between 10.57 and 12.64 mg kg-1 in YL, whereas in ML it changed from 18.51 to 23.97 mg kg-1 (Fig. 3d). The average Sb content, increased from 0.11 mg kg to 1 in the 1st spring tea to 0.14 mg kg-1 in autumn tea in YL, whereas in terms of ML, the Sb content exhibited the same trend

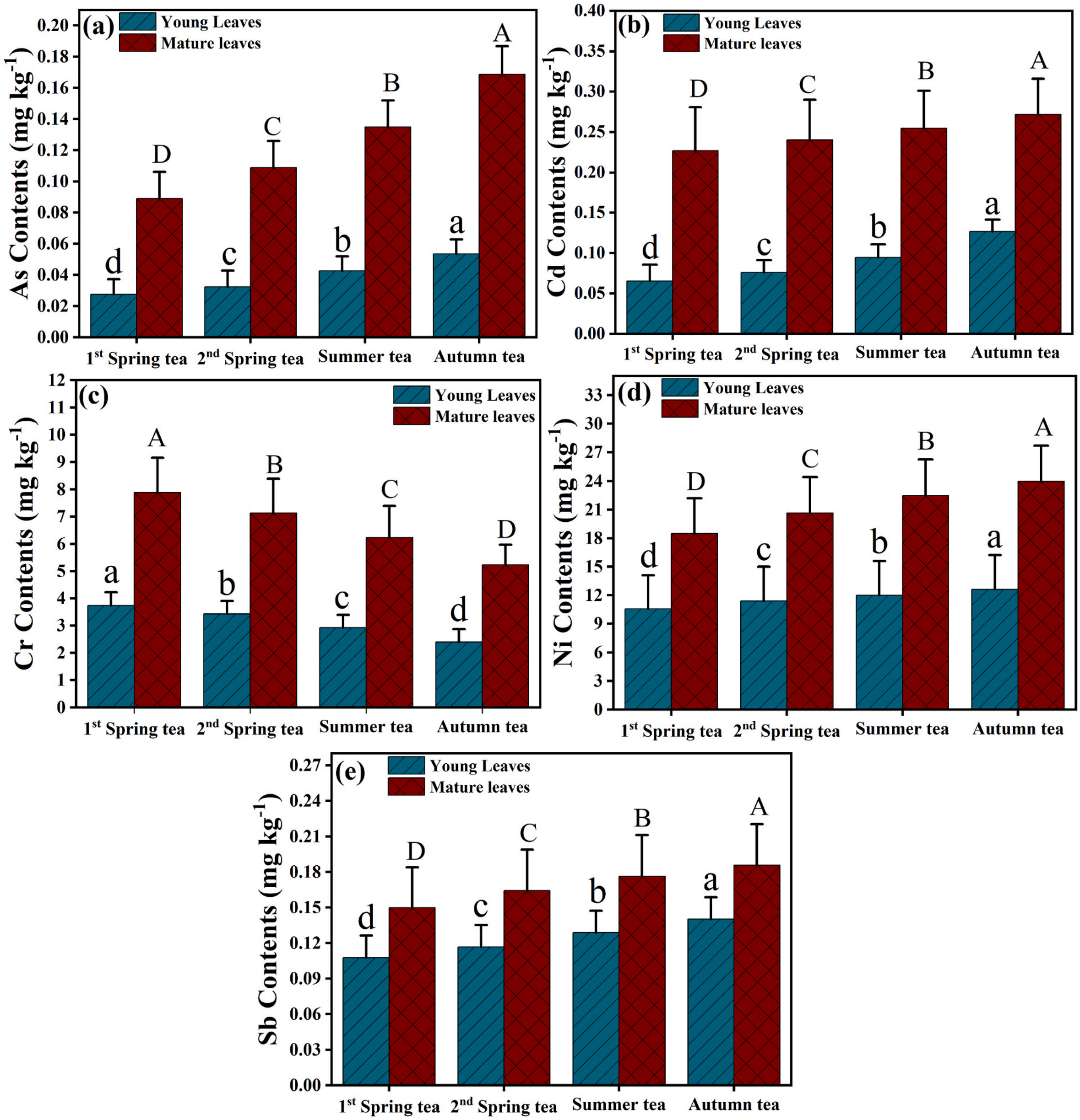

Fig. 3. Concentrations of heavy metals (As, Cd, Cr, Ni and Sb) in young leaves (YL) and mature leaves (ML) (mg kg^{-1}) over the four growing seasons, the 1st spring, the 2nd spring, summer and autumn. The different lowercases and uppercases indicated that nutrient concentrations in YL and ML significantly differed between each season, respectively ($p < 0.05$).

and increased from 0.15 mg kg to 1 in the 1st spring to 0.19 mg kg-1 (Fig. 3e).

