

Lead contamination in different varieties of tea plant (*Camellia sinensis* L.) and factors affecting lead bioavailability

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Abstract

BACKGROUND: There has been increasing concern in recent years about the concentration of lead (Pb) in tea. However, little research has been done to address questions concerning the distribution of Pb in different varieties of tea plant and the differences among tea plant varieties in their uptake and accumulation of Pb from the soil. Therefore the aim of this study was to investigate the accumulation of Pb in different tea plant varieties and the factors affecting Pb bioavailability.

RESULTS: Three patterns of Pb distribution could be observed in different plant parts among the eight tea varieties surveyed, as well as a linear relationship between the Pb concentration in fine roots and the exchangeable Pb fraction in the corresponding soil. The uptake of Pb by fine roots increased significantly as the soil pH decreased. The average ratios of Pb concentration in fine roots to those in young stems and young leaves were 5.18 and 31.80 respectively. In fine roots the Pb concentration varied from 22.7 to 61.6 mg kg⁻¹.

CONCLUSION: The results indicated that the uptake, transport and accumulation of Pb by tea plant organs were strongly governed by soil conditions and tea variety, thus providing tea producers with useful information on variety selection for the production of quality teas containing low levels of Pb.

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Keywords: lead; tea plant varieties; soil pH; sequential extractions

INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most popular non-alcoholic beverages in the world because of its taste, aroma and low caffeine content.¹ It has frequently been reported that drinking tea is beneficial to human health owing to tea's antimutagenic, anticarcinogenic and antioxidant effects.² However, various reports have noted the potential health implications of trace metals in tea, particularly since the tea bush is known to accumulate such metals.^{3,4} For example, some researchers found that Chinese green tea possessed the highest contents of heavy metals among tested tea brands.⁵ Another investigation revealed that among 57 tested tea samples marketed in Beijing (China) the concentration of lead varied from 0.198 to 6.345 mg kg⁻¹ dry weight.⁶

Lead (Pb) is a physiological and neurological toxin that can affect almost every organ and system in the human body.⁷ Rapid industrialisation over the last two decades in China has resulted in an increased Pb burden on the environment. Recent surveys⁸ on tea products in some areas of China have revealed an increasing number of cases (16.8%) exceeding the former maximum permissible concentration (MPC) for Pb of 2 mg kg⁻¹ dry weight, as set by the Chinese Ministry of Health.⁹ Moreover, Pb concentration in tea products is increasing annually. Although Pb intake from drinking tea is generally very low at the former MPC of 2 mg kg⁻¹,¹⁰ sometimes tea made from leaves higher in Pb concentration (~3.9 mg kg⁻¹) can exceed the 0.01 mg kg⁻¹ limit set for drinking water in China.¹¹ Therefore effects on human health from the consumption of Pb through drinking tea have attracted

much attention among both consumers and producers.¹² Also, Pb concentration in tea has become an issue that affects trade and consumer confidence.

Many factors can influence the concentration of Pb and other metals in both tea plants and final tea products, including the variety of tea plant, soil/atmospheric conditions, the maturity of raw materials used and the processing of leaves.¹³ For instance, different clones of black tea were found to contain different aluminium (Al) concentrations,¹⁴ while the concentrations of fluoride and Al in tea plants were reported to differ significantly among Zhe-nong113, Zhe-nong121, Zi-sun and Bi-feng.¹²

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Tea is one of the most important cash crops worldwide, particularly in China. However, the rapid industrialisation of China over the past two decades has caused increased contamination of the environment by heavy metals, especially Pb, which has inevitably led to increased contamination of tea plants. Since many tea varieties are planted in China, it is important to understand the differences among tea plant varieties and genotypes in terms of Pb uptake and translocation. However, current research is mostly concerned with the concentration of Pb or other heavy metals in tea. There are few studies on the distribution of Pb in different varieties of tea plant or on the differences among tea varieties regarding their uptake and accumulation of Pb from the soil.

Therefore the aim of the present study was to investigate the accumulation of Pb in different varieties of tea plant as well as the factors affecting Pb bioavailability. The investigated tea bushes of the different varieties were all planted at the same site and subjected to similar cultivation practices, thus making it possible to identify the effects of tea variety on the uptake of Pb. Moreover, the sequential extraction method used can yield valuable information on changes in the chemical association and availability of Pb, both of which are caused by the interaction between different tea plant varieties and the soil. It is also expected that the results of the present study will provide useful information on variety selection for the production of quality teas containing low levels of Pb. Based on this study, it will be possible to choose a particular tea plant variety that takes up less Pb as a main cultivar. On the other hand, the knowledge of particular varieties that take up more or less Pb than others will be useful to meet our objectives for the next stage of study on the different mechanisms of uptake, distribution and localisation of Pb within tea plants.

MATERIALS AND METHODS

Field location and soil properties

In the city of Hangzhou (Zhejiang, China) the annual mean precipitation is 1400–1700 mm and the annual mean temperature is 17–18 °C, a climate suitable for the growth of tea. The study site is located at Zhejiang University Tea Plantation in Hangzhou. The tea garden, which contains 41 different varieties, is a variety resources nursery subordinate to Zhejiang University Tea Research Institute. All these varieties were planted at about the same time more than 10 years ago. Tea plants of the same variety were planted in two rows 30–40 cm apart, with a space of 1 m between varieties. The total area allocated for each variety was 10–15 m². Different plots of all varieties were arranged in a completely randomised block design. In this plantation, cultivation and management were kept the same; therefore we randomly collected and composited four soil samples from unplanted areas in the tea garden. Selected physicochemical properties of the soil are shown in Table 1.

