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Accumulation and distribution of arsenic and cadmium by tea plants*

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Abstract: It is important to research the rules about accumulation and distribution of arsenic and cadmium by tea plants, which will give us some scientific ideas about how to control the contents of arsenic and cadmium in tea. In this study, by field investigation and pot trial, we found that mobility of arsenic and cadmium in tea plants was low. Most arsenic and cadmium absorbed were fixed in feeding roots and only small amount was transported to the above-ground parts. Distribution of arsenic and cadmium, based on their concentrations of unit dry matter, in tea plants grown on un-contaminated soil was in the order: feeding roots>stems≈main roots>old leaves>young leaves. When tea plants were grown on polluted soils simulated by adding salts of these two metals, feeding roots possibly acted as a buffer and defense, and arsenic and cadmium were transported less to the above-ground parts. The concentration of cadmium in soil significantly and negatively correlated with chlorophyll content, photosynthetic rate, transpiration rate and biomass production of tea plants.

Key words: Tea plant, Arsenic (As), Cadmium (Cd), Absorption, Accumulation
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INTRODUCTION

Tea is a popular drink and famous for its function on health benefits. In recent years, the food safety problem of tea has been concerned especially about the lead content, but the contents of arsenic (As) and cadmium (Cd) were less concerned about. There is little information on heavy metals such as As and Cd in tea plants, and there are still no national standards to limit their contents. However, the average contents of As and Cd in tea that tested recently were twice those of ten years ago (Shi *et al*., 2007). During 1997 to 1998, the laboratory of Tea Research Institute of Chinese Academy of Agricultural Sciences had tested 328 tea samples which come from the main tea producing areas of China, such as Zhejiang, Jiangsu, Anhui and Guizhou Provinces, and so on, and found that the average content of Cd was 0.06 mg/kg, and its ranges were between ND (not detectable) and 1.59 mg/kg, in which 94.2% were less than 0.20 mg/kg and 4.6% were between 0.20 and 0.50 mg/kg; and that the average content of As was 0.30 mg/kg, and its ranges were between ND and 2.0 mg/kg, in which 84.1% were less than 0.50 mg/kg and 13.6% were between 0.50 and 1.00 mg/kg. And in the year of 2004, 2307 tea samples which come from the main tea producing areas of China had been tested, it was found that the average content of Cd was 0.10 mg/kg, and its ranges between ND and 7.22 mg/kg, in which 58.3% were less than 0.20 mg/kg and 40.9% were between 0.20 and 0.50 mg/kg; and that the average content of As was 0.65 mg/kg, and its ranges between ND and 2.00 mg/kg, in which 62.9% were less than 0.50 mg/kg and 30.1% were between 0.50 and 1.00 mg/kg. These data showed that the contents of As and Cd in tea were just going up constantly. Now the exact reasons about such changes are still not clear. However, we know that the heavy metals from soils and water can be passed to various organisms through the food chains and lead to various diseases in humans, such as cancer and oaf. As accumulation might cause some hazards to the residents who had ingested young fronds of this

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fern as part of the daily diet (Chang *et al*., 2005), so it is important to study the rule about accumulation and distribution of As and Cd by tea plants. In this study, the distribution and uptake of As and Cd in the tea plants were investigated.

MATERIALS AND METHODS

To investigate the absorption and distribution of Cd and As in tea plants, pot experiment was conducted by adding known amount of their salts to the soil. A red soil was collected from a tea field located in Lanxi County of Zhejiang Province. The contents in the soils were (pH 6.0): 1.86% organic matter, 77 mg/kg available K and 20 mg/kg available P. Total and extractable Cd by HCl (0.1 mol/L) were 1.48 and 0.019 mg/kg, respectively, while total and extractable As were 9.47 and 0.10 mg/kg, respectively. The soil was air-dried, crushed to pass 2-mm sieve, and thereafter placed in pots. Each pot had 10 kg soil and was grown with 6 young plants (Longjing 43) in October of 1999. Each pot was thinned to 4 plants. In late February of 2000, Cd as $CdCl_2$ in solution was added to the pots at doses of 0, 6 and 20 mg/kg (hereafter referred to as CK, Cd1 and Cd2, respectively). As in the form of Na_2HAsO_4 was applied to the pots in the same time at 0, 50 and 200 mg/kg (hereafter referred to as CK, As1 and As2, respectively). There were 4 replications for each Cd and As treatment.