3.3. Estimated daily intake (EDI)

Heavy metals present in tea leaves do not completely leach into tea infusions. As a result, the metal transfer rate (TR) from tea leaves into infusions should be taken into account (Zhang et al., 2018a). In the current study, a leaching experiment to assess the transfer of heavy metals from tea leaves into infusions was not conducted. Consequently, reported TR values were utilized to estimate the Estimated EDI in this research. The TR values for As, Cd, Cr, Ni and Sb were 23.83%, 14.18%, 11.45%, 67.71% and 11.78%, respectively (Zhang et al., 2018a). Table 2 shows the computed EDI values (mg kg^{-1} bw d^{-1}) of heavy metals from tea infusion consumption. The average EDI values (mg kg^{-1} bw d^{-1}) of As, Cd, Cr, Ni and Sb for the ML infusion were higher than those for the YL infusion. The results further revealed that the EDI values (mg kg^{-1} bw d^{-1}) for heavy metals in both categories (YL and ML) of leaves increased from the 1st sprig to the autumn season, except for Cr, which decreased over the growing seasons.

3.4. Overall risk of metals and hazard indexes

Fig. 4 shows the computed THQs of As, Cd, Cr, Ni and Sb, as well as the accumulative HI values for YL and ML infusions from the four growing seasons (the 1st spring, the 2nd spring, summer and autumn) of 87 investigated elite tea cultivars. THQ of individual metal and accumulative HI values for As, Cd, Cr, Ni and Sb were all less than one for both the YL and ML infusions, indicating that the five heavy metals will not result in adverse health effects for adults via daily tea consumption. The average THQs of each metal related to ingesting YL infusion were rated in detail as follows: 0.082 (Summer) > 0.076 (the 2nd spring) > 0.067 (the 1st spring) > 0.027 (autumn), similarly, the rankings for ML infusion were as follows: 0.176 (autumn) > 0.162 (summer) > 0.147

Table 2
Estimated daily intake (EDI) (mg kg^{-1} bw d^{-1}) of (As, Cd, Cr, Ni and Sb) for adults associated with the consumption of infusions of young leaves (YL) and mature leaves (ML) from the 1st spring tea, the 2nd spring tea, Summer tea and autumn tea.

Season and leaf type	As	Cd	Cr	Ni	Sb
1st spring tea-YL	1.312E-07	1.512E-06	6.961E-05	0.001165	2.065E-06
2nd spring tea-YL	1.258E-06	1.758E-05	6.388E-05	0.001258	2.238E-06
Summer tea-YL	1.652E-06	2.188E-06	5.452E-05	0.001225	2.470E-06
Autumn tea-YL	2.074E-06	2.927E-06	4.472E-05	0.000159	2.689E-06
Mean-YL	1.279E-06	2.096E-06	5.818E-05	0.000977	2.365E-06
1st spring tea-ML	3.452E-06	5.244E-06	0.0001471	0.002041	2.874E-06
2nd spring tea-ML	4.224E-06	5.550E-06	0.0001330	0.002276	3.154E-06
Summer tea-ML	5.242E-06	6.588E-07	0.0001163	0.002477	3.383E-06
Autumn tea-ML	6.547E-06	6.273E-06	9.765E-05	0.002643	3.563E-06
Mean-ML	4.866E-06	5.737E-06	1.235E-04	0.002359	3.243E-06

The mean represents the average value of the four seasons.

