

Soil Cu contamination destroys the photosynthetic systems and hampers the growth of green vegetables

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Abstract

Soil metal contamination leads to a decrease in a yield of crops and is a threat to human health. In the present study, the properties (*i.e.*, photosynthetic pigments, gas-exchange parameters, chlorophyll fluorescence, biomass, leaf area, leaf mass per area) of three green vegetables (*i.e.*, *Brassica chinensis*, *Chrysanthemum coronarium*, *Brassica alboglabra*) grown under various Cu treatments [0, 200, 400, and 600 mg(Cu) kg⁻¹] were measured and analysed. The results showed that soil Cu contamination resulted in the damage of photosynthetic pigments, negative effects on gas exchange, and hampered growth of all three vegetables. However, it did not significantly influence PSII functions of the three vegetables. It indicates that soil Cu contamination negatively affected photosynthesis particularly due to stomatal factors, but not due to the damage of photosynthetic apparatus.

Additional key words: fluorescence kinetics; green vegetable; metal contamination; photosynthesis; soil Cu contamination.

Introduction

Soils are often polluted by metals due to developing industry (Chen *et al.* 2015) and agriculture (Zeng *et al.* 2007), and the population expansion (Meng *et al.* 2014). The investigation of heavy metal contents in soils is a basis for promoting the development of vegetable production with high quality and efficiency. Zeng *et al.* (2007) reported that Zn, Cr, and Cu occur in relatively high concentrations in lands utilized for the vegetable production, and that the highest concentration of Cu occurs in greenhouse soils. Heavy metal pollution in greenhouse soils becomes a serious problem (Zupančič 2016). Since Cu is not a biodegradable chemical, the manure containing Cu, which is used in vegetable lands, brings a potential risk

to soil causing Cu contamination (Lin *et al.* 2015). Although Cu is an essential element for plant growth and development, *e.g.*, plays role in many physiological processes, such as photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein metabolism, *etc.*, it is toxic to plants at a high concentration, and an accumulation of Cu in agricultural products leads even to a threat to human health (Xu *et al.* 2006). Generally, the Cu content in leaves changes from 5 to 25 mg kg⁻¹ (Huang 2004), and its content varies between 0.64–1.28 mg(Cu) l⁻¹ in human blood (Wang 2004).

Brassica chinensis is popular in southern China and southeast Asia. Being winter-hardy, *Brassica* varieties

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Abbreviations: ABS/RC – absorption; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO₂ concentration; DF_{abs} – driving forces; DI₀/RC – dissipation at t = 0; E – transpiration rate; ET₀/RC – electron transport at t = 0; F_m – maximum Chl fluorescence intensity; F₀ – minimum Chl fluorescence intensity; F_v/F_m – maximum yield of PSII photochemistry; g_s – stomatal conductance; HCu – high Cu contamination; LA – leaf area; LCu – low Cu contamination; LMA – leaf mass per area; MCu – middle Cu contamination; OEC – oxygen-evolving complex; PI_{abs} – photosynthetic performance index; P_N – net photosynthetic rate; Q_A – primary bound plastoquinone; Q_B – secondary bound plastoquinone; RC – reaction center; TR₀/RC – energy flux for trapping at t = 0; φE₀ – probability that an absorbed photon will move an electron into the electron transport chain; φP₀ – maximum quantum yield of primary photochemistry; ψE₀ – efficiency with which a trapped exciton can move an electron into the electron transport chain.

