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Critical factors determining fluoride concentration in tea leaves produced from Anhui province, China

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ABSTRACT

This study investigated the fluoride present in tea plants (*Camellia sinensis (L.)* O. Kuntze) and its relationship to soils, varieties, seasons and tea leaf maturity. The study also explored how different manufacturing processes affect the leaching of fluoride into tea beverages. The fluoride concentration in the tea leaves was significantly correlate to the concentration of water-soluble fluoride in the soil. Different tea varieties accumulated different levels of fluoride, with varieties, Anji baicha having the highest and Nongkang zao having the lowest fluoride concentration. In eight different varieties of tea plant harvested over three tea seasons, fluoride concentration were highest in the summer and lowest in the spring in china. The fluoride concentration in tea leaves was directly related to the maturity of the tea leaves at harvest. Importantly, the tea manufacturing process did not introduced fluoride contamination. The leaching of fluoride was 6.8% and 14.1% higher in black and white tea, respectively, than in fresh tea leaves. The manufacturing step most affecting the leaching of fluoride into tea beverage was withering used in white, black and oolong tea rather than rolling or fermentation. The exposure and associated health risks for fluoride concentration in infusions of 115 commercially available teas from Chinese tea markets was determined. The fluoride concentration ranged from 5.0 to 306.0 mg kg^{-1}, with an average of 81.7 mg kg^{-1}. The hazard quotient (HQ) of these teas indicated that there was no risk of fluorosis from drinking tea, based on statistical analysis by Monte Carlo simulation.

1. Introduction

Tea, prepared from the leaves of the tea plant, is one of the most widely consumed non-alcoholic beverages in the world. Drinking tea frequently has been reported to have beneficial effects on human health (Forester and Lambert, 2011; Khokhar and Magnusdottir, 2002; Thielecke and Boschmann, 2009). There is a large variety of commercial teas available, classified based on the degree of fermentation: non-fermented tea (green tea and white tea), semi-fermented tea (yellow tea and oolong tea) and fully fermented tea (black tea and dark tea) (Alcazar et al., 2007; Zhao et al., 2011; Zheng et al., 2015). Generally speaking, most teas (green and black) use the young, tender shoots of the tea plant. For dark tea, the mature tea leaves are used.

Tea plants accumulate environmental fluoride absorbed from the air and soil and up to 98% of it in the leaves (Gago et al., 2014; Janiszewska and Balcerzak, 2013; Malinowska et al., 2008). Thus, tea leaves are usually very high in fluoride. Several factors can influence the concentration of fluoride in tea leaves, including soil characteristics, environmental conditions, tea varieties and the maturity of harvested tea leaves (Alvarez-Ayuso et al., 2011; Fernandez-Caceres et al., 2001).

Soil conditions influence fluoride uptake by the tea plant in several ways. The optimum soil pH range for tea plants cultivation is 5.0–5.6, whereas, lower pH facilitates the accumulation of fluoride (Fung and Wong, 2002; Mehra and Baker, 2007). Previous investigations have found that the uptake of fluoride by tea plants was linearly related to the concentration of fluoride in the soil (Fung et al., 1999). However, others have reported that plants take up only the water-soluble fluoride from the soil and that the fluoride in the plants is mainly derived from the water-soluble fluoride in the soil (Chavoshi et al., 2011; Larsen and Widdowson, 1971). Thus, most of the research has focused on the effect of soil properties, such as soil pH, organic matter and cation exchange capacity, on water-soluble fluoride present in the soil (Barrow and Ellis, 1986; Jha et al., 2011; Loganathan et al., 2006). Few publications have reported the direct relationship between tea plant fluoride levels and soil properties.

Although there were reports about the different varieties of tea plant possessed different abilities to uptake and accumulate

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fluoride from soil and air (Ruan and Wong, 2001; Shu et al., 2003), however, this aspect studies were not sufficient thus far. Moreover, few studies have been reported the effect of seasons on the concentration of fluoride in tea leaves.

It has been shown that most of the fluoride was present in the cell walls and the soluble fractions of the tea leaves, and that vacuoles contained 98.0% of the total fluoride in tea leaf protoplasts (Gao et al., 2014). Since different kinds of tea undergo different manufacturing processes, there are different degrees of destruction of the cell structures in the tea leaves. Up to now, there has been no comprehensive research about how the manufacturing process affects the leachable fluoride in the six kinds of tea.

While humans can and do uptake fluoride from various food products, tea infusions are considered the second highest source, after drinking water. Fluoride exhibits both beneficial and toxic effects to the human body. For example, appropriate intake of fluoride is beneficial for preventing dental caries and for development of strong bones (Fernandes et al., 2014). However, excessive intake over the long term may lead to the development of dental fluorosis in children or even skeletal fluorosis in both children and adults (Cai et al., 2015; Cao et al., 2003; Wong et al., 2003). The consumption of large amounts of tea can pose a potential health risk, as the fluoride in tea is easily released during infusion (Cao et al., 2004, 2006; Heikens et al., 2005; Sofuoglu and Kavcar, 2008).