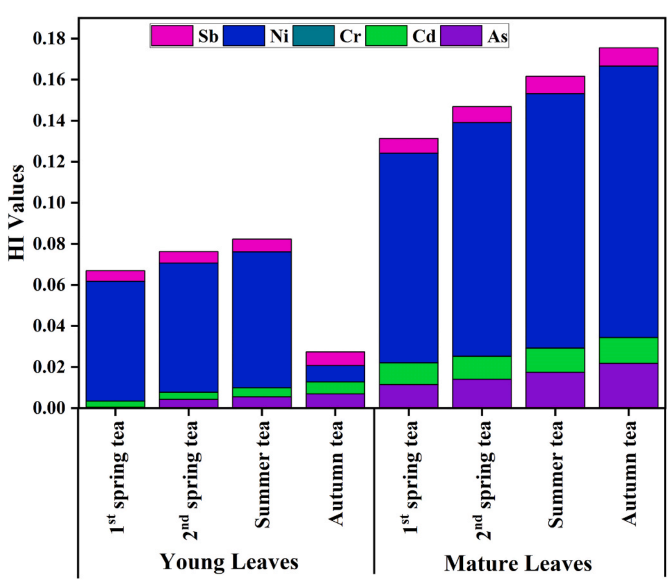

Fig. 4. The calculated target hazard quotients (THQ) of As, Cd, Cr, Ni, and Sb and the accumulative hazard indexes (HI) for adults associated with the consumption of infusions of young leaves of tea (YL) and mature leaves (ML) from the four seasons, the 1st spring tea, the 2nd spring tea, summer tea and autumn tea.

(the 2nd spring) > 0.131 (the 1st spring) indicating a significant difference between the YL and ML.

3.5. Correlation of scrutinized nutrients between the two categories of leaves over the entire growing season

The correlation among the studied elements is presented in Fig. 5. Significant highly positive correlation was observed for the contents of Al-YL with Al-ML, B-ML, Ca (YL and ML), Mn-YL, Cr-ML and Sb-ML, while with Cu (YL and ML), Zn (YL and ML), the correlation was significantly negative. Correspondingly, Al-ML was found significantly positive correlated with B-ML, Ca (YL and ML), Fe-ML, Mn-ML and Ni-YL while with B-YL, Co (YL and ML), Cu (YL and ML), Mg (YL and ML) and Zn (YL and ML) significantly negative. Similarly, the correlation of B-YL with Mn-YL, Mg-ML, Na-YL, Zn-YL, Ca-YL, Co-YL, Co-ML, Cu-YL and Cu-ML was found significantly positive whereas with Mn-ML, Ni-YL, B-ML and Ca-ML was significantly negative. B-ML was observed positively correlated with Ca-ML, Fe-ML, Mn-ML and As-YL while negatively correlated with Co-YL, Co-ML, Cu-YL, Cu-ML, Mg-ML, Zn-YL, Zn-ML and Sb-YL. Further, Ca-YL was found significantly positive correlated with Fe-YL, Mn-YL and Cr-ML, on the other hand, Ca-ML was positively correlated with Fe-ML, Mn-ML and Sb-YL while found negative with Mg-ML, Zn-YL, Zn-ML, Cd-YL, Co-YL, Co-ML, Cu-YL, Cu-ML. Co-YL was observed extremely positive significant with Co-ML, Cu (YL & ML), Fe-YL, Mn-YL, Mg (YL & ML), Na-ML, Zn-YL and Cd-YL while tremendously negative significant with Ni-YL, Ni-ML and Sb-ML. For the concentrations of Co-ML, it was observed that the contents of Cu, Fe, Mn, Mg, Zn in YL and Cu, Mg, in ML were found to be expressively positive correlated with Co-ML whereas they had a negative correlation with Ni-YL and Sb-ML. Besides, for the rest of the analyzed elements such as Fe, Mn, Mg, Na, Zn, As, Cd, Cr, Ni and Sb, moderate or weak correlations were observed in YL/ML as shown in Fig. 5. By using the pearson distance measurement method between different tea cultivars, a separate heatmap was created to visualize the concentrations of analyzed nutrients in the two categories of tea leaves and the influences of different seasons on these nutrients (Fig. S1). The heatmap patterns illustrated that the leaf category significantly influenced the concentration, accumulation, and distribution of each nutrient. Furthermore, the dynamic variations within these categories were influenced by seasonal fluctuations. For instance, the distinct colors observed for each nutrient, leaf type, and season on the x-axis, with each cultivar represented on the y-axis of the heatmap, indicate the variation of each nutrient within a particular leaf category and season.

The color key at the top of the heatmap describes the scores (red indicates higher and orange indicates lower) of the analyzed nutrients.