Collection of root zone soil and plant samples

In October 2007, samples of different tea plant parts and corresponding root zone soil samples (0–20 cm depth) were collected from the tea garden. The soil samples were taken at a distance of about 2 cm from the roots of different varieties. From healthy individuals of each variety, samples of young leaves (~1 month of age), mature leaves (grown in the previous year), young stems, mature stems, fine roots (fibrous roots), coarse roots (lateral roots) and seeds were collected. Three replicates of all samples of soil and tea plant tissues were collected. All tea

Table 1. Analysis of selected soil properties^a

| Sampling position | pH | Organic matter (g kg ⁻¹) | Total Pb (mg kg ⁻¹) |
|-------------------|-------------|--------------------------------------|---------------------------------|
| 1 | 5.75 ± 0.03 | 12.3 ± 0.5 | 25.14 ± 0.20 |
| 2 | 6.06 ± 0.02 | 12.5 ± 0.1 | 29.25 ± 0.84 |
| 3 | 5.95 ± 0.02 | 12.2 ± 0.2 | 25.63 ± 0.41 |
| 4 | 6.15 ± 0.02 | 12.0 ± 0.2 | 27.56 ± 1.35 |
| Average | 5.98 | 12.3 | 26.90 |

^a Results are reported as mean ± standard error (three replicates).

plant samples were first washed thoroughly with tap water to remove dust and dirt and then washed a further three times with deionised water. Afterwards the samples were oven dried at 80 °C to constant weight and ground to a fine powder for subsequent analyses. The soil samples were air dried at room temperature and passed through a 2 mm sieve prior to chemical analyses.

Chemical analyses

Each tea plant sample was accurately weighed (5.000 g) into a crucible and heated in a muffle furnace at 500 °C for 6 h. The resulting ash was dissolved in 2 mL of 6 mol L⁻¹ HCl. Care was taken to ensure that all the ash came into contact with the acid. The acid solution in the crucible was then diluted to 25 mL with deionised water. Pb concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; IRIS/AP, Thermo Jarrell Ash Corporation, Franklin, MA, USA). The detection limit for Pb was 0.004 mg L⁻¹. Blanks and a standard reference tea material (GB07605-GSV-4, Institute of Geophysical and Geochemical Sciences, Chinese Academy of Geological Sciences, Langfang, Hebei, China) with a certified Pb concentration of 4.4 ± 0.2 mg kg⁻¹ were included for quality control.

For the assessment of soil total Pb, each soil sample was first digested with a mixed solution of concentrated HNO₃/HCl/HF. Pb concentration was then determined by ICP-AES. Soil pH was determined using a combined glass electrode in 1:2.5 (w/v) soil/water suspension.¹⁵ Soil organic matter was analysed by the dichromate method.¹⁶

Fractionation of Pb in soil

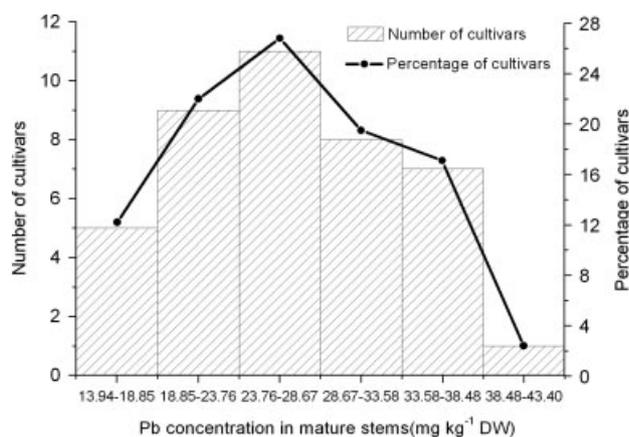
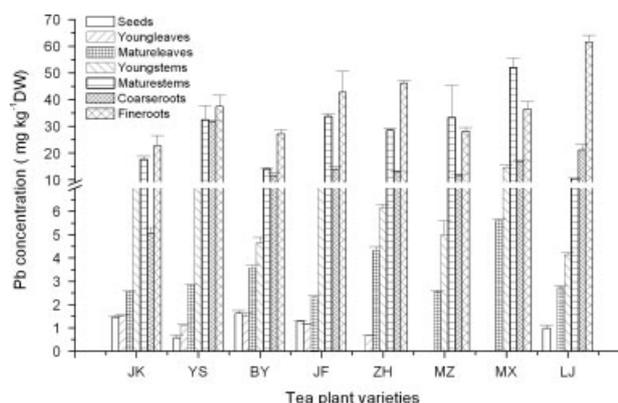
Pb in the soil can be divided into five fractions, i.e. exchangeable, carbonate-bound, organic matter-bound, oxide-bound and residual fractions. The revised sequential extraction method of Tessier *et al.*¹⁷ was used as detailed in Table 2.¹⁸ Briefly, each air-dried soil sample was accurately weighed (2.000 g) into a 50 mL polypropylene centrifuge tube with a screw cap and extracted successively. After each extraction the suspension was centrifuged at 6500 × g for 15 min and the supernatant was passed through Whatman No. 42 filter paper. Pb concentration in the extracts was determined by ICP-AES. The residual fraction of Pb in the soil was calculated by subtracting the sum of extractable Pb from the total concentration of Pb in the soil.