The purposes of the present study were to (i) investigate the effects of planting conditions in the accumulation of fluoride by tea plants, (ii) explore the effect(s) of different manufacturing processes on the amount of leaching of fluoride and (iii) estimate the health risks resulting from exposure to fluoride via tea drinking for adults.

2. Materials and methods

2.1. Sampling and sample pre-treatment

2.1.1. Sample collection

2.1.1.1. The effect of soil on fluoride concentration of tea leaves. Twenty six tea plantations located in the main tea production regions in Anhui province of southeast China, were chosen for this study (Fig. A.1). In each tea plantation, five sampling sites (about 500 g each) were collected randomly from each site to the 0–20 cm depth. For the collection of tea leaves, about 100 g of tea leaves (one bud and two leaves) were collected from each sampling sites. All soil samples and all tea leaves from each sampling site were pooled, respectively, before analysis.

2.1.1.2. The effect of variety, season and tea leaf maturity on fluoride concentration in tea leaves. At Anhui agricultural university Tea Research Institute, 8 different varieties of tea plant, namely Huangshan zaoya, Longjing 43, Wuniu zao, Pingyang tezao, Shucha zao, Nongkang zao, Baihao zao, Anji baicha, were chosen to study the effect of variety and season on fluoride concentration in tea leaves. Fresh tea leaves (one bud and two leaves) were plucked in spring (April), summer (June) and autumn (September) of 2014 from 8 varieties of tea. From 4 varieties, namely Longjing43, Shuchazao, Nongkangzao and Huangshan zaoya tea leaves were harvest at different ages to test fluoride concentration as a factor of maturity. Tea samples were harvested and divided into the following parts: the bud, first leaves, second leaves, third leaves, fourth leaves, fifth leaves and sixth leaves.

2.1.1.3. The effect of manufacturing process on fluoride concentration in tea leaves. Hand-plucked tea samples (one bud and two leaves; growing at Shucheng plantation) from the variety Longjing 43 were processed according to the six protocols shown in the flowchart (Fig. A.2) to yield six kinds of tea: green, black, oolong, white, yellow and dark. Three separate tea samples from each manufacturing step were collected.

2.1.1.4. Health risk assessment. A total of 115 tea specimens were purchased from the Chinese supermarkets during 2014–2015, including 21 green tea, 23 black tea, 15 yellow tea, 25 white tea, 19 oolong tea and 12 dark tea. The risks to human health from chronic exposure to the fluoride were assessed.

2.1.2. Sample pre-treatment

All tea leaf samples were unwashed and were directly oven-dried at 75 °C for 24 h to a constant weight. The tea leaves were ground to pass through a 40 mesh sieve. Soil samples were air-dried, ground, and passed through a 2-mm nylon sieve. An aliquot of each soil sample was ground to finer particle sizes with a carnelian mortar and passed through a 0.149-mm nylon sieve.

2.2. Analysis

All analysis of tea leaves and soil samples outlined below were replicated three times.

2.2.1. Analysis of fluoride in tea leaves

The total fluoride in tea leaves was analyzed according to the method (McQuaker and Gurney, 1977).

The water-soluble fluoride concentration in tea products and tea leaves was measured according to the method, which involved extraction with boiling ultra-pure water for 0.5 h, and testing with an ion selective electrode (AQSIQ, 2008).

2.2.2. Soil analysis

Total soil fluoride (T-F) was obtained by the NaOH fusion method (McQuaker and Gurney, 1977), Soil water-soluble fluoride (WS-F) was extracted in 1:5 deionized water (An et al., 2015), and the concentration of fluoride was measured using the F-ion selective electrode method. The cation exchange capacity (CEC) was measured by using the ammonium acetate procedure (Hendershot and Duquette, 1986). Soil pH was determined by a pH meter in a water suspension using a solid liquid ratio of 1:2.5 (Yu et al., 2006). The total phosphorus (T-P) was determined by a H_2SO_4–$HClO_4$ digestion method (Sparks et al., 1996), Soil bioavailable P (Av-P) was extracted using 0.05 mol L^{-1} $NaHCO_3$ (based on pH) (Shi et al., 2008). The T-P and Av-P concentration was determined with the molybdenum-blue method. The soil organic matter content (OM) was measured using potassium bichromate oxidation process (Shi et al., 2007).

2.2.3. Heath risk assessment

The risk to individual health from ingestion of the fluoride in tea was assessed based on a hazard quotient (HQ). The equations are as follows(USEPA, 1992, 1999):

$$HQ = ADD/RfD \quad (1)$$

$$ADD = \frac{C \times IR}{BW} \quad (2)$$

where ADD is the chronic daily intake of fluoride (mg kg^{-1} day^{-1}); RfD is the daily intake reference dose (6×10^{-2} mg kg^{-1} day^{-1}) suggested by the United States Environmental Protection Agency (USEPA, 2000); C is the mean concentration of water-soluble fluoride in tea (mg kg^{-1}, dry weight); IR is the consumption rate of tea (11.4 g $person^{-1}$ day^{-1}) reported by (Wu,