4. Discussion

The tea plant (*Camellia sinensis*) is recognized as an Al-hyperaccumulator. It may accumulate up to 3000 mg kg^{-1} of Al in old leaves without exhibiting hazardous symptoms (Ruan and Wong, 2004). Al stands out as the most distinctive inorganic element in tea plants, with concentrations in tea leaves notably higher compared to those in other plants (Fung et al., 1999). According to (Shu et al., 2003), the Al content of the sixth leaves of Sichuan Qunti in Gaoxian plantation was found to be 13-fold greater than that of the bud with two leaves, suggesting that mature leaves were the primary contributors to the high Al content in tea. Similar findings were noted in this study, with the Al content being higher in the ML compared to the YL (Fig. 1). Dynamic variations were noticed in the content of Al both in YL and ML over the growing seasons, as Al increased between the 1st and 2nd spring, decreased in summer and highly increased in the autumn season. These findings align with a previous study which observed a continuous deposition of Al in tea leaves, with its concentration increasing over time (Huang et al., 2021). Our findings are further supported by (Zaman et al., 2023), who reported significant variations in elemental concentrations in plant tissues influenced by atmospheric element levels, pH of the growth medium, presence or absence of complexing agents, plant species, and the specific part of the plant under investigation.

Mn is a trace element essential for the growth and development of tea plants, playing a critical physiological role in various plant functions. In normal tea plant growth, young tissue has a lower Mn level, than aged tissue, which has an increased Mn content. Old leaves have the highest Mn content, while buds, stems, and roots have the lowest. In the present study, the Mn content was found to be higher in ML compared YL. However, interestingly, throughout the growing seasons, the content of Mn was found to have an increasing tendency, whereas, in ML, it was

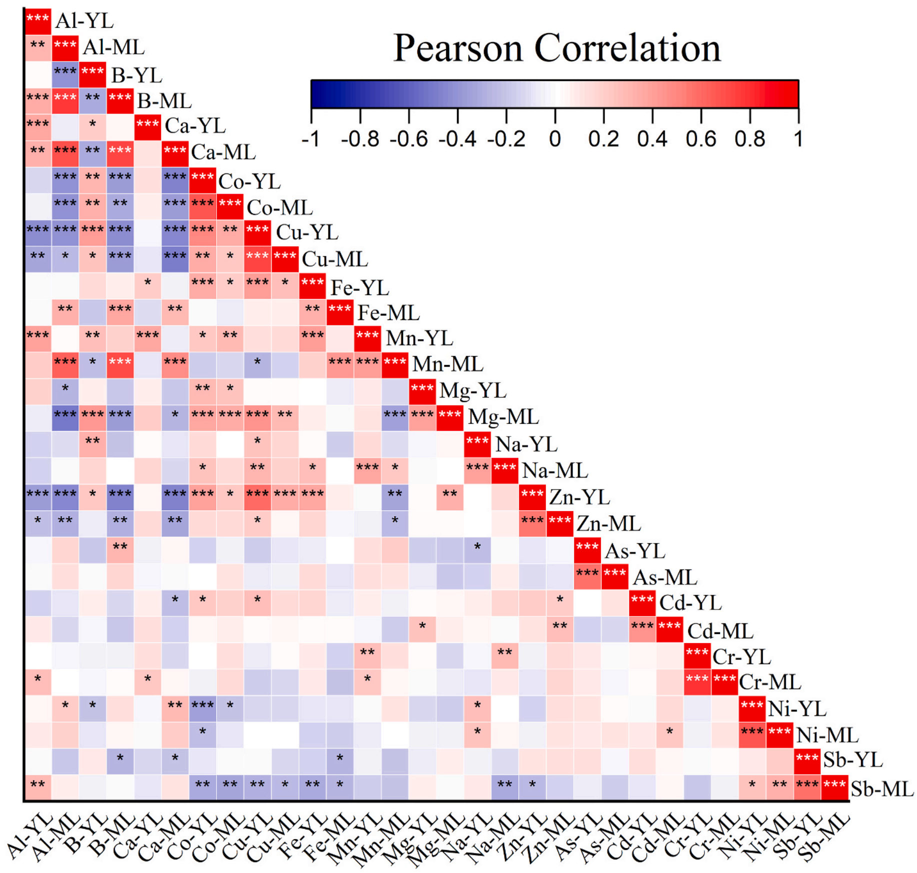

Fig. 5. Correlogram of contents (Al, Mn, Mg, B, Ca, Cu, Co, Fe, Na, Zn, As, Cd, Cr, Ni, and Sb) identified in the two types of leaves, i.e., young leaves (YL) and mature leaves (ML) in 87 tea (*Camellia sinensis*) cultivars over the four growing seasons, the 1st spring, the 2nd spring, summer and autumn. The colored gradient legends represent the coefficient of correlation r values from −1.0 (blue) to +1.0 (red). The significant correlation was at a level of ($p^{*}<0.05$), ($p^{**}<0.01$) and ($p^{***}<0.001$). All coefficients were computed by the pearson possible pairs of variables in the matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