Statistical analysis

All analyses of different tea plant parts and soil samples were replicated three times, with results presented as the average of the three replicates. Data were expressed on a dry weight (DW) basis. Statistical analyses, including multiple linear regression and

Table 2. Sequential extraction procedure¹⁸

| Fraction | Extractant | Operational conditions |
|----------------------|--|--|
| Exchangeable | 0.5 mol L ⁻¹ Mg(NO ₃) ₂ | 25 °C, shaken for 2 h, liquid/soil = 20 : 2 |
| Carbonate-bound | 1 mol L ⁻¹ NaOAc + HOAc (pH 5) | 25 °C, shaken for 2 h, liquid/soil = 20 : 2 |
| Oxide-bound | 0.175 mol L ⁻¹ (NH ₄) ₂ C ₂ O ₄ + 0.1 mol L ⁻¹ H ₂ C ₂ O ₄ | 25 °C, heated for 2 h in bath at 85 °C; addition of water, shaken for 2 h; liquid/soil = 20 : 2 |
| Organic matter-bound | 0.02 mol L ⁻¹ HNO ₃ + 300 mL L ⁻¹ H ₂ O ₂ , 0.8 mol L ⁻¹ NH ₄ OAc (0.0075 mol L ⁻¹ HNO ₃) | 25 °C, addition of 3 mL of HNO ₃ and 5 mL of H ₂ O ₂ , heated in bath at 80 °C; evaporation of water, addition of 20 mL of NH ₄ OAc containing HNO ₃ , shaken for 1 h |

**Figure 1.** Cultivar distribution according to Pb concentration in mature stems of tea plants.**Figure 2.** Concentration of Pb in different organs of eight tea plant varieties. Data columns and vertical bars represent mean ($n = 3$) and standard deviation respectively.

analysis of variance, were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Pb in tea bushes

According to the variation in Pb concentration in mature stems of all 41 tea plant varieties, the numerical distribution of stem tissue Pb was calculated, with the result shown in Fig. 1. Wide variation in Pb content existed in mature stems; for example, five varieties (12.2%) ranged from 13.9 to 18.9 mg Pb kg⁻¹, 11 varieties (26.8%) ranged from 23.8 to 28.7 mg Pb kg⁻¹ and only one variety (2.4%) exceeded 38.5 mg Pb kg⁻¹.

Based on these results for Pb concentrations in mature stems of different varieties of tea plant and the consideration of whether the tea plant variety is a main cultivar in Zhejiang province, eight varieties, namely Jin-feng (JF), Bi-yun (BY), Mao-xie (MX), Mei-zhan (MZ), Zheng-he (ZH), Yin-shuang (YS), Long-jing43 (LJ) and Jiu-keng (JK), were chosen for further research. The Pb concentrations in various tea plant parts of these eight varieties are presented in Fig. 2 (no Pb was detected in young leaves of MX and MZ; there were no young leaf samples for LJ and no seed samples for MX, MZ and ZH).

The average ratios of Pb concentration in fine roots to those in young stems and young leaves were 5.18 and 31.8 respectively. In fine roots the Pb concentration varied from 22.7 to 61.6 mg kg⁻¹. The ratio of Pb concentration in mature leaves to that in young leaves ranged from 1.7 to 6.5. Generally, the Pb concentration decreased markedly from the roots to the seeds of the tea plant; however, it was found that there were three patterns of Pb

distribution in different organs among the eight varieties. For LJ (group A) the Pb concentration in various organs showed the following order: fine roots > coarse roots > mature stems. For JK, YS, BY, JF and ZH (group B) the Pb concentration in various organs showed approximately the same order: fine roots > mature stems > coarse roots. For MX and MZ (group C) the Pb concentration in various organs showed a different order: mature stems > fine roots > coarse roots. Statistical analysis indicated that the difference in Pb concentrations among varieties also varied in different organs (data not shown). Moreover, the Pb concentration in aerial parts of MX was the highest among the eight varieties. Statistical analysis showed that there was a significant difference between MX and the other seven varieties in terms of Pb concentration in different organs, including coarse roots, fine roots, mature leaves and young leaves.

Correlation coefficients among the concentrations of Pb in different parts of the tea plant are presented in Table 3, from which it is seen that the correlation between Pb concentrations in different organs differed with their relative position. There was a highly significant positive correlation between the Pb concentration in mature stems and that in young stems. In contrast, the Pb concentration in fine roots was negatively and significantly correlated with that in young leaves. A significant and negative correlation was also detected between the Pb concentration in coarse roots and that in seeds.