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are increasingly grown in northern Europe. Considering convenience and practical factors, residents of suburban and urban areas in China often cultivate *B. chinensis* for their own consumption in nearby patches of idle lands (Fu and Wang 2015). *Chrysanthemum coronarium* is native to Mediterranean and Asia and used as a food and ornamental plant (Miyazawa and Alaoui 2015). *C. coronarium* is also used as traditional medicine due to its antibacterial activity (against *Klebsiella pneumonia* with low sensitivity) by its essential oil (Moussaoui and Alaoui 2016). *Brassica alboglabra* is native to coastal southern China and western Europe. *B. alboglabra* has become established as an important food crop plant because of its large food reserves and rich in essential nutrients (Guo *et al.* 2016). The above three green vegetables could provide carbohydrates, proteins, and less fat for human, they could also supply rich nutrients, *e.g.*, vitamins A, C, K, B₆, folate, calcium, and calories. Therefore, farmers in southern China often cultivate the green vegetables as commercial agricultural products. Many farmers cultivate the green vegetables in a greenhouse. Thus, the effect of soil Cu contamination on the green vegetables becomes a serious scientific issue; *e.g.*, Zeng *et al.* (2007) reported that the highest concentration of Cu occurs in greenhouse vegetable land soils comparing to other types of vegetable plantation land patterns. Additionally, soil metal contamination has been paid great attention in recent decades (Planquart *et al.* 1999, Lin *et al.* 2015, Zupančič 2016, Stolpe *et al.* 2017). However, in different countries, the limit values of soil heavy metal pollutants are different (Zeng *et al.* 2007). Researching on the effect of soil metal contamination on vegetables is a basis for assessment of heavy metal pollution in greenhouse soils (Zupančič 2016) and for allowing national governments to set soil pollution limits (Zeng *et al.* 2007).

The effect of metal(s) on plant has been widely reported, *e.g.*, chromate on *Spirodela polyrhiza* (Susplugas

et al. 2000, Appenroth *et al.* 2001), Cu on *Oriza sativa* (Xu *et al.* 2006), Cu on *Lemna minor* (Razinger *et al.* 2007), Cd, Cu on *Lemna minor* (Razinger *et al.* 2010), Cd and Cu on *Lemna minor* (Razinger *et al.* 2012), heavy metals (Cu, Cr, Ni, *etc.*) on *Wedelia trilobata* (Lin *et al.* 2012a) or *Alternanthera philoxeroides* (Lin *et al.* 2012b), Cd on *W. trilobata* (Shi *et al.* 2014), Pb on *B. chinensis* (Fu and Wang 2015, Tang *et al.* 2015). The pollutants commonly significantly affect glutathione, antioxidant enzymes, glutathione reductase, guaiacol peroxidase, and catalase in plants (*e.g.*, Razinger *et al.* 2007). It is also known that photosynthesis is inhibited by heavy metals, and PSII is considered as the main target of heavy metals (Susplugas *et al.* 2000, Appenroth *et al.* 2001). Chlorophyll (Chl) fluorescence of plants changes after exposure to Cu (Razinger *et al.* 2007, 2010, 2012).

However, up till now, not much is known about the toxic effect of Cu on three common green vegetables (*B. chinensis*, *C. coronarium*, *B. alboglabra*). Because of this limited knowledge, in the present study, we focused on the physio-ecological index [*i.e.*, pigments, net photosynthetic rate (P_N)] and the plant growth properties [*i.e.*, leaf biomass, leaf area (LA), and leaf mass per area (LMA)] of the three vegetables grown in soil with varying degree of Cu contamination by pot-culture experiments. We also focused on the alteration and function of PSII through analysis of the Chl *a* fluorescence transient in the vegetables grown at different Cu concentrations by pot-culture experiments. We also analysed gas-exchange characteristics, such as stomatal conductance (g_s) and the intercellular CO₂ concentration (C_i), in the plants grown at varying degrees of soil Cu contamination. Our results may provide not only a basis for understanding the effect of pollutant on a process of photosynthesis in vegetables, but a robust basis for vegetable land soils management, especially, for greenhouse soils.

