Lead Absorption by Tea Leaves and its Distribution in Tea Plants

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Abstract: Background: Automobile exhaust, tire wear and drifting dust resulted from urbanization are new source of lead into tea plant to threaten human health by food chain. Despite of a large amount of atmospheric lead falls onto the soil, the floating particle can also be absorbed directly by tea leaves. **Purpose:** To evaluate the storage of lead by tea leaves when they were contaminated and the element movement in the plant as well as the possible mechanism referring to the process. **Methods:** After applying lead solution on leaves of *Camellia sinensis* (L.) O. Kuntze cultivar Fuyun No. 6 for 10 months, lead content in the leaf, stem and root was determined by GFAAS and lead distribution in different tissues of the three plant organs was investigated by Environmental scanning electron microscopy (ESEM) coupled with energy dispersive X-ray microanalysis (EDXA). **Results:** Lead captured directly by tea leaves increased greatly with the application onto the blades without showing any morphological symptoms or retardant growth. Lead entered tea leaves through symplast and apoplast pathways, and moved downwards actively to the root via the phloem system. **Conclusions** Lead captured by tea leaves constitutes contaminants in the product and we argue for the limit of atmospheric lead in tea gardens.
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1. Introduction

Literatures have proved that crops like ryegrass, wheat and barley that grow in neutral or alkaline soil obtain lead (Pb) mainly from atmosphere because in such soil Pb tends to combine with mineral or organic compounds to form complex which is insoluble to enter the plants [1-5]. As for *Camellia sinensis* (L.) O. Kuntze (tea plants), scientists believe soil is the source for Pb into the plants because the soil is acidic and Pb is released to be uptaken by plants' roots. Based on this thought China government has limited the amount of Pb in the soil but never in the air for tea production [6-8]. In resent years researchers have noticed that Pb content in tea leaves rises greatly if tea trees are growing close to expressways [9-11]. According to them, automobile exhaust, tire wear and drifting dust release Pb in the air, fall onto the soil and then tea plant roots absorb it and transport to the leaf [9,10]. Because tea plants are evergreen and have a great deal of organic compounds in their leaves [12,13], we assume that Pb from the air might combine directly with the chemicals in leaves to form Pb complex. In addition, tea plants grow usually for decades to provide fresh leaves as the raw material for tea beverage production, Pb could accumulate in leaves year after year. However, even though Wang et al. have recently applied plastic film to decrease Pb in young leaves by 13%-46% [14], few studies have reported on the direct effect of atmosphere on leaf Pb rising because the atmospheric Pb can also enter the plant through the soil. The lack of nuclear technology makes it difficulty to trace Pb in the system from the air to the plant.

Since the invention of environmental scanning electron microscopy (ESEM), plant structure can be observed in vapor at atmospheric pressures neither with dry processing nor gold coating in specimen preparation due to the unique vacuum system of this equipment. When ESEM is fixed with energy dispersive X-ray analysis (EDXA), chemical nature of a structure can be revealed [15]. Scientists successfully applied ESEM with EDXA to demonstrate silica deposition in rice and sorghum with fresh, unfixed, hydrated samples [16,17].

This article intended to examine Pb absorption by tea leaves on a stimulation experiment, in which Pb was applied to tea leaves as a solution instead of gas. It also applied ESEM with EDXA to investigate the absorption and storage of Pb by tea leaves, as well as the transport of Pb from leaf to stem and root.

2. Material and Methods

Plant culture

In early spring sand was sifted through a 0.3mm sieve, immersed in 10% (v/v) HNO_3 for 24h to remove background Pb, rinsed with tapping water

till pH back to neutral. Earthenware pots were cleaned and charged with 6 kg sand each. Seeds of *Camellia sinensis* (L.) O. Kuntze cultivar Fuyun No. 6 were washed in detergent, sterilized in 0.1% (w/v) $HgCl_2$, rinsed and immersed in constant dripping water for 7d. The seeds were covered in the pot with sand and sprayed with water irregularly to keep moisture. The experiment was done in the laboratory of tea biotechnology in Anhui Agricultural University, Hefei city. The laboratory was on the sunny side with natural light, temperature and humidity.