The chlorophyll and the photosynthetic rate were determined in the following way: choose 10 mature leaves of the same age in every pot, and test four points by random in every leaf with SPAD meter for chlorophyll (Japan) and CID-310 photosynthesis meter (USA).

Young shoots were regularly collected from the plants. Old leaves, stems, feeding roots and main roots were also collected each time when young shoots were plucked. Plant samples were thoroughly rinsed first with tap water and then with deionized water. The young shoots were first treated in a kitchen microwave oven to inactivate enzymes and then dried in an oven at 80 °C. Other plant samples were dried directly in the oven after rinsing with water. Plant samples were ground for further analysis. Soil samples were collected from each pot, air-dried and ground to pass a 0.01-mm sieve.

In addition to the pot experiment, plant samples were also collected from tea fields located within the authors' institute. We chose different tea garden with different varieties and age, and the samples were collected just like those in the pot experiment.

Total As and Cd contents in the soil were digested by HCl-HNO_3-$HClO_4$ (according to GB 15618-1995) and the extractable form of Cd was extracted by 0.1 mol/L HCl and the extractable form of As was extracted by 0.5 mol/L $NaHCO_3$. Plant samples were digested with HNO_3 in a microwave digestion system (Mars 5, CEM Corp., USA). As and Cd in soil and plant samples were determined in an ICP-OES (IRIS/AP, Thermo Jarrel Ash, USA). Reference standards of tea (GBW07605, GSV-4) and soil (GBW07405) were analyzed similarly as a quality control.

RESULTS AND DISCUSSION

Distribution of As and Cd in tea plants

Tea plants grown on un-contaminated soil showed similar As and Cd distribution. Concentrations of As and Cd in feeding roots were 2~50 times higher than those in stems or main roots, 5~100 times higher than those in old leaves, and 25~600 times higher than those in young shoots (Table 1), suggesting that feeding roots were the main accumulation organ. The concentration of As and Cd in tea plants was in the order: feeding roots>stems≈main roots>old leaves>young shoots.

Table 1 also showed that there was large difference of As and Cd concentrations among cultivars, which maybe caused by different growth environments. However, Table 1 shows that the concentrations of As and Cd in tea plants of different tea gardens reduced from the feeding roots to young shoots, which reflected the course of absorption and accumulation of As and Cd in tea plants. The main roots and stems were the main channels of As and Cd transmission in tea plants, and also the main accumulation parts.

Concentrations of As and Cd in tea plants

The results of pot experiment showed that the concentrations of As and Cd in tea plants increased

remarkably by the addition of As and Cd salts (Tables 2 and 3). However, the increase of their concentrations in different organs varied largely. For example, As concentration in feeding roots was increased to 344 mg/kg (about 100 times that of CK) when 50 mg/kg As was added to the soil, while As concentration in young shoots increased to 11.2 mg/kg (about 20 times that of CK). And the As contents in main roots, stems and old leaves were increased significantly. When 200 mg/kg As was added, concentrations of As in feeding roots, main roots, stems, old leaves and young shoots increased to 1193, 62.4, 39.4, 38.7 and 22.1 mg/kg, respectively.

When 50 mg/kg Cd (Cd1) was added, Cd concentration increased by about 90 times in feeding roots and by about 200 times in young shoots compared to CK. In the treatment of Cd2, concentrations of Cd increased by about 300 times in feeding roots and by about 40 times in young shoots. The results showed that As and Cd accumulation in different organs varied largely when external salts were added to the soil. The absolute accumulation was the strongest in feeding roots and was to a much less degree in young shoots. The distribution of As and Cd, based on their concentrations of unit dry matter, in tea plants grown on contaminated soil, was in the following order: feeding roots>main roots>stems≈old leaves>young leaves. This showed that the mobility of As and especially of Cd in tea plants was low. Most As and Cd appeared to be fixed in roots and only limited amount was translocated to the above-ground parts. Compared with plants on contaminated soil, their roots possibly acted as a buffer and hold back the contaminations transported to above-ground parts. This finding is in line with those observed in other plants such as wheat and maize (Tan *et al*., 1994).