Materials and methods

Plant materials and pot-culture experiments: Seeds of the three green vegetables were purchased from *Fuzhou Golden Seed Co., Ltd.* (No. 495, Fuzhou, Fujian province, China). The pot-culture experiments were carried out in a greenhouse at the Fuqing Branch of Fujian Normal University from October to December in 2015. The pot-culture experiments were repeated twice. The seeds were sterilised with 0.1% HgCl₂ for 10 min, followed by washing and soaking in distilled water for 8 h, and then they were sown in sterilized silica culture. Then, one of plants with three leaves was transplanted into a pot (diameter: 200 mm, height: 200 mm) with about 2 kg soils. The properties of the soil (Lin *et al.* 2015) were: pH 6.4, 2.24 g(total N) kg⁻¹, 24.2 g(available N) kg⁻¹, 1.15 g (total P) kg⁻¹, 9.03 mg(available P) kg⁻¹, 68 mg(total potassium) kg⁻¹, and 21.7% of clay particles. The soil Cu background value was 127.17 mg kg⁻¹ (Lin *et al.* 2012a,

2012b). In order to simulate the pollution of soil, three Cu treatments were designed according to Chinese environmental quality standard for soils (GB 15618-1995). Before being filled into pots, air-dried soil was added with 0 (as control), 200 (low Cu contamination, LCu), 400 (middle Cu contamination, MCu), or 600 mg kg⁻¹ (high Cu contamination, HCu) of CuSO₄·5H₂O. Each treatment had 15 replicates.

Photosynthetic pigments: Measurements of photosynthetic pigments were performed with three replicates on 10 December 2015. Photosynthetic pigments were extracted according to Arnon (1949). Fresh leaf (the third from bottom) samples (0.2 g), which were randomly selected, were ground using mortar and pestle in 20 ml of 80% acetone (v/v), and then filtered through No. 2 *Whatman* filter paper. Chl *a*, Chl *b*, carotenoids (Car), and

total pigments were measured at 470, 646, and 663 nm, using UV spectrophotometer *Model 1801* (Beifen-Ruili Analytical Instrument Co. Ltd, Beijing, China) according to Lichtenthaler and Wellburn (1983).

Photosynthetic capacity: The measurements of photosynthetic capacity were performed twice (corresponding to two pot-culture experiments). Each time, seven healthy and fully developed leaves (the third from bottom) were randomly selected in order to measure photosynthetic capacity. The P_N [irradiance of 1,000 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$, 25°C, and 360–380 ppm CO_2] was monitored on the leaf with *CIRAS-2* portable photosynthesis system (*PP Systems*, USA) with an LED radiation source. Once the steady state was reached, recording was made and stored. At the same time, the C_i , g_s , and transpiration rate (E) were recorded. The measurement of photosynthetic capacity was performed between 9:00 and 16:00 h (solar time).

Chl *a* fluorescence and JIP: In the second pot-culture experiments, seven healthy and fully developed leaves (the third from bottom) were randomly selected per treatment in order to measure Chl *a* fluorescence. Chl *a* fluorescence was measured at room temperature (22°C) using a plant efficiency analyser (*Handy-PEA* fluorometer, *Hansatech Instruments*, UK) with protocol [*i.e.*, the sample was illuminated with 2-s pulse of excitation light intensity of 3,000 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$] from 8 to 10 December 2015. Leaves were adapted in darkness for 20 min before measurements. The F_0 (minimum Chl fluorescence intensity), F_m (maximum Chl fluorescence intensity), F_v/F_m (maximum yield of photochemistry, where $F_v = F_m - F_0$) were recorded and stored. At the same time, PI_{abs} (photosynthetic performance index), ϕE_0 (probability that an absorbed photon will move an electron into the electron transport chain), ϕP_0 (maximum quantum yield of primary photochemistry), ψE_0 (efficiency with which a trapped

exciton can move an electron into the electron transport chain), ABS/RC (absorption), ET_0/RC (electron transport at $t = 0$), DI_0/RC (dissipation at $t = 0$), TR_0/RC (energy flux for trapping at $t = 0$), and DF_{abs} (driving forces) were recorded and stored. Fluorescence measurements were performed around noon (11:00–12:00 h, solar time). At the same time, the JIP-test was performed. All the fluorescence transients were recorded in a time span from 10 μs to 2 s with a data acquisition rate of 10 μs for 2 s. Detailed JIP-test see Susplugas *et al.* (2000) or Appenroth *et al.* (2001).