When 2nd leaf grew, 4 seedlings with the same size were remained. Every 7d the pot was added 1 L balanced nutrition solution with pH 5.5 [18]. Leaves were applied on the both blades by $Pb(CH_3COO)_2 \cdot 3H_2O$ solution with a soft brush 3 times a day. The concentration was 0, 7, 14, 42, 70 or 98 μg L^{-1} Pb respectively. New leaves were also treated with Pb solution. Treatment 98 μg L^{-1} Pb had 7 pots, treatment 0 μg L^{-1} Pb had 3 pots, and the other treatments had 6 pots each.

Lead determination by GFAAS

After 10 months of Pb application on tea leaves, samples of root, stem and leaf were cleaned with deionized water, over-dried at 80℃, ground and screened through a 200 mesh sieve.

Leaf sample of each treatment was also prepared by the same procedure as above but not cleaned by deionized water.

Sample of 1.000 g was carbonated on an electric-hot plate and dry-ashed in a marval furnace (sx-2-4-10, Factory of Shanghai Experimental Electric Oven, China) at 500℃ for 6h. When it was cool, the sample was added 1ml diluted HCl solution (1:1, v/v, 37% HCl to water) and heated on the electric-hot plate to boil. This procedure was repeated untill the sample solution became clear. The sample solution was set to 10ml with deionized water and Pb concentration was determined by GFAAS (SOLAARM6, Thermo Electron Corporation, America).

Every assay had 3 replicas of 1 pot each. The data was processed by excel software and correlation analysis was done by IBM SPSS Statistics.

Lead Detection by ESEM with EDXA

Pb relocation in leaf, stem and root was investigated by an ESEM (XT30 ESEM-TMP， Philips, Holand) fitted with an EDXA system (INCA300, Oxford, England). Fresh freehand sections of leaf, stem and root from 98 μg L^{-1} Pb treatment were rinsed with deionised water and fixed on a copper stage by double-sided adhesive tape. ESEM working condition was 5 to 8℃ temperature, 20 kV accelerating voltage, 3.8 τ to 6.1 τ chamber pressure, and 6.5 nm resolving capability. Pb X-ray intensity in a section was scanned either in linear or area profile. The contents of Pb were estimated semi-quantitatively by X-ray counts emitted during the raster scanning.

Leaves of 0 and 98 μg L^{-1} Pb treatments were also observed under Stereoscopy (Motic, SMZ-143 SERIES, China) fitted with camera (Sony, DSC-F717, Japan).

3. Results

Lead injury to leaf

Although tea seedling did not show any injuries or symptoms in growth or color change, stereoscopy observed dark Pb compounds gathered in leaf veins and corroded epidermal cells to make leaf surface extremely rough (Figure 1).

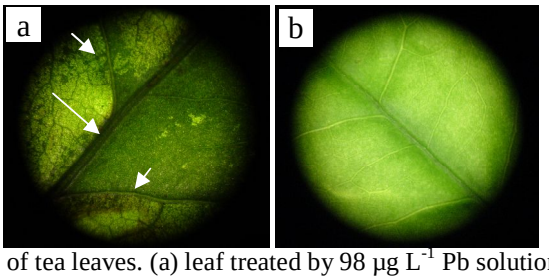

Figure 1. Stereoscopic images of tea leaves. (a) leaf treated by 98 μg L^{-1} Pb solution was rough and dark, arrows indicating veins with Pb in them. (b) leaf treated by H_2O was smooth and bright.

ESEM revealed that the upper epidermis of the leaf was damaged much more seriously by Pb than the lower (Figure 2). On the upper epidermis fragments of different sizes covered over the whole blade and no cells could be distinguished [Figure 2(a)]. While on the lower epidermis only little fragments spotted and epidermal cells and guard cells kept clear cell image [Figure 2(b)], indicating that the lower blade of the tea leaf had a certain way to resist Pb stress.

