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Effects of high soil lead concentration on photosynthetic gas exchange and chlorophyll fluorescence in *Brassica chinensis* L.

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ABSTRACT

This study determined the effects of high soil lead concentration on photosynthetic gas exchange and chlorophyll fluorescence in *Brassica chinensis* L. Results showed the net photosynthetic rate, the maximum PSII quantum yield, photochemical quenching, and quantum yield of PSII photochemistry continuously increased until lead concentration reached 600 mg/kg. These parameters slightly decreased when lead concentration reached 900 mg/kg and significantly decreased when reached or exceeded 1200 mg/kg. As lead concentration increased, stomatal conductance and transpiration rate decreased; minimum fluorescence increased to different degrees; intercellular CO_2 concentration initially decreased, increased, and then sharply decreased; and nonphotochemical quenching initially decreased and then increased. Therefore, soil treatment with 900 mg/kg lead can only slightly affect *B. chinensis*, whereas those with ≥ 1200 mg/kg can significantly affect this crop.

Keywords: photosynthetic physiology; lead stress; suburban or urban area; vegetable consumption; food safety

The rapid development of agriculture, industry, and urbanization over the past decades has increased the occurrence of lead leakage into soil in many regions of China. Lead concentration in some suburban or urban soils has reached several hundred or even thousand mg/kg (Lin et al. 2007). Lead in soil can enter plant cells and produce a wide range of toxic effects on the plants (Ahmad et al. 2011), and inhibit enzyme activities, and perturb mineral nutrition and water balance (Sharma and Dubey 2005). These disorders upset normal physiological activities and reduce plant photosynthesis (Sharma and Dubey 2005, Ahmad et al. 2008). Moreover, lead contamination in soil poses a serious threat to the health of animals and humans through the food chain (Ahmad et al. 2011). Therefore, lead contamination in soil has become a major ecological concern and attracted considerable attention.

Vegetable consumption is a primary pathway of human exposure to heavy metals (Chang et al. 2014). However, considering convenience and practical factors, residents of suburban and urban areas in China often cultivate *Brassica chinensis* L. for their own consumption in nearby patches of idle lands that are likely to have been seriously polluted by lead (Hu and Ding 2009). *B. chinensis* has a certain tolerance to lead and grows well even in soils contaminated with some degrees of lead concentrations without any visible nonspecific symptoms (i.e., ≤ 500 mg/kg) (Wang et al. 2009). Hence, people tend to neglect the possible health risk of consuming *B. chinensis* grown in soils with serious lead contamination. In addition, the effects of growing *B. chinensis* in soils contaminated with high lead concentrations remain poorly understood.

In this study, suburban or urban soils with serious lead contamination were prepared by adding lead at different concentrations into soil. The effects of these lead-contaminated soils on the photosynthetic gas exchange and chlorophyll fluorescence in *B. chinensis* were investigated. The results of this study provided insights into the food safety problem brought about by consuming *B. chinensis* grown in suburban or urban soils with serious lead contamination.

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MATERIAL AND METHODS

Plant materials and growing conditions. All field experiments were conducted in the rain-proof sun shed of Jiangsu University, China, from November 2013 to April 2014. *B. chinensis* cv. Shanghai Green was used as the plant material in the experiment. Seeds of *B. chinensis* were sown in plastic pots (20 cm × 25 cm) each filled with soils treated with lead at different concentrations. Seedlings were gradually reduced to one seedling per pot, and 60% relative soil moisture in each pot was maintained and normal fertilization was conducted during the experiment. Soil treatments with three replicates consisted of a CK without lead (T_0) and five treatments with lead at concentrations of 300, 600, 900, 1200, and 1500 mg/kg soil dry weight (designated as T_{300}, T_{600}, T_{900}, T_{1200}, and T_{1500}, respectively). The different lead concentrations were obtained by mixing different amount of $Pb(NO_3)_2$ power into soils with a background concentration: lead 25.6 mg/kg, available P 38.52 mg/kg, available K 76 mg/kg, and available N 585.82 mg/kg.