Biomass, LA and LMA: Seven plants were randomly selected to measure biomass, LA and LMA. LA was measured based on image processing according to Lü *et al.* (2010). Leaf image segmentation was implemented using scanner (*HP LaserJet 1536dnf MFP*, Guangzhou, China). The LA calculation equation is as follows:

$$\text{LA} = \text{Sf} \times \frac{\text{Pl}}{\text{Pf}} \quad (1)$$

where, Sf denotes the square, Pl denotes the pixel number of leaf object in the image, and Pf denotes the pixel number within the square in the image. Leaf samples were dried at 65°C until no further reduction in mass, and the dry mass (DW) was measured by weighting method. The following equation was applied to estimate LMA:

$$\text{LMA} = \frac{\text{DW}}{\text{LA}} \quad (2)$$

Statistical analysis: One-way analysis of variance (*ANOVA*) was conducted with the *SPSS 16.0*. The data (\pm standard error) were tested for normality (*Kolmogorov-Smirnov's* test) and homogeneity (*Levin's* test). Evaluation of significance of the data between the groups of tested parameters was done through *Duncan's* test ($P < 0.05$).

Results

Photosynthetic pigments: Concentrations of Chl *a* and total pigments of Cu-contaminated plants were all lower than that of control (Fig. 1). Overall, all of the Chl *a* and total pigments declined along with increasing soil Cu contamination. In *B. chinensis*, the concentrations of Chl *b* and Car in Cu-contaminated groups were also all lower than that of control. The concentrations of Car showed no difference between the LCu (200 mg kg^{-1}) and the control, or between the MCu (400 mg kg^{-1}) and the HCu (600 mg kg^{-1}), however, the concentrations of Car in the MCu and HCu were both different from the LCu or the control. In *C. coronarium*, the concentrations of Chl *b* in Cu-contaminated groups were also all significantly lower than that of control. Overall, the concentrations of Chl *b* declined with increasing soil Cu contamination, however, the concentrations of Car ascended along with increasing soil Cu. And, the concentrations of Car were not different

between LCu, MCu groups and the control, but remarkably increased in the HCu group. In *B. alboglabra*, the concentrations of Chl *b* decreased with increasing soil Cu contamination, but the HCu (600 mg kg^{-1}). The concentration of Car was not significantly affected by soil Cu contamination.

Gas-exchange parameters: Compared with the control, all P_N declined in the Cu-contaminated groups (Table 1). With increasing soil Cu contamination, all P_N showed apparently decreasing trends. The E of *C. coronarium* showed no significant difference between the control and different Cu-contaminated groups. The E of *B. chinensis* and *B. alboglabra* both showed no consistent upward or downward trends with increasing Cu contamination. The g_s showed consistent downward trend with the increasing Cu contamination. With the increasing Cu contamination,