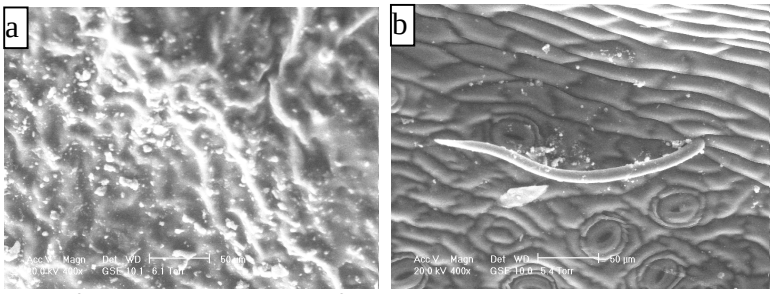

Figure 2. ESEM images of Pb contaminated tea leaf. (a) upper epidermis was etched and cells did not have clear boundaries. (b) lower epidermis had clear epidermal cells and guard cells

Lead absorption and storage by leaves

GFAAS determination showed that Pb content in tea leaves increased with the application of Pb on leaves (Figure 3), and the coefficient correlation was significantly high (r=0.979, p=0.004). Leaves rinsed with deionized water had lower Pb than those without being washed from 5.00% to 17.12%. The results indicate that tea leaves can capture Pb by ways of adsorption and absorption which supported our hypothesis.

Figure 3 displays that Pb absorbed by tea leaves transported to the stem and root especially at high Pb application, the correlation coefficients were significant (stem, r=0.980, p=0.003; root, r=0.973, p=0.005). The data in Figure 3 suggest that low concentration of outside Pb tends to store in tea leaves and may also move to the stem and root when outside Pb is much. For instance, Pb content in the leaf was 3.4 times that in the root at Pb 7 μg L^{-1}, but only about 0.47 at Pb 98 μg L^{-1}.

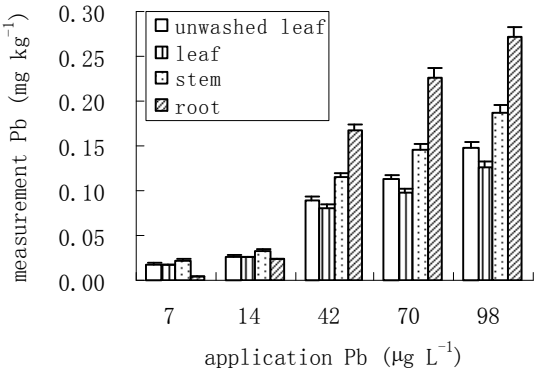

Figure 3. Pb content in leaf, stem and root after Pb application on tea leaves

If the concentration of Pb in leaf, stem and root of every treatment was averaged, the ratio of each organ to the average could present the tendency of Pb partition among the three organs as in Table 1.

Table 1. Ratio of organ Pb to the average

Pb treatment (μg L^{-1})	Leaf	Stem	Root
7	1.96	0.69	0.35
14	0.93	1.21	0.86
42	0.67	0.95	1.38
70	0.63	0.93	1.45
98	0.65	0.96	1.39

Ratios in table 1 are relatively stable when the treatment Pb exceeded certain amount, although the exact amount of Pb is increasing. This might suggest the tea leaves have a stable storage of Pb and will output the excess Pb to the root.