Photosynthetic gas exchange parameters. Photosynthetic gas exchange parameters were measured from 09:00 to 11:00 on three selected clear days (March 12, 13, and 15, 2014) when *B. chinensis* was at fast growth phase with leaf number 15. Three matured leaves of *B. chinensis* were selected from each treatment group. The net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate-photosynthetic photon flux density (P_n-PPFD, Cond-PPFD, c_i-PPFD, and T_r-PPFD) response curves were determined across a PPFD coverage range of 0–1800 μmol/m^2/s under air temperature of 25°C (set with the instrument) and ambient CO_2 concentrations (370–380 μmol/m^2/s) and by using a LI-6400 Portable Photosynthesis System (LI-COR 6400, Lincoln, USA).

Chlorophyll fluorescence parameters. The chlorophyll fluorescence of leaves of *B. chinensis* was measured using an imaging pulse-amplitude-modulated fluorometer (Heinz Walz, Effeltrich, Germany) on March 14, 2013. After a dark-adaptation of 30 min, the minimum fluorescence (F_0), maximum fluorescence (F_m), and maximum PSII quantum yield (F_v/F_m) were automatically determined using ImagingWin (Walz, Germany). Then kinetic induction fluorescence was conducted, and the quantum yield of PSII photochemistry (ΦPSII), nonphotochemical quenching (NPQ), and photochemical quenching (qP) were automatically calculated. Finally, rapid light curve (RLC) measurements were conducted with photosynthetic active radiation (PAR) values ranging from 0–1176 μmol/m^2/s. The apparent electron transport rate (ETR) at a given actinic illumination was also automatically calculated (Fu et al. 2012, Guo et al. 2012).

Statistical analysis. Statistical analysis was performed by using one-way ANOVA followed by least significant difference (*LSD*) test at different significance levels.

RESULTS AND DISCUSSION

Photosynthetic gas exchange. Among the photosynthetic gas exchange parameters, P_n is the most important in determining the influence of different environmental stresses on photosynthesis (Olszewski et al. 2014). As shown in Figure 1a, the P_n-PPFD response curves of *B. chinensis* under the different treatments rapidly increased with increasing PPFD, slowly peaked, and then slowly decreased. During the entire light response, the mean P_n in T_{300} and T_{600} increased by 6.1% and 0.2%, respectively, compared with that in T_0. Meanwhile, the mean P_n in T_{900}, T_{1200}, and T_{1500} decreased by 3.9, 28.0, and 40.6%, respectively, compared with that in T_0. No significant difference in the mean P_n was detected between T_0 and T_{300} ($P = 0.068$), T_{600} ($P = 0.124$), and T_{900} ($P = 0.082$). By contrast, the mean P_n of T_0 significantly differed from those of T_{1200} ($P = 0.012$) and T_{1500} ($P = 0.000$). The above results show the photosynthetic capacity of *B. chinensis* enhanced in soils treated with lead at 300 and even 600 mg/kg but slightly decreased in soils treated with lead at 900 mg/kg. Serious photosynthetic inhibition and thus visible nonspecific symptoms were observed after treatment with lead at ≥ 1200 mg/kg.

Cond and T_r, reflecting the degree of stomatal opening and closing from different aspects, are often related. Similar to the P_n-PPFD response curves, both Cond-PPFD and T_r-PPFD response curves in each treatment initially increased rapidly, gradually peaked, and then gradually decreased with increasing PPFD (Figures 1b,c). The mean Cond and T_r during the entire light response process gradually