Soil characteristics

Selected physical and chemical properties of the corresponding soil are shown in Table 4. The pH of all root zone soil samples was less than 4.7, with the lowest value reaching 4.27, indicating

Table 3. Pearson's correlation coefficients between Pb concentrations in different parts of tea plant^a

| Tea plant organ | Seeds | Young leaves | Mature leaves | Young stems | Mature stems | Fine roots |
|-----------------|----------------|----------------|---------------|----------------|--------------|------------|
| Young leaves | 0.808 | | | | | |
| Mature leaves | 0.295 | -0.560 | | | | |
| Young stems | -0.423 | -0.180 | 0.639 | | | |
| Mature stems | -0.436 | -0.682 | 0.546 | 0.846** | | |
| Fine roots | -0.503 | -0.913* | 0.022 | -0.125 | -0.139 | |
| Coarse roots | -0.938* | -0.332 | 0.004 | 0.210 | 0.186 | 0.479 |

^a Significant relationships are presented in bold: * $P < 0.05$; ** $P < 0.01$.

Table 4. Average values of soil pH, organic matter content and total Pb concentration in root zone soil of different varieties of tea plant^a

| Tea plant variety | pH | Organic matter (g kg ⁻¹) | Total Pb (mg kg ⁻¹) |
|-------------------|---------------|--------------------------------------|---------------------------------|
| JK | 4.45 ± 0.01c | 17.4 ± 0.2de | 29.13 ± 1.77a |
| YS | 4.65 ± 0.02a | 18.2 ± 0.2d | 23.34 ± 0.43c |
| BY | 4.39 ± 0.01d | 17.0 ± 0.3e | 22.01 ± 0.30c |
| JF | 4.29 ± 0.01ef | 18.0 ± 0.3d | 21.88 ± 0.90c |
| ZH | 4.27 ± 0.01f | 22.0 ± 0.3b | 21.97 ± 0.41c |
| MZ | 4.55 ± 0.03b | 20.9 ± 0.2c | 25.44 ± 1.80abc |
| MX | 4.40 ± 0.02d | 23.5 ± 0.2a | 29.18 ± 2.92a |
| LJ | 4.31 ± 0.01e | 20.6 ± 0.6c | 26.71 ± 0.53ab |
| Average | 4.41 | 19.7 | 24.96 |

^a Results are reported as mean ± standard error (three replicates). Different letters within a column indicate significant ($P < 0.05$) differences between soil samples.

that the root zone soil in the tea garden was severely acidified (compared with data in Table 1). According to the difference analysis of the soil pH among the soil samples corresponding to the eight varieties, there was a significant difference between YS and the other varieties, which was also true of MZ and JK. The organic matter content in the root zone soil ranged from 17.0 to 23.5 g kg⁻¹, with an average of 19.7 g kg⁻¹. Once again, based on the analysis of the soil organic matter content, it was found that considerable differences existed between MX or ZH and the other seven varieties. The total Pb concentration in the root zone soil ranged from 21.9 to 29.2 mg kg⁻¹, with an average of 25.0 mg kg⁻¹. The mean Pb concentration in natural soils of Zhejiang province is 26.6 ± 13.3 mg kg⁻¹.¹⁹ This indicates that the soil in the tea garden is not contaminated with Pb.

Chemical fractionation of Pb

The concentration and proportion of Pb in different fractions of tea plant root zone soil are shown in Figs 3(a) and 3(b) respectively. The distribution of root zone soil Pb was similar for all tea plant varieties, with Pb being present predominantly in the residual and oxide-bound fractions. The average proportions of residual and oxide-bound Pb were 72.6 and 23.6% respectively. These values suggest that hydrous iron (Fe) and Al oxides and clay minerals play a major role in the immobilisation of Pb in the soil. The highest proportion of exchangeable Pb was found in LJ soil (0.9%), followed by JF (0.9%), ZH (0.8%) and MX (0.4%) soils, with YS (0.1%) and MZ (0.1%) soils having the lowest measurable proportion of