Contents of extractable of As and Cd in the soil increased significantly by the addition of As and Cd to the soil. Concentrations of As and Cd in young shoots increased linearly with their extractable levels in the soil (Fig.1). Concentrations of As and Cd did not vary significantly between two sampling dates while those of extractable As and Cd in the soils decreased slightly in the second sampling date. Larger slopes of regression curves were observed from the second sampling date, which might be a result of accumulation of As and Cd in the plants by constant absorption from the soil.

Table 1 Concentrations of As and Cd in tea plants grown in field

Cultivar	Cd (mg/kg)					As (mg/kg)				
	FR	MR	Stems	ML	YS	FR	MR	Stems	ML	YS
Jiuken1	6.0±0.09	0.14±0.01	0.41±0.09	0.07±0.01	0.02±0.01	4.57±0.19	0.24±0.05	0.46±0.02	0.18±0.08	0.04±0.02
Jiuken2	1.5±0.04	0.26±0.00	0.48±0.03	0.24±0.06	0.10±0.01	0.54±0.02	0.38±0.18	0.36±0.11	0.05±0.00	0.02±0.02
Longjing 43	4.3±1.05	0.21±0.01	0.43±0.08	0.08±0.04	0.03±0.02	2.06±0.85	0.52±0.17	0.61±0.11	0.26±0.10	0.07±0.05
Biyun	6.1±0.21	0.15±0.01	0.41±0.01	0.15±0.01	0.06±0.01	1.54±0.02	0.24±0.01	0.37±0.01	0.07±0.05	0.03±0.02
Yinshuang	1.5±0.13	0.08±0.03	0.38±0.11	0.08±0.04	0.04±0.02	1.19±0.36	0.46±0.21	0.94±0.30	0.27±0.10	0.05±0.03

FR: Feeding roots; MR: Main root; ML: Mature leaves; YS: Young shoot

Table 2 Concentration (mg/kg) of As in tea plants as affected by addition of the As salt

Treatment	Feeding roots	Main root	Stems	Mature leaves	Young shoot
CK	3.9±0.4	3.8±0.6	7.0±0.8	0.4±0.3	0.7±0.2
As1	1344±24	32.5±1.3	21.4±1.3	15.8±1.4	11.1±0.8
As2	2913±55	62.4±2.5	39.4±1.2	38.7±1.1	22.1±0.8

Table 3 Concentration (mg/kg) of Cd in tea plants as affected by addition the Cd salt

Treatment	Feeding roots	Main root	Stems	Mature leaves	Young shoot
CK	1.12±0.26	0.47±0.08	0.70±0.08	0.42±0.27	0.02±0.00
Cd1	91.0±3.5	5.6±1.4	3.3±1.0	4.4±0.1	3.0±0.3
Cd2	293±17	96±1	6.6±0.9	7.6±0.1	5.6±0.9

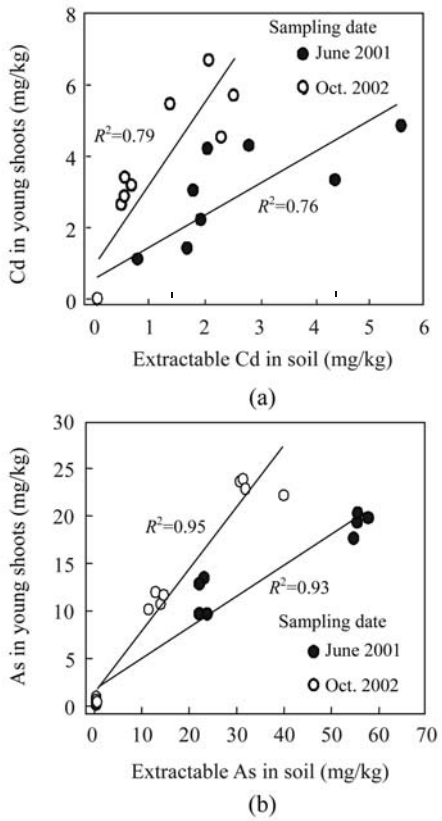

Fig.1 Relationship of extractable As (a) and Cd (b) in soil with As (a) and Cd (b) concentrations in young shoots