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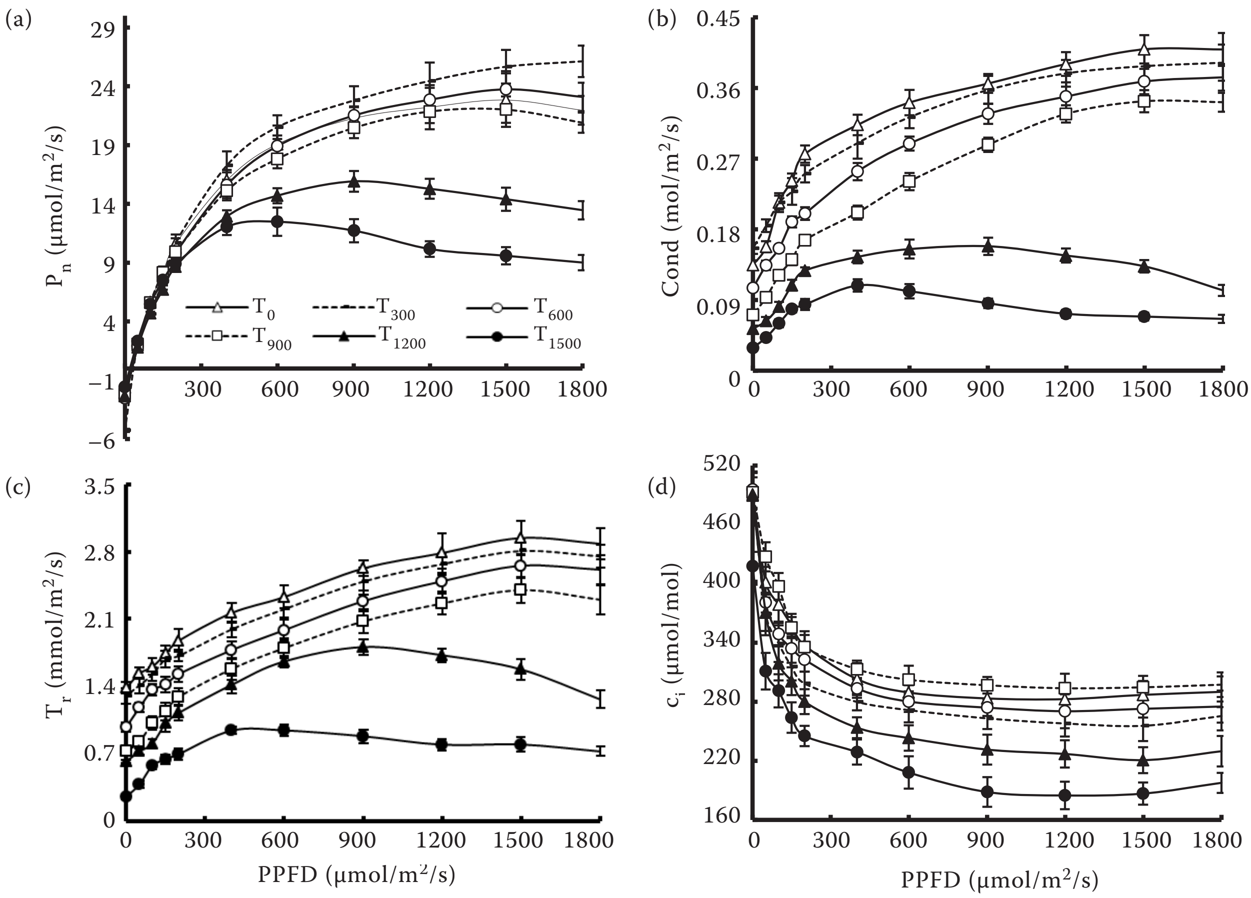

Figure 1. Response curves of various photosynthetic parameters to photosynthetic photon flux density (PPFD) for different treatments. P_n – photosynthetic rate; Cond – stomatal conductance; T_r – transpiration rate; c_i – intercellular CO_2 concentration; T_0 – without lead; T_{300} – 300, T_{600} – 600, T_{900} – 900, T_{1200} – 1200, T_{1500} – 1500 mg/kg soil dry weight

decreased with increasing lead concentration in the soils. Compared with T_0, the mean Cond in T_{300}, T_{600}, T_{900}, T_{1200} and, T_{1500} decreased by 2.8, 15.6, 28.2, 60.8 and 75.5%, respectively (P = 0.101, 0.027, 0.002, 0.000 and 0.000, respectively); the mean T_r in above treatments decreased by 5.5, 15.1, 27.1, 42.5 and 68.1%, respectively (P = 0.059, 0.046, 0.003, 0.000 and 0.000, respectively). In the present study, the two parameters decreased with increasing lead concentration in the soils regardless of the increase or decrease in P_n. By contrast, different results were observed in maize under lead stress (Ahmad et al. 2011), which maybe resulted from the differences in lead concentration setting in the respective test.