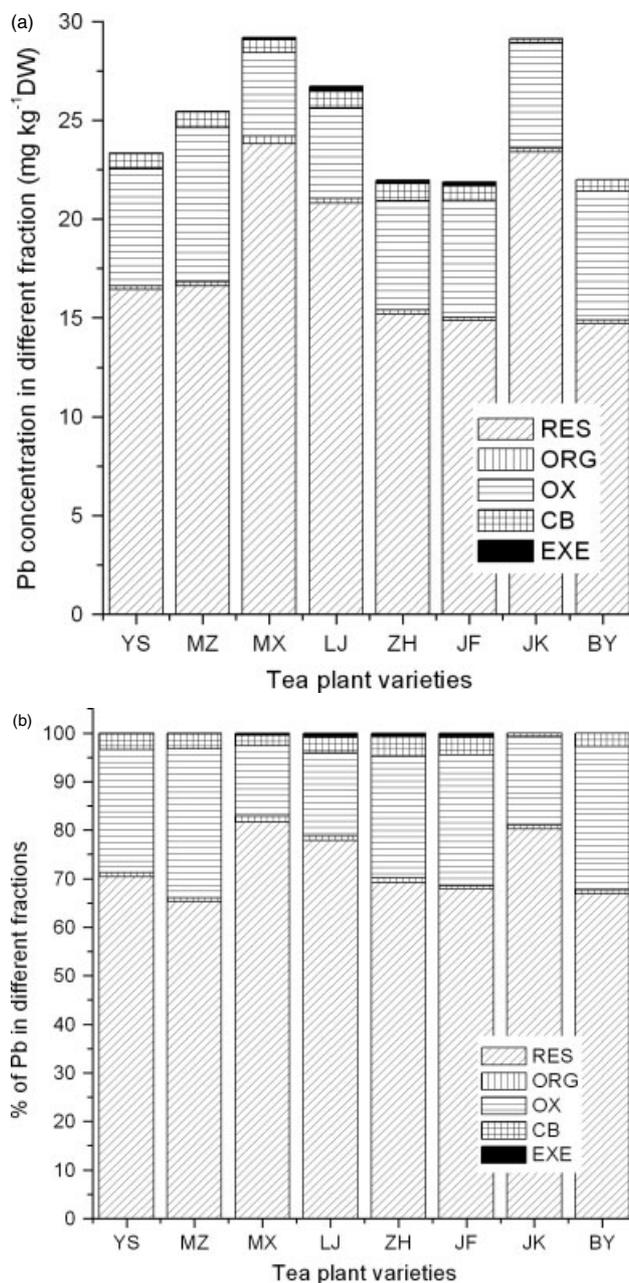


Figure 3. (a) Concentration and (b) percentage of Pb in different fractions of tea plant root zone soil: EXE, exchangeable Pb; CB, carbonate-bound Pb; ORG, organic matter-bound Pb; OX, oxide-bound Pb; RES, residual Pb.

exchangeable Pb. No exchangeable Pb was detected in JK soil or BY soil.

Correlation coefficients between the Pb fractions of root zone soil and the soil properties are presented in Table 5. The exchangeable Pb concentration in root zone soil was significantly and negatively correlated with the soil pH ($P < 0.05$), suggesting that the concentration of exchangeable Pb increases with decreasing soil pH. This indicates that the soil pH has an important role in controlling the solubility of Pb. Tea plantation can acidify the soil and thus enhance metal mobility. However, the organic matter-bound Pb concentration in root zone soil was significantly and positively correlated with the soil organic matter content ($P < 0.05$). The residual Pb concentration in root zone soil was

Table 5. Correlation coefficients between soil properties and Pb fractions of root zone soil^a

| Soil property | EXE | CB | ORG | OX | RES |
|----------------|----------------|--------|---------------|--------|----------------|
| pH | -0.731* | -0.185 | -0.089 | 0.430 | 0.038 |
| Organic matter | 0.437 | 0.454 | 0.722* | -0.351 | 0.324 |
| Total Pb | -0.094 | -0.536 | 0.686 | -0.474 | 0.972** |

^a EXE, exchangeable Pb; CB, carbonate-bound Pb; ORG, organic matter-bound Pb; OX, oxide-bound Pb; RES, residual Pb. Significant relationships are presented in bold: * $P < 0.05$; ** $P < 0.01$.

mainly affected by the soil total Pb concentration, increasing with increasing total content of Pb ($P < 0.01$).

Most correlations of Pb concentrations in tea plant organs with soil properties and Pb fractions were generally poor (Table 6). Heavy metals, including Pb, are associated with various soil components in different ways, determining their mobility and availability. Exchangeable forms are considered readily mobile and available to plants, while other forms – precipitated as carbonate, complexed with organic matter and occluded in Fe or manganese (Mn) – can be considered relatively active or firmly bound. From Table 6 we can see that there is a significant correlation between exchangeable Pb and the Pb content in fine roots, indicating that exchangeable Pb is the primary bioavailable fraction in the soil for tea plant uptake. Although it did not reach the level of statistical significance, there was still a moderately positive correlation between carbonate-bound Pb and the Pb content in fine roots.

DISCUSSION

Pb distribution in different tea plant varieties

From Fig. 2 we can see that there were three patterns of Pb distribution in different organs among the eight varieties, which were all planted at the same site and subjected to similar cultivation practices. For group A the Pb concentration decreases from the roots to the aerial parts, which shows that Pb is retained in the root system. For groups B and C, Pb is accumulated in the stems to a certain extent, which shows that the tea plant varieties in these two groups can accumulate Pb in their aerial parts. We also found that the Pb concentration in the aerial parts of MX is the highest among the eight tea plant varieties. Furthermore, the difference between MX and the other varieties in terms of the Pb concentration in different plant organs is significant. This indicated that there

might exist inherent differences in the uptake, translocation and accumulation of Pb among the eight varieties, especially MX. We concluded from our research that MX is unsuitable for planting in an area where the Pb concentration is relatively high. Generally speaking, the concentration of a particular element in different plant parts can reflect the uptake, translocation, retranslocation and utilisation of that element. With regard to different tea plant varieties, plant factors such as root exudates, root surface area, root absorption ability, mycorrhisation and transpiration rate can affect both Pb availability in the soil and total Pb uptake by the root.²⁰ On the other hand, differences in the translocation of Pb are possibly related to the differences in the form of Pb existing in different tea plant varieties. It has been reported that ions and low-molecular-weight complexes of Pb are mobile in plants.²¹ Thus the distribution difference of Pb in tea plants may occur in the levels of uptake or in the transportation from root to stem or from stem to leaf.