observed that the Mn content frequently decreased throughout the growing seasons (Fig. 1). Similar results were found by (Demir and Bostan, 2018), which revealed that the content of Mn has changed with the harvest season, particularly in the old tea leaves. In terms of Mn enrichment in tea leaves, it is possible to assume that the mechanism of Mn enrichment by old tea leaves is due to the following factors: 1) It can be attributed to the very acidic soil in tea plantations (pH = 4.30), because Mn mobility is strong in acidic soil, especially at pH values below 4.5. The concentration of accessible Mn in soil has been shown to rise significantly as soil pH decreases (Ishibashi et al., 2004; Zhang et al., 2018b); this encourages increased Mn absorption by tea plant roots. In particular, a very acidic environment (pH 4.5) might encourage the spontaneous rearrangement of valence electrons in hausmannite and manganite in soil, resulting in Mn^{2+} and birnessite (Ishibashi et al., 2004). 2) the high translocation factor of manganese (Mn) in tea plants facilitates its mobility from the roots to the aboveground sections and leaf tissues through the transpiration stream (Ruan et al., 2000). 3) As documented by Xu et al. (2006) on the hyperaccumulator plant (*Phytolacca acinosa*), once Mn reached the old tea leaves, it could not be easily remobilized through the phloem to other organs (e.g., young leaves, shoot, and new branch). 4) The great ability of old tea leaves to accumulate Mn can be related to the functions of genes involved in Mn distribution, absorption, translocation, and transformation in tea plants Li et al. (2019). Likewise, the Mg content in ML was found to be higher compared to YL, and in both YL and ML, the Mg content exhibited an increase with seasonal fluctuations. However, the average content of Mg was higher than Mn in the studied cultivars and this can be attributed to the fact that nutrients such as N, P, K and Mg are highly mobile in the tea plant tissue and are translocated from old leaves to young leaves by (Singh and Mann, 2012).

The average contents of B, Ca, Co, Cu, Fe, Na, and Zn are shown in Fig. 2. In a broad context, the concentration ranges of elements found in the leaves of the 87 analyzed cultivars closely aligned with the values observed in previous investigations (Fernández-Cáceres et al., 2001; Han et al., 2005; Marcos et al., 1998; Moreda-Pineiro et al., 2003; Yemane et al., 2008). Through statistical analysis, it was intriguing to observe significant differences in the levels of the majority of elements in tea leaves across various cultivars. The contents of all these elements were presented in mg kg^{-1} and according to the average levels of these elements, they were ranked as follows, Ca > Na > Fe > B > Zn > Cu > Co. These variations can be attributed to the previous studies (Han et al., 2006; Jin et al., 2008; Ruan and Wong, 2001) that documented that a variety of variables, including soil qualities (such as mineral composition, pH, cation exchange capacity, and particle size), environmental conditions (such as temperature, precipitation, moisture and air quality), and tea plant characteristics, influence the elemental contents of the tea plants (e.g. cultivar, biological status, etc.). In the current study, the 87 cultivars were grown in the same location and exposed to the same soil and environmental circumstances (Zaman et al., 2022b). Consequently, variations in the element composition of tea leaves among cultivars were mainly attributed to differences between cultivars. Tea cultivars contain wide differences in element concentrations, owing to variability caused by cross-pollination (Bieleski, 1994).

The ML has much higher concentrations of As, Cd, Cr, Ni and Sb than the YL. The explanation for this might be that, in terms of the same heavy metal, the YL and ML have distinct physiological requirements and absorption mechanisms. Similarly, certain non-nutritive elements (e.g., As, Cd and Cr) have been found to accumulate more in older leaves than in younger ones (Zhang et al., 2018b). Furthermore, the actual mobility of elements from old to new plant parts via the phloem has been observed to be element-dependent by Xu et al. (2006). In contrast to other nutrients with great mobility in plant tissues (e.g., N, P, K, and Zn), heavy metals in plants may not be reallocated and remobilized. As a result, the heavy metals in tea plant leaves accumulated as the leaves became older, Y. Zhang et al. (2020). Fig. 3 shows that both in YL and ML, the content of heavy metals had an increasing tendency over the growing seasons. These results are consistent with a previous study (Peng et al., 2018), which reported that the concentrations of heavy metals such as Al, Mn, Pb, and Cd in tea leaves increase as they mature. The increase between the bud and the 6th leaf depends on the variety too. For these four heavy metals, there were substantial positive correlations between leaf maturity and accumulation. In contrast, the content of Cr had a decreasing pattern with seasonal fluctuation as the Cr levels decreased both in YL and ML. This can be attributed to the immobile characteristic because there is minimal bioconcentration or biomagnification of chromium since the majority of the biosphere is reducing for chromium and is relatively immobile (Kimbrough et al., 1999). Furthermore, a correlogram and heatmap were used to depict the natural variations, seasonal dynamics, and correlations among all analyzed nutrients in both leaf categories across the four growing seasons of each cultivar. This visualization highlighted the significance level of these correlations through different color patterns.