All the c_i-PPFD response curves rapidly decreased with increasing PPFD, gradually decreased, and then slightly increased (Figure 1d). The mean c_i during the entire light response process in T_{900} was higher by 3.8% (P = 0.091) than that in T_0, whereas those in T_{300}, T_{600}, T_{1200}, and T_{1500} were lower by 9.1, 4.8, 14.4 and 26.3% than that in T_0 (P = 0.052, 0.061, 0.035 and 0.003, respectively). c_i is mainly affected by CO_2 consumption during photosynthesis and by the degree of stomatal openness (Kosobrukhov et al. 2004). It is the result of the combined effect of Cond and P_n. Thus, the decrease in c_i in T_{300} and T_{600} can be attributed to the decrease in Cond and to the increase in CO_2 consumption for photosynthesis. The increase in c_i in T_{900} can be mainly attributed to the decrease in CO_2 consumption for photosynthesis, and non-stomatal limitation is the major factor responsible for P_n reduction. The decrease in c_i in T_{1200} and T_{1500} can be mainly attributed to the decrease in Cond, and stomatal limitation is the major factor responsible for P_n reduction. Therefore, the effect of stomatal closure seems to be a main factor limiting photosynthesis at high concentration of lead in soil. Some studies showed that high concentra-

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Table 1. Minimum fluorescence (F_0), maximum fluorescence (F_m), and maximum PSII quantum yield (F_v/F_m) of leaf samples from different treatments after dark adaptation

	T_0	T_{300}	T_{600}	T_{900}	T_{1200}	T_{1500}
F_0	0.2936[a]	0.2995[a]	0.2980[a]	0.3002[a]	0.2995[a]	0.2961[a]
F_m	0.0725[b]	0.0716[b]	0.0725[b]	0.0806[ab]	0.0956[a]	0.102[a]
F_v/F_m	0.753[a]	0.761[a]	0.757[a]	0.732[ab]	0.681[b]	0.656[b]

T_0 – without lead; T_{300} – 300, T_{600} – 600, T_{900} – 900, T_{1200} – 1200, T_{1500} – 1500 mg/kg soil dry weight

tion of lead in root level can cause a decline in Cond by increasing the secretion of abscisic acid resulting in the stomata closure, and indirectly lead to a decline in photosynthetic assimilation (Sharma and Kumar 2002).

Chlorophyll fluorescence. The measurement of chlorophyll fluorescence is a quantitative, noninvasive, rapid, and powerful method to assess the characteristics of the photosynthetic apparatus and the extent to which plants suffer from different environmental stresses and nutrient deficiencies (Calatayud et al. 2006, Živčák et al. 2014).

A loss of PSII reaction centers is associated with an increase in F_0 (Fu et al. 2012). Table 1 shows F_0 of leaves in T_{300}, T_{600}, T_{900}, T_{1200}, and T_{1500} were 2.0, 1.5, 2.2, 2.0, and 2.1% higher than that in T_0. However, no significant difference in F_0 was detected between any two treatments. So it was conclude that the lower degrees of loss of PSII reaction centers occurred in different lead treatments. Compared with T_0, F_m of leaves in T_{300} decreased by 1.2%, whereas those of leaves in T_{900}, T_{1200}, and T_{1500} increased by 11.2, 31.9, and 40.7%, respectively; F_v/F_ms in T_{300} and T_{600} increased by 1.1% and 0.5%, respectively, whereas those in T_{900}, T_{1200}, and T_{1500} decreased by 2.8, 9.6, and 12.9%, respectively; significant differences in F_m and F_v/F_m were observed between T_0 and T_{1200}/T_{1500} but not between T_0 and $T_{300}/T_{600}/T_{900}$. F_v/F_m reflects the potential photochemical efficiency of the active center of PSII in the dark. Although some studies show that the parameter F_v/F_m is often insensitive to stress (Živčák et al. 2008), other studies show the F_v/F_m of a plant often declines when the plant is exposed to environmental stresses (Calatayud et al. 2006, Fu et al. 2012). These contradictory results may be due to differences among different plants. Judged from the second view above, the change trends of both F_0 and F_v/F_m under the different treatments reflected that a slight stress occurred in T_{900}, a serious stress occurred in T_{1200} and T_{1500}, and no stress occurred in T_{300} and T_{600}.