Lead distribution in leaf

Figure 4(a) and (c) are the transection of Pb-polluted tea leaf and its line scan match of Pb through the main vein. The upper epidermis had Pb of 9 counts and the lower had 3 counts, showing more Pb on the upper part than on the lower, which explained photos in Figure 2 that upper epidermis was corroded while the lower was not. Parenchyma cells near the upper epidermis also had more Pb than those near the lower epidermis. For the xylem tissue, which was about 280 μm thick, two patterns of Pb counts were distinguished. The upper half could be named the rising pattern and the lower half the smooth pattern. The smooth pattern extended from the lower xylem to the phloem and l layer of parenchyma cells inside the lower epidermis. Because in this typical vascular conductive structure xylem occupied the largest area and had more Pb than the phloem, it is possible that phloem exported Pb and xylem imported Pb.

On the lower epidermis leaf, Pb scattered uniformly on epidermal hair, cells, guard cells and stomata [Figure 4 (b) and (d)], hinting Pb moved effectively in the lower epidermis. Interesting thing was that the lower epidermis was near the phloem and the upper epidermis near the xylem. These results extended the previous findings that in tea leaves Pb was stored in the xylem and transported by the phloem conductive system.

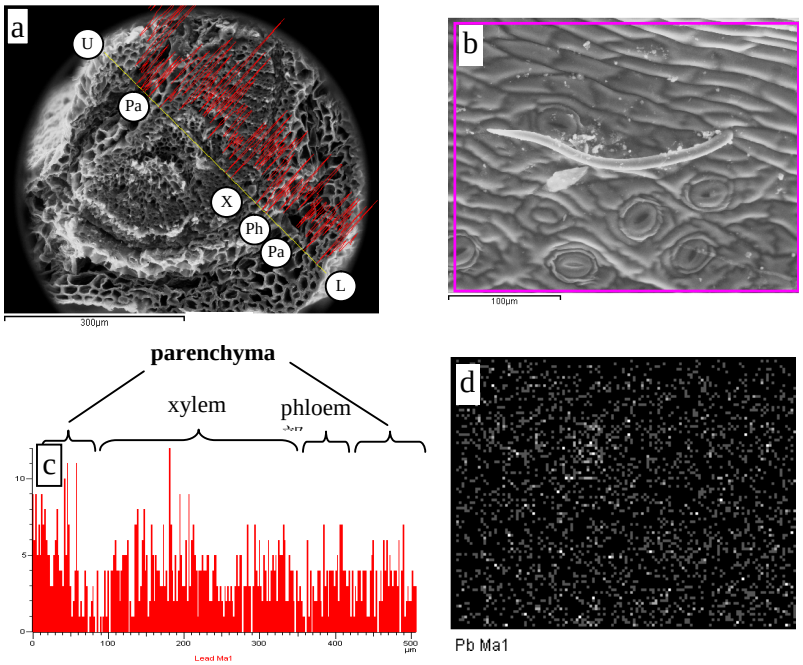

Figure 4. Pb distribution in different tissues of *C. Sinensis* leaf revealed by ESEM with EDXA. (a) Transverse section with line scan of Pb. U, upper epidermis; L, lower epidermis; Pa, parenchyma cells; Ph, phloem; X, xylem. (b) Epidermal cells, guard cells and a hair. (c) Line scan match of (a) showing Pb intensity from upper epidermis to lower epidermis through the main vein. Vertical scale is Pb counts and horizontal scale is the distance from the upper epidermis. (d) Area scan match of the rectangle in (b) showing Pb intensity in hair, guard cells and epidermal cells.