Contents of chlorophyll, photosynthetic rate and transpiration rate of plants receiving external As and Cd salts

The contents of chlorophyll, stomatal conductance, photosynthetic rate and transpiration rate of old leaves of the same age were measured (Fig.2). Addition of 50 mg/kg As (As1) significantly decreased the photosynthetic and transpiration rates. However, increasing application amount at As2 did not affect these parameters. Chlorophyll contents were reduced at As2. Chlorophyll contents and stomatal conductance were reduced by the addition of 0.50 mg/kg Cd (Cd1), while the photosynthetic and transpiration rates were only significantly affected by the addition of higher amount of Cd (Cd2). These results could be explained by the following researches. Cd directly or indirectly inhibits physiological processes such as respiration, photosynthesis, water relations and gas exchange (van Assche and Clijsters, 1990). Cd may be preferentially accumulated in chloroplasts. Photosynthesis is inhibited by CO_2-fixation, stomatal conductance, chlorophyll synthesis, electron transport and enzymes of the Calvin cycle (Ernst, 1980). Changes in cellular metabolism can be observed even at low level of Cd before visual symptoms become evident (Lagriffoul *et al*., 1998). Also, it could be

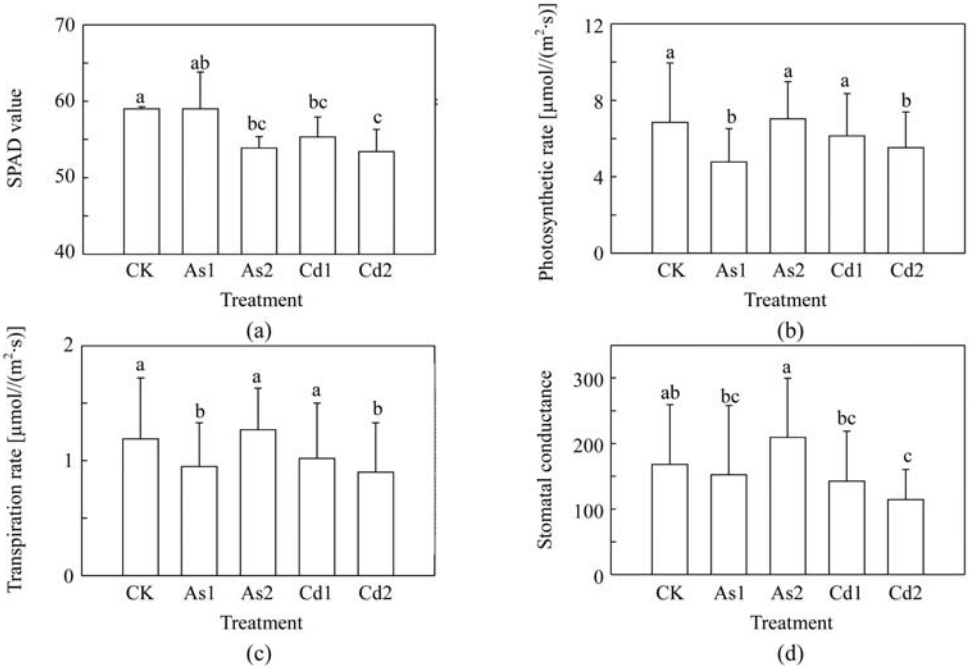

Fig.2 Chlorophyll content (a), photosynthetic rate (b), transpiration rates (c) and stomatal conductance (d) of plants receiving As and Cd salts

Different lowercase letters mean significant different (P<0.05)

found that the tea plants could resist higher concentration of As than that of Cd. Qian *et al*.(2006) had found the same results on the duckweed. At the same concentration, the single-toxicity order of Cd and As to the chlorophyll of duckweed was Cd>As.