The light energy absorbed by leaves can be used to drive photosynthesis, and excess energy can be dissipated as heat or re-emitted as chlorophyll fluorescence. However, the total amount of chlorophyll fluorescence is very small, and large portions of the absorbed light are used to drive photosynthesis (qP) or are dissipated as heat in fluorescence quenching (NPQ) (Ralph and Gademann 2005). Similar to qP, the proportion of light absorbed by the chlorophyll associated with PSII used in photochemistry is reflected by ΦPSII. Figure 2 shows that the qP and ΦPSII of leaves initially increased slightly and then decreased, whereas the NPQ of leaves showed an opposite trend with increasing lead concentration in the soils. Compared with T_0, the qPs of leaves in T_{300} and T_{600} increased by 5.6% and 1.9%, respectively, whereas those of leaves in T_{900}, T_{1200}, and T_{1500} decreased by 3.7, 12.1, and 26.3%, respectively; the ΦPSIIs of leaves in T_{300} and T_{600} increased by 15.2% and 3.7%, respectively, whereas those of leaves in T_{900}, T_{1200}, and T_{1500} decreased by 16.0, 22.4, and 26.3%, respectively; the NPQs of leaves in T_{300} and T_{600} decreased by 8.6% and 0.7%, respectively, whereas those of leaves in T_{900}, T_{1200}, and T_{1500} increased by 39.6, 44.4, and 76.8%, respectively. Significant differences in qP and ΦPSII were detected between T_0 and T_{1200}/T_{1500} but not between T_0 and $T_{300}/T_{600}/T_{900}$; while significant differences in NPQ were observed between T_0 and $T_{900}/T_{1200}/T_{1500}$ but not between T_0 and T_{300}/T_{600}. Therefore, it was concluded that the more absorbed light was used to drive photosynthesis while the less absorbed light was dissipated as heat when the added lead in the soils did not exceed 600 mg/kg. When the added lead in the soils exceeded 900 mg/kg, the proportion of absorbed light for driving photosynthesis

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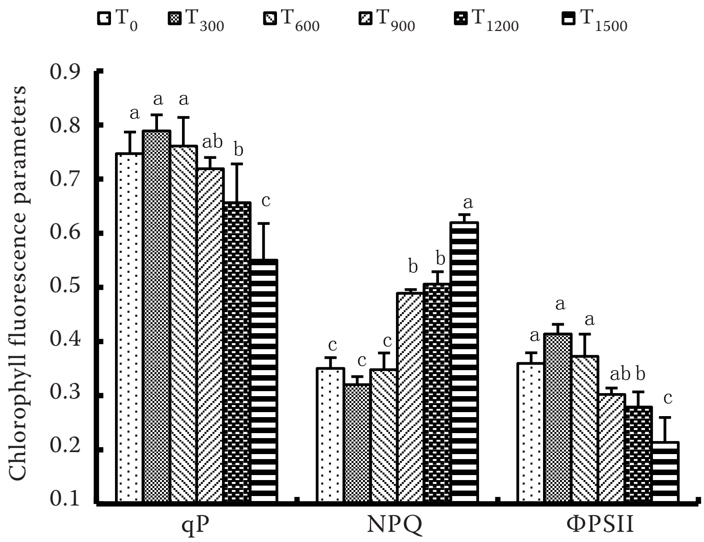

Figure 2. Photochemical quenching (qP), non-photochemical quenching (NPQ), and quantum yield of PSII photochemistry (ΦPSII) of leaf samples from different treatments under actinic illumination. T_0 – without lead; T_{300} – 300, T_{600} – 600, T_{900} – 900, T_{1200} – 1200, T_{1500} – 1500 mg/kg soil dry weight

drastically decreased, whereas that of absorbed light for dissipating as heat drastically increased.