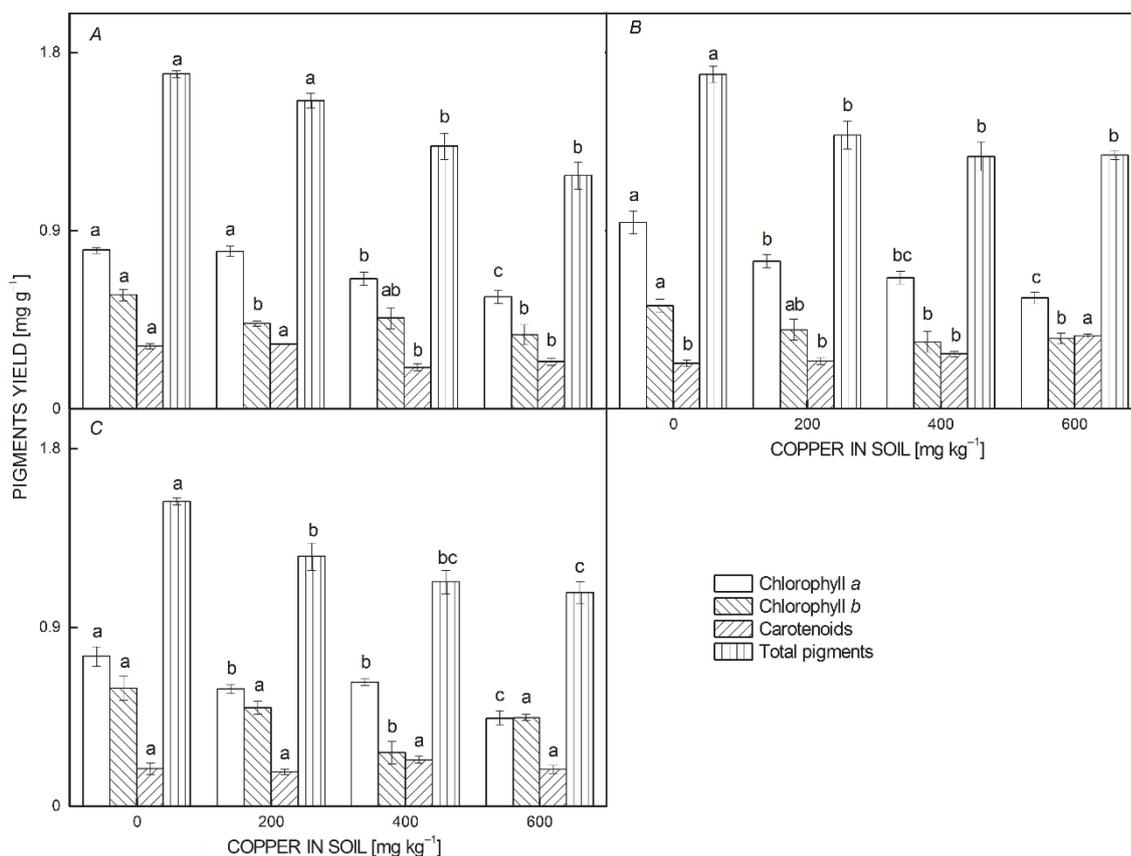


Fig. 1. Effects of soil Cu contamination on the pigment contents of *Brassica chinensis* (A), *Chrysanthemum coronarium* (B), and *Brassica alboglabra* (C). Different lowercase letters show significant difference between different Cu-contaminated groups at $P < 0.05$, and, error bars show standard error (SE), $n = 3$.

the C_i also showed consistent downward trends, except for the data measured in *B. chinensis* on 3 November, and except for the data measured in *B. alboglabra* on 4 November 2015.

Chl fluorescence: The parameters of Chl *a* fluorescence changed with increasing Cu contamination, but the change had no statistical significance (Fig. 2). In *B. alboglabra*, with increasing soil Cu contamination, the PI_{abs} , F_0 , F_m , F_v , F_v/F_m , ϕP_0 , ϕE_0 , ψE_0 , DF_{abs} all declined in the MCu plants compared with the control; most of parameters slightly increased at the HCu. The ABS/RC showed consistent upward trends; the TR_0/RC and DI_0/RC all increased from the control to MCu, but declined again at the HCu. ET_0/RC and F_v/F_m did not show any consistent trends. It indicates that the values of Chl *a* fluorescence parameters were not significantly different between control and Cu-contaminated plants.

OJIP: All leaves showed normal Chl *a* fluorescence, OJIP transient, and the curve started from the initial F_0 intensity and increased to a peak of P or F_m (Susplugas *et al.* 2000, Appenroth *et al.* 2001). Two intermediate steps J (about 2 ms) and I (about 30 ms) appeared between O and P.