Statistical analyses (Table 3) demonstrated that the Pb concentration in fine roots was negatively and significantly correlated with that in young leaves. A similar correlation was also detected between the Pb concentration in coarse roots and that in seeds. These results revealed that in tea plants the absorbed Pb was mainly accumulated in roots, with a minimal amount transferred to stems and leaves. Pb has been reported to move predominantly into the root apoplast and thereby in a radial manner to cross the cortex and accumulate near the endodermis, which to some degree functions as a barrier to the movement of Pb between roots and shoots. This may in part explain why plant roots can generally take up quite a large amount of Pb from the soil while restricting greatly the translocation of Pb to aerial parts.²²

Factors affecting Pb in tea plants

It is generally agreed that the optimal range of soil pH for tea plant growth is 5.0–5.6.²³ It has also been proven that tea plantation can cause soil acidification. A survey conducted in 2000 showed that 44% of tea garden soils in Zhejiang, Anhui and Jiangsu provinces had a pH below 4.0, compared with only 13% in 1990.²⁴ This large increase is likely due to acid secretions from tea plant roots, the formation of H⁺ during nitrification of ammonia delivered in the form of nitrogenous fertilisers, and the application of physiologically acid fertilisers.^{25–27}

From Tables 1 and 4 we can see that the organic matter content in the tea garden subsurface root zone soil is significantly higher than that in the background soil, which is probably a result of root exudates, the annual application of organic fertilisers and

Table 6. Correlation coefficients between Pb concentrations in different tea plant organs and soil properties and Pb fractions^a

| Soil property | Seeds | Young leaves | Mature leaves | Young stems | Mature stems | Fine roots | Coarse roots |
|---------------|--------|----------------|---------------|-------------|--------------|----------------|--------------|
| pH | -0.567 | 0.312 | -0.231 | 0.121 | 0.194 | -0.514 | 0.407 |
| OM | -0.530 | -0.938* | 0.684 | 0.420 | 0.573 | 0.382 | 0.100 |
| Total Pb | 0.039 | 0.537 | 0.205 | 0.399 | 0.151 | -0.146 | -0.173 |
| EXE | -0.233 | -0.724 | 0.085 | 0.000 | 0.002 | 0.920** | 0.195 |
| CB | -0.560 | -0.810 | 0.067 | -0.146 | 0.197 | 0.686 | 0.520 |
| OX | 0.288 | 0.293 | -0.479 | -0.501 | -0.089 | -0.499 | -0.191 |
| ORG | -0.126 | 0.003 | 0.787* | 0.646 | 0.445 | 0.142 | 0.096 |
| RES | 0.022 | 0.494 | 0.286 | 0.467 | 0.130 | -0.037 | -0.121 |

^a OM, organic matter; EXE, exchangeable Pb; CB, carbonate-bound Pb; ORG, organic matter-bound Pb; OX, oxide-bound Pb; RES, residual Pb. Significant relationships are presented in bold: * $P < 0.05$; ** $P < 0.01$.

the accumulation of tea biomass through the incorporation of tea prunings and old leaves. Lu and Liang²⁸ reported that the amount of organic acids in tea plant soils could reach a level 48 times greater than that in soils of other common crops. In this study we found a positive relationship between organic matter content and organic matter-bound Pb. In the past, different opinions were held about the potential role of organic matter in changing Pb bioavailability. Several studies have suggested that addition of organic matter would immobilise Pb by forming strong complexes with soil Pb.^{29,30} In contrast, there have also been numerous studies reporting that addition of chelates or low-molecular-weight organic acids to the soil could enhance the solubility of Pb.³¹

In order to assess the potential contamination of the food chain by heavy metals, many studies have been conducted in recent years to identify and evaluate the bioavailability of trace elements in the soil.^{32–35} Here we evaluated the bioavailability of Pb in tea garden soils. The method of sequential extraction can provide some insight into the chemical association and availability of soil Pb. The variation in Pb fractions in terms of concentration and proportion (Fig. 3) may be the result of plant physiology, growth of roots, rate of metabolism, distribution of roots and micro-organisms in the root zone. However, roots can change the major physical and chemical characteristics of root zone soil, which may in turn change the speciation and chemical characteristics of trace metals.³⁶ As a consequence, the change in Pb fractions may result from root-induced changes in dissolved organic carbon, redox potential and microbial activity near the root.

Previous research has shown that exchangeable and carbonate fractions appear to be readily bioavailable.³⁷ Our study also showed a significant correlation between the exchangeable fraction of Pb and the Pb content in fine roots and a moderately positive correlation between the carbonate-bound fraction of Pb and the Pb content in fine roots. Research supports the fact that metal bioavailability is affected by the following factors: soil cation exchange capacity, texture, clay mineralogy, redox potential, organic content, metal binding by root exudates, root-induced pH changes, root-induced microbial activities as well as levels of other elements present in the soil.^{38,39} Among these factors, some researchers consider pH to be the most important.⁴⁰ Here we found that acidification of the tea garden soil was very significant, as the pH of all root zone soil samples was less than 4.7, with the lowest being pH 4.3. Acidic conditions favour the solubilisation of Pb from the solid phase of soils.⁴¹ In addition, metals associated with oxides can be released under acidic or reducing conditions, and root-induced changes in pH and redox potential (Eh) can thereby play a role in the bioavailability of trace metals in the soil.⁴² Previous research also showed that the amount of available metals increased significantly when the soil pH decreased.⁴³ Thus it is not surprising to find that the exchangeable fraction of Pb in tea plant soils was negatively related to the soil pH. Nevertheless, the effect of pH on the availability of soil heavy metals was found to be much smaller when the soil pH was greater than 4.4.⁴⁴ Therefore it is important to maintain the soil pH at levels greater than 4.4. For instance, regular application of a small quantity of lime materials may be an efficient way to maintain the soil pH at levels greater than 4.5, and this in turn can decrease the impact of heavy metals on tea plants. Research has shown that liming resulted in a decrease in the proportion of Pb in the exchangeable and carbonate-bound fractions in the soil, with a concurrent increase in the fractions bound to Fe and Mn oxides and residues.⁴⁵ Another alternative approach is to apply