The apparent ETR is closely related to photosynthetic activity and is an approximation of the rate of electrons pumped through the photosynthetic chain (Ralph and Gademann 2005). Figure 3 shows that all rapid light-response curves of ETRs under different treatments rapidly increased and then slightly declined at the different PARs with increasing PAR. At low-range PAR, the light-response curves of all treatments closely overlapped at corresponding determined time periods. With increasing PAR, different degrees of separation of ETR light-response curves were observed under the different treatments. The mean ETR during the entire light response process in T_{300} was the highest among the different treatments and 9.3% higher than that in T_0 (P = 0.078). Compared with T_0, ETRs in T_{600}, T_{900}, T_{1200}, and T_{1500} were lower by 7.9, 15.2, 30.4, and 49.2%, respectively (P = 0.071, 0.029, 0.002 and 0.000, respectively). The ETRs of *B. chinensis* decreased to different degrees when the added lead in the soils reached 600 mg/kg. It indicates that ETR is more sensitive to lead stress than the parameters of F_v/F_m, qP, ΦPSII, and P_n.

In conclusion, treated soils with lead at concentrations up to 600 mg/kg increased P_n, qP, and ΦPSII to some degrees. However, these parameters decreased when the added lead reached 900 mg/kg. Thus, *B. chinensis* can grow well not only in soils contaminated with lead at ≤ 500 mg/kg (Wang

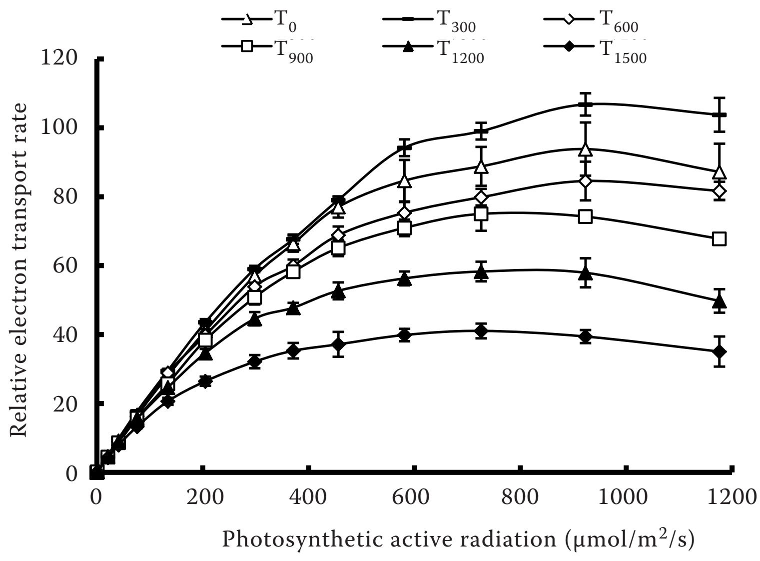

Figure 3. Light-response curves of electron transport rate (ETR) of leaf samples from different treatments. T_0 – without lead; T_{300} – 300, T_{600} – 600, T_{900} – 900, T_{1200} – 1200, T_{1500} – 1500 mg/kg soil dry weight

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et al. 2009) but also at 600 and even 900 mg/kg. Serious photosynthetic inhibition accompanied with some visible nonspecific symptoms in *B. chinensis* only occurred when the added lead reached or exceeded 1200 mg/kg. Therefore, *B. chinensis* has strong tolerance to high lead concentrations, and consuming *B. chinensis* grown in suburban or urban soils in China poses a serious threat to human health.

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