A decrease in the F_m level appeared in *B. alboglabra* and *C. coronarium* in Cu-contaminated plants (Fig. 3). On the contrary, an increase in the F_m level could be seen in *B. chinensis* at the Cu contamination. It verified that the photosynthetic apparatus of all three vegetables was not significantly influenced by the soil Cu contamination.

Biomass, LA and LMA: The effects of soil Cu contamination on the biomass are shown in Fig. 4A. LCu and MCu (200 and 400 mg kg⁻¹, respectively) contamination had promoted the biomass of *C. coronarium* compared with the control, however, the HCu (600 mg kg⁻¹) decreased the biomass of *C. coronarium*. The LCu (200 mg kg⁻¹) increased the biomass of *B. chinensis* compared with the control, but without any statistical significance. The biomass of *B. chinensis* and *B. alboglabra* both showed downward trends with the increasing Cu contamination. The biomass of *B. chinensis* at HCu significantly decreased.

The effects of soil Cu contamination on the LA of vegetables are shown in Fig. 4B. The LA of *C. coronarium* and *B. alboglabra* both increased at the LCu compared with the control, but it had no statistical significance. LA of all plant types descended with the increasing Cu

Table 1. Effects of soil Cu contamination on gas-exchange parameters of vegetables. E – transpiration rate, g_s – stomatal conductance, P_N – net photosynthetic rate, C_i – intercellular CO_2 concentration. Data show means \pm SE and at the same row, the differential *lowercase letters* indicate differ significantly at $P < 0.05$, $n = 7$.