Plant growth and concentrations of quality-related components in young shoots

No toxicity symptom of added Cd and As was observed in plants receiving external salts. The biomass production and chemical compounds relating to quality were also not influenced by the addition of As to soil (Table 4). Biomass production was not affected by low level of Cd (Cd1), but the quality-related components were significantly reduced by Cd1 treatment, and the biomass production and the quality-related components were significantly negatively influenced by Cd2 treatment. Especially the contents of amino acid in the tea shoots were significantly reduced, and the ratio of tea polyphenol to amino acid significantly increased in Cd2 treatment (Table 5).

Table 4 Biomass production of tea plants receiving external Cd and As in pot experiment

Treatment	Biomass production (g/plant)					
	Buds weights	Old leaves	Feeding roots	Coarse roots	Main roots	Stems
CK	7.32±1.71^{a}	18.73±4.63^{a}	19.84±7.97^{a}	12.92±2.93^{a}	26.60±5.79^{a}	73.25±2.86^{a}
As1	5.83±2.07^{a}	18.23±2.28^{a}	20.04±4.61^{a}	11.09±2.34^{b}	19.42±3.09^{b}	72.58±0.07^{a}
As2	7.73±2.70^{b}	18.09±2.00^{a}	19.18±1.43^{a}	11.68±1.55^{b}	32.95±4.56^{c}	72.91±8.53^{a}
Cd1	6.26±1.85^{a}	18.40±1.41^{a}	18.58±1.74^{a}	10.27±0.82^{c}	25.19±1.82^{a}	75.01±8.44^{a}
Cd2	4.48±1.88^{c}	18.13±1.46^{a}	16.57±3.14^{b}	11.16±0.61^{b}	32.96±8.07^{c}	74.55±12.2^{a}

Different superscript letters following data in the same columns indicate significant difference between treatments

Table 5 Quality-related chemical components in young shoots of tea plants receiving external Cd and As salts

Treatment	Caffeine (%)		Amino Acid (%)		Polyphenols/free amino acid	
	Sept., 2001	Oct., 2002	Sept., 2001	Oct., 2002	Sept., 2001	Oct., 2002
CK	1.37±0.01^{a}	1.06±0.01^{a}	1.69±0.01^{A}	1.46±0.01^{A}	12.93±0.02^{A}	12.76±0.19^{AB}
As1	1.61±0.02^{b}	0.91±0.26^{a}	1.20±0.02^{B}	1.24±0.04^{B}	19.79±0.48^{B}	14.81±1.94^{BC}
As2	1.41±0.09^{c}	0.81±0.01^{b}	1.78±0.01^{A}	1.57±0.01^{A}	13.73±0.08^{AC}	10.18±0.35^{A}
Cd1	1.24±0.01^{d}	0.93±0.04^{a}	1.44±0.01^{C}	1.06±0.11^{C}	15.13±0.13^{C}	17.82±1.40^{C}
Cd2	1.03±0.02^{e}	0.72±0.05^{b}	0.64±0.01^{D}	0.64±0.01^{D}	35.50±0.52^{D}	25.31±2.33^{D}

Different lowercase superscript letters mean significant different (P<0.05); Different capitals superscript letters mean extremely significan different (P<0.01)

CONCLUSION

1. Concentrations of As and Cd in tea plants from high to low levels follow the order: feeding roots>stems≈main roots>old leaves>young leaves.

2. Roots preserve the absorption of most As and Cd under the condition of addition of external As and Cd to the soil, which might provide mechanism to prevent them from being transferred to the above-ground part.

3. Concentrations of As and Cd in young shoots were linearly correlated with their extractable levels in the soil.

4. The addition of As and Cd, depending upon their doses, reduced chlorophyll content, stomatal conductance, photosynthetic rate and transpiration rate, leading to decrease of biomass production of the plant.

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