phosphorus fertiliser to fix Pb in the soil, thus decreasing Pb bioavailability.⁴⁶

CONCLUSION

It was found that the uptake, transport and accumulation of Pb by tea plants are to some degree governed by soil properties and the tea plant variety. The bioavailability of soil Pb can be affected by the activity of roots in the root zone soil. Root exudates of tea plants contain organic compounds that may be of different kinds and quantities. Excretion products such as acetic, oxalic, citric and tartaric acids and polysaccharides are able to form complexes and chelates with metal ions, thus modifying the fixation and mobility of soil heavy metals. As far as the eight tea plant varieties studied here are concerned, we advise that MX not be planted in an area with relatively high soil Pb concentration. Furthermore, the different plant distribution of Pb observed when MX is compared with the other varieties might be caused by a different interaction mechanism between roots and soil, and this different interaction mechanism might have originated from root exudation processes. Further research concerning this aspect is expected to be undertaken in the next stage.

ACKNOWLEDGEMENTS

We thank Dr Xiaochang Wang (Zhengjiang University Tea Research Institute) for collecting samples and Dr Yuanzhi Shi (Key Laboratory of Tea Chemical Engineering, China Ministry of Agriculture) for sample analysis. This work was supported by the National Natural Science Foundation of China (40871108, 40432004 and 20677050).

REFERENCES

- 1 Mondal TK, Bhattacharya A, Laxmikumaran M and Ahuja PS, Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell Tissue Organ Cult* **76**:195–254 (2004).
- 2 Khokhar S and Magnusdottir SGM, Total phenol, catechin and caffeine contents of teas commonly consumed in the United Kingdom. *J Agric Food Chem* **50**:565–570 (2002).
- 3 Bosque MA, Schuhmacher M, Domingo JL and Llobet JM, Concentrations of lead and cadmium in edible vegetables from Tarragona province, Spain. *Sci Total Environ* **95**:61–70 (1990).
- 4 Wong MH, Fung KF and Carr HP, Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. *Toxicol Lett* **137**:111–120 (2003).
- 5 Al-Oud SS, Heavy metal contents in tea and herb leaves. *Pak J Biol Sci* **6**:208–212 (2003).
- 6 Qin F and Chen W, Lead and copper levels in tea samples marketed in Beijing, China. *Bull Environ Contam Toxicol* **79**:247–250 (2007).
- 7 Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological Profile for Lead*. US Department of Health and Human Services, Public Health Service, Atlanta, GA (1999).
- 8 Shi YZ and Wu X, Lead concentration of tea in China and suggestions for improving tea quality. *J Tea* **26**:128–129 (2000). (in Chinese).
- 9 Chinese Ministry of Health, *Hygienic Standard for Tea*. GB9679-88 (1988).
- 10 Han WY, Zhao FJ, Shi YZ, Ma LF and Ruan JY, Scale and causes of lead contamination in Chinese tea. *Environ Pollut* **139**:125–132 (2006).
- 11 Yang F and Li YP, The effects of different marinating methods on solubility of the lead in tea leaves. *Chin J Health Lab Technol* **11**:349 (2001). (in Chinese).
- 12 Ruan JY and Wong MH, Accumulation of fluoride and aluminium related to different varieties of tea plant. *Environ Geochem Health* **23**:53–63 (2001).
- 13 Jin CW, He YF, Zhang K, Zhou GD, Shi JL and Zheng SJ, Lead contamination in tea leaves and non-edaphic factors affecting it. *Chemosphere* **61**:726–732 (2005).
- 14 Owuor PO, Gone FO, Onchiri DB and Jumba IO, Levels of aluminium in green leaf of clonal teas, black tea and black tea liquors, and effects