Species	Date	Gas-exchange parameter	Cu exposure [mg kg ⁻¹ (soil)]			
			0 mg kg ⁻¹	200 mg kg ⁻¹	400 mg kg ⁻¹	600 mg kg ⁻¹
<i>C. coronarium</i>	2 nd Nov.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	10.0 \pm 0.7 ^a	9.4 \pm 0.4 ^a	9.7 \pm 0.6 ^a	8.8 \pm 0.6 ^a
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	2.8 \pm 0.2 ^a	3.0 \pm 0.2 ^a	2.7 \pm 0.3 ^a	2.0 \pm 0.2 ^b
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	2,153.4 \pm 701.4 ^a	1,437.4 \pm 390.1 ^{ab}	944.1 \pm 236.0 ^b	447.4 \pm 77.0 ^b
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	360.6 \pm 3.1 ^a	362.3 \pm 3.1 ^{ab}	346.3 \pm 7.8 ^{bc}	339.1 \pm 5.7 ^c
	8 th Dec.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	12.9 \pm 1.6 ^a	7.3 \pm 0.4 ^b	6.6 \pm 0.4 ^b	8.0 \pm 1.0 ^b
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	2.9 \pm 0.2 ^a	3.0 \pm 0.1 ^a	3.0 \pm 0.1 ^a	3.1 \pm 0.1 ^a
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	2,120.9 \pm 1,300.1 ^a	953.9 \pm 92.8 ^a	982.7 \pm 61.9 ^a	866.1 \pm 63.7 ^a
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	391.6 \pm 7.4 ^a	381.9 \pm 2.0 ^a	381.1 \pm 1.7 ^a	373.6 \pm 3.6 ^a
<i>B. chinensis</i>	3 rd Nov.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	17.4 \pm 1.1 ^a	8.2 \pm 0.7 ^c	11.4 \pm 0.9 ^b	6.3 \pm 1.1 ^c
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	1.0 \pm 0.2 ^{ab}	0.6 \pm 0.0 ^b	1.2 \pm 0.1 ^a	0.7 \pm 0.1 ^b
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	274.4 \pm 98.3 ^a	101.0 \pm 9.2 ^b	253.9 \pm 32.0 ^{ab}	115.4 \pm 13.7 ^{ab}
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	215.6 \pm 51.0 ^c	267.0 \pm 7.4 ^b	309.6 \pm 7.6 ^a	300.4 \pm 20.1 ^a
	9 th Dec.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	14.3 \pm 1.1 ^a	10.6 \pm 0.8 ^b	8.3 \pm 0.4 ^{bc}	6.9 \pm 1.1 ^c
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	2.5 \pm 0.1 ^a	2.0 \pm 0.1 ^b	2.2 \pm 0.1 ^{ab}	2.1 \pm 0.1 ^b
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	826.9 \pm 103.0 ^a	557.7 \pm 80.6 ^b	719.4 \pm 58.8 ^{ab}	604.0 \pm 41.1 ^b
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	373.3 \pm 2.1 ^a	354.7 \pm 4.6 ^c	363.7 \pm 1.4 ^{bc}	366.1 \pm 4.1 ^a
<i>B. alboglabra</i>	4 th Nov.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	16.5 \pm 0.6 ^a	12.8 \pm 0.7 ^b	10.8 \pm 1.2 ^b	11.9 \pm 0.6 ^b
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	0.9 \pm 0.1 ^b	1.3 \pm 0.1 ^a	1.1 \pm 0.0 ^a	1.1 \pm 0.0 ^a
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	326.7 \pm 39.5 ^c	756.7 \pm 77.5 ^a	481.9 \pm 23.9 ^b	467.0 \pm 31.6 ^b
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	326.0 \pm 4.1 ^c	359.3 \pm 4.7 ^a	347.7 \pm 5.4 ^{ab}	342.0 \pm 3.5 ^b
	10 th Dec.	P_N [$\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$]	14.8 \pm 1.3 ^a	11.7 \pm 1.0 ^{ab}	9.0 \pm 1.6 ^{bc}	6.1 \pm 0.5 ^c
		E [mmol(H ₂ O) m ⁻² s ⁻¹]	3.8 \pm 0.3 ^a	2.4 \pm 0.2 ^b	1.8 \pm 0.3 ^{bc}	1.3 \pm 0.1 ^c
		g_s [mmol(H ₂ O) m ⁻² s ⁻¹]	705.9 \pm 133.0 ^a	287.4 \pm 42.9 ^b	199.4 \pm 36.3 ^b	179.3 \pm 22.9 ^b
		C_i [$\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$]	354.7 \pm 6.8 ^a	314.6 \pm 11.1 ^b	304.4 \pm 5.5 ^b	320.7 \pm 7.8 ^b

contamination, and the LA of *C. coronarium* and *B. chinensis* at HCu significantly decreased.

The effects of soil Cu contamination on the LMA are shown in Fig. 4C. The LMA of *C. coronarium* and *B. alboglabra* were not significantly affected by Cu contamination. The LMA of *B. chinensis* at MCu and HCu significantly decreased.

Discussion

Photosynthetic pigments: Heavy metals reduce accumulation of photosynthetic pigments (Rai *et al.* 2016). At a cellular level, an excess of heavy metals can cause damage leading to the formation of reactive oxygen radicals or the interaction with proteins impairing key cellular processes (*e.g.*, photosynthesis), inactivating enzymes, and disturbing protein structure (Rai *et al.* 2016, Vilas *et al.* 2016). Chl content is indicative of the plant metabolisms efficiency and health status, whereas Car plays a major role in photoprotection and defending plant cell from oxidative stress (Kopsell *et al.* 2007). Photosynthetic pigments are useful for stress resistance and may be partly disappeared because that they participate in radical ions elimination and energy dissipation (Wilson *et al.* 2006).

Previous studies have shown that pollutants lead to

It indicates that soil Cu contamination significantly decreased the yields of *B. chinensis* and *C. coronarium* at high Cu concentrations and significantly increased the yields of *C. coronarium* at