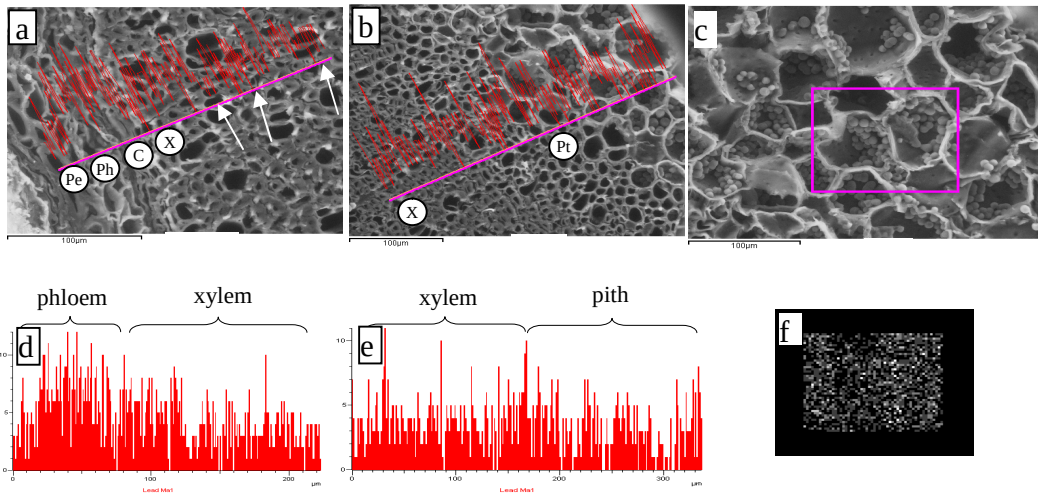

Figure 5. Pb distribution in different tissues of *C. Sinensis* stem revealed by ESEM with EDXA. (a) Transection of stem with line scan of Pb from periderm to xylem. Pe, periderm; Ph, phloem; C, cambium; X, xylem. (b) Transection of stem with line scan of Pb from xylem to pith. Pt, pith. (c). Transection of stem showing pith. (d) Line scan match of (a) showing Pb intensity from periderm to xylem. Vertical scale is Pb counts and horizontal scale is the distance from periderm. (e) Line scan match of (b) showing Pb counts from xylem to pith. Horizontal scale is the distance from xylem. (f) Area scan match of the rectangle in (c) showing Pb intensity in storage particles and other components in pith cells.

Lead distribution in stem

In the transsection of the stem there were five tissues including from outside the periderm, phloem, cambium, xylem and pith (Figure 5 a, b and c). Because the sample was too large in this case, continuous photos were taken. Two patterns of Pb intensity could be seen in the stem [Figure 5 (a), (b), (d), (e)]. One was the parabola pattern in the phloem of about 80 m thick with low Pb of 3 counts at both ends, and high Pb of 12 counts in the middle. The other was the smooth pattern in the xylem and pith. Arrows in Figure 5(a) did not show more Pb in vessel cavities than the other area indicated that they could not be the special reception site of Pb from the leaf, nor did the pith cells in Figure 5(c) and (f). Because of the larger area, the xylem and pith might be the storage place for Pb in the stem.

Lead distribution in root

Figure 6(a) showed the transcetion of the root included tissues from outside epidermis, cortex, endodermis, phloem, cambium and xylem. The cortex held more than half of the root space. Figure 6(a) and (b) showed that Pb in the cortex was very dense. Interestingly, endodermis acted as a boundary for Pb distribution outside and inside. Pb inside decreased from the phloem to the xylem. The cortex t was the storage place of Pb in the root, instead of the xylem as in the leaf and stem.

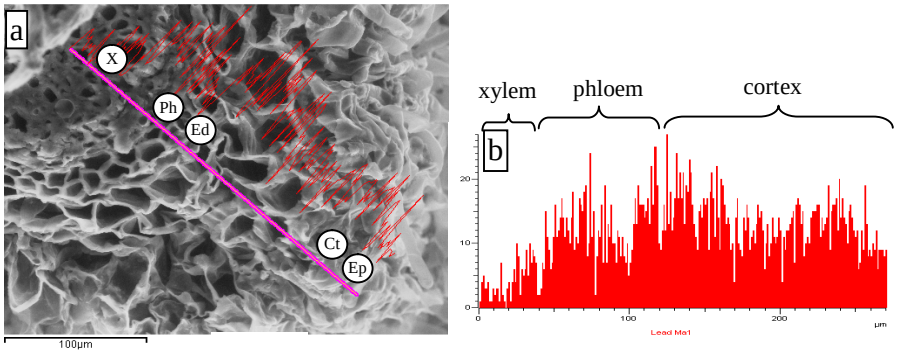

Figure 6. Pb distribution in different tissues of *C. Sinensis* root revealed by ESEM with EDXA. (a) Transection of root with line scan of Pb from xylem at center to periphery endodermis. Ep, epidermis; Ct, cortex; Ed, endodermis; Ph, phloem; X, xylem. (b) Line scan match of (a) showing Pb counts from xylem to epidermis. Vertical scale is Pb counts and horizontal scale is the distance from xylem.