The computed HI values for As, Cd, Cr, Ni and Sb indicated that these five heavy metals may not induce harmful health consequences in humans when ingested through YL and ML infusions. The THQ and HI values were used to calculate the non-carcinogenic impacts of heavy metals on human health when they were consumed in the form of fruits, vegetables, and drinks (de Oliveira et al., 2018; Peng et al., 2018). according to a prior study (Huang et al., 2018), heavy metals in tea had fewer health hazards than rice and vegetables. In Taiwan, the HI values for As, Cd, and Cr exposure in three varieties of tea (green, black, and oolong) were all less than one, suggesting no hazard to human health (Shen and Chen, 2008). Even though the health risk of heavy metal intake through tea consumption was very low, there are additional ways that can play an essential role in human heavy metal exposure (Hadayat et al., 2018). Consequently, additional research on dietary composition and the presence of heavy metals in food is necessary to evaluate associated health risks. Furthermore, the findings of the current evaluation may be subject to considerable uncertainty. For a long time, tea infusion consumption was thought to be the sole method for people to be exposed to heavy metals (Li et al., 2015). Other dietary sources of heavy metals, such as drinking water, vegetables, fruits and cereals, might have a part in developing the potential health hazards. Previous research found that the health hazards caused by heavy metals varied by crop type, with solanaceous fruiting vegetables posing the lowest risk and grains (e.g., rice, corn, and wheat) posing the most (Zheng et al., 2020). Furthermore, the possible health consequences of heavy metals change according to the age range of exposed receptors. In particular, THQs of heavy metals, exhibited a decreasing sequence of toddlers > children > adults > adolescents (Karimyan et al., 2020). Furthermore, the same heavy metal transfer rates were utilized to compute the EDI for the YL and ML infusions, but there may be deviations from the real scenario. As a result, the findings of the current assessment should be considered preliminary. Further research into various routes of heavy metal exposure and the development of in vitro digestive models for humans are necessary.

5. Conclusions

We identified natural variations and significant dynamic changes in hyperaccumulators, minerals and heavy metal content for 87 five-year-old elite tea cultivars in two leaf categories, namely young leaves and mature leaves, during the spring, summer, and autumn seasons ($p < 0.05$). Accessions differences, as well as discernible tendencies, were discovered for the dynamic fluctuations in hyperaccumulators, minerals and heavy metal concentrations in the two types of leaves across the entire growing season. In addition, the contents of all analyzed nutrients were significantly higher in ML than in YL. The concentrations of As, Cd, Cr, Ni, and Sb in all tea samples were within the WHO and Chinese regulatory limits. Exposure to five heavy metals (As, Cd, Cr, Ni, and Sb) and the average THQ and HI values of young and mature leaves were less than one, indicating that intake of tea leaf infusions would not result in health concerns for adults.

Although a large number of study findings, further research is desired to fully understand the science of tea plant minerals accumulation, micronutrient absorption specifications and the impact of minerals on human health from frequent tea drinking in all tea-growing nations. Future studies should focus on identifying soil nutrient status, tea plant production potential, individual nutrient requirements, the role of hyperaccumulators, minerals, heavy metals and their ratios that would contribute the most to tea production with diversified tea commodities to promote tea plantation sustainability.

CRediT authorship contribution statement

Fawad Zaman: Writing – review & editing, Writing – original draft, Conceptualization. **Wajid Ali Khattak:** Investigation, Data curation. **Muhammad Ihtisham:** Data curation. **Muhammad Ilyas:** Data curation. **Ahmad Ali:** Writing – review & editing, Conceptualization. **Abbas Ali:** Data curation. **Haroon Khan:** Writing – review & editing, Data curation. **Khalid Ali Khan:** Conceptualization, Writing – review & editing. **Dejiang Ni:** Conceptualization. **Hua Zhao:** Conceptualization. **Fu-Sheng Chen:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declared that they have no competing financial interest or personal relationship that could have appeared to influence the work reported in the article.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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