- of rates of nitrogen fertilizers on the aluminium black tea contents. *Food Chem* **35**:59–69 (1990).
- 15 Bao SD, *Analysis of Soil and Agricultural Chemistry*. China Agriculture Press, Beijing, pp. 30–34 (2000).
- 16 Liu GS, *Soil Physical and Chemical Analyses and Soil Profile Description*. Chinese Standards Publisher, Beijing, p. 226 (1996).
- 17 Tessier A, Campbell PGC and Bisson M, Sequential extraction procedure for the speciation of particulate trace metals. *Anal Chem* **51**:844–851 (1979).
- 18 Tu C, Zheng CR and Chen HM, Effect of applying chemical fertilizers on forms of lead and cadmium in red soil. *Chemosphere* **41**:133–138 (2000).
- 19 Li JX, Yang XE, He ZL, Jilani G, Sun CY and Chen SM, Fractionation of lead in paddy soils and its bioavailability to rice plants. *Geoderma* **141**:174–180 (2007).
- 20 Davies BE, Lead, in *Heavy Metals in Soils*, ed. by Alloway BJ. Blackie Academic and Professional, London, pp. 206–223 (1995).
- 21 Yang JR, Bao ZP and Zhang SQ, The distribution and binding of Cd and Pb in plant cell. *Chin Environ Sci* **13**:263–268 (1993). (in Chinese).
- 22 Verma S and Dubey RS, Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci* **164**:645–655 (2003).
- 23 Othieno CO, Soil, in *Tea: Cultivation to Consumption*, ed. by Willson KC and Clifford MN. Chapman and Hall, London, pp. 137–192 (1992).
- 24 Han WY, Ruan JY, Lin Z, Wu X, Xu YW, Shi YZ, et al, The major nutritional limiting factors in tea soils and development of tea speciality fertilizers series. *J Tea Sci* **22**:70–74 (2002). (in Chinese).
- 25 Liang YR, Zhao QQ, Lu JL and Liu ZS, Effect of pruned tea leaf and various nitrogen fertilizers on soil pH and activated aluminum content. *J Tea* **26**:205–208 (2000). (in Chinese).
- 26 Ruan JY, Ma LF and Shi YZ, Properties of rhizosphere soil of tea plants and the impact of nitrogen fertilizer. *J Tea Sci* **23**:167–170 (2003). (in Chinese).
- 27 Zhang ZG, Wu Y and Xie DT, On acidification of tea garden soils in Chongqing. *J Southwest Agric Univ* **26**:15–17 (2004). (in Chinese).
- 28 Lu LJ and Liang YR, Characteristics of tea plant roots and management of tea gardens. *Tea Sci Technol* **14**:1–5 (1994). (in Chinese).
- 29 Nelson WO and Campbell PGC, The effects of acidification on the geochemistry of Al, Cd, Pb and Hg in freshwater environments: a literature review. *Environ Pollut* **71**:91–130 (1991).
- 30 Geebelen W, Vangronsveld J, Adriano DC, Carleer R and Clijsters H, Amendment-induced immobilization of lead in a lead-spiked soil: evidence from phytotoxicity studies. *Water Air Soil Pollut* **140**:261–277 (2002).
- 31 Chen YX, *Environmentology*. China Environmental Science Press, Beijing, p. 397 (2001).
- 32 Quevauviller P, Van Der Sloot HA, Ure A, Muntau H, Gomez A and Rauret G, Conclusions of the workshop: harmonization of leaching/extraction tests for environmental risk assessment. *Sci Total Environ* **178**:133–139 (1996).
- 33 Kennedy VH, Sanchez AL, Oughton DH and Rowland AP, Use of single and sequential chemical extractants to assess radionuclide and heavy metal availability from soils for root uptake. *Analyst* **122**:89–100 (1997).
- 34 Maiz L, Esnaola MV and Millan E, Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure. *Sci Total Environ* **206**:107–115 (1997).
- 35 Zhang SZ and Shan XQ, Is the bioavailability index applicable for trace elements in different types of soil? *Chem Speciat Bioavail* **12**:117–123 (2000).
- 36 Romkens PFAM, Bouwman LA and Boon GT, Effect of plant growth on copper solubility and speciation in soil solution samples. *Environ Pollut* **106**:315–321 (1999).
- 37 Kabala C and Singh BR, Fractionation and mobility of copper, lead and zinc in soil profiles in the vicinity of a copper smelter. *J Environ Qual* **30**:485–492 (2001).
- 38 Merry RH, Tiller KG and Alston AM, The effects of contamination of soil with copper, lead and arsenic on the growth and composition of plants. *Plant Soil* **95**:115–128 (1986).
- 39 Ernst WHO, Bioavailability of heavy metals and decontamination of soils by plants. *Appl Geochem* **11**:163–167 (1996).
- 40 Tiller KG, Heavy metals in soil and their significance. *Adv Soil Sci* **9**:113–142 (1989).
- 41 Kabata-Pendias A, *Trace Elements in Soils and Plants* (3rd edn). CRC Press, Boca Raton, FL, p. 413 (2001).
- 42 Marschner H and Romheld V, Root-induced changes in the availability of micronutrients in the rhizosphere, in *Plant Roots – the Hidden Half* (2nd edn), ed. by Waisel Y, Eshel A and Kafkafi U. Marcel Dekker, New York, NY, pp. 557–579 (1996).
- 43 Lindsay W, *Chemical Equilibria in Soils*. Wiley and Sons, New York, NY, p. 449 (1979).
- 44 Zhang MK and Fang LP, Tea plantation-induced activation of soil heavy metals. *Commun Soil Sci Plant Anal* **38**:1467–1478 (2007).
- 45 Han WY, Shi YZ, Ma LF, Ruan JY and Zhao FJ, Effect of liming and seasonal variation on lead concentration of tea plant (*Camellia sinensis* (L.) O.Kuntze). *Chemosphere* **66**:84–90 (2007).
- 46 Hettiarachchi GM and Pierzynski GM, Soil lead bioavailability and *in situ* remediation of lead-contaminated soils: a review. *Environ Prog* **23**:78–93 (2004).