Dynamic movement of Pb

When the highest Pb counts in the xylem and phloem were connected from the leaf, stem to the root, a dynamic relocation of Pb absorbed by leaf in tea seedlings was obtained (Figure 7). Pb in the xylem was relatively stable around 10 counts, while in the phloem it kept increasing dramatically from 7 counts in leaf to 12 in stem and 25 in root. These results suggest that in tea seedling the xylem could not be the path of Pb from the leaf to the root, but the phloem is possible the conducting system. The rising tendency of Pb counts in the phloem was entirely consistent with that of Pb concentrations in the leaf, stem and root, indicating the Pb accumulation in root from the leaf through the xylem.

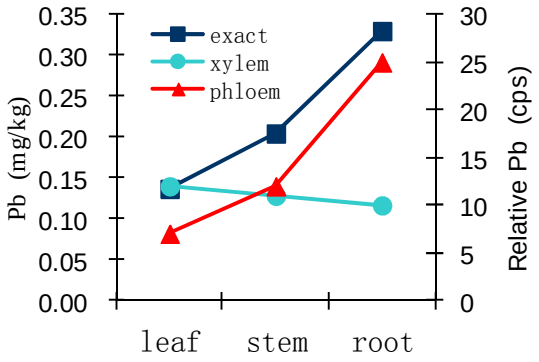

Figure 7. Pb in leaf, stem and root

4. Discussions

The main aim of this paper is to estimate the storage of Pb by tea leaves. Han et al. stated that the

atmosphere beside a highway contained Pb 14.2 μg m^{-3} and that the automobile exhaust contained Pb 7.3 to 14.0 μg m^{-3} [11]. In this experiment we set the least Pb concentration as 7μg L^{-1} for the consideration that leaves were treated Pb solution 3 times a day and this period together was about 1min. Because there are 1440 minutes in a day, Pb solution in the experiment was condensed 1000 times to imitate the possible Pb amount fallen on the leaf from the air. Chinese state limits Pb to 5 mg kg^{-1} in tea leaves product [19]. In this experiment tea leaves treated with Pb 7 μg L^{-1} accumulated it 0.018 mg kg^{-1}. Although this amount was only 0.365% of the state limit, it would be 10.95%-14.60% in real production because tea plants usually grow for about 30-40 years, excluding the part from the root back to the leaf. This figure is in the range of Wang et al.'s 13% to 46% [14] in real tea gardens. The reason for Wang et al.'s greater data might be that theirs include the part from the soil. In addition, our treatments imply that more Pb from the air would greatly increase the leaf capture of this heavy metal. Therefore it is necessary for the government to propose a standard of air Pb contaminants.

Because water washed away part of Pb captured by tea leaves, we concluded that Pb entered tea leaves by apoplast and symplast pathways. The results of etched upper epidermal and corrosion resistant of lower epidermis to Pb implied that Pb might transport through the phloem system, which goes with and the knowledge of elements movement absorbed by plant leave [20]. The flow against Pb concentration in the phloem system from the leaf, stem to the root leads to the active mechanism of Pb movement downwards.

Reliance on these measures must be further confirmed, however, because Pb treatment in this experiment is not the real tea production air situation. It would be beneficial to replicate this study on large scales. It would also be necessary to measure the flow of Pb between the air and tea plants over decades instead of tea seedlings in only one year to estimate more accurately the risk of Pb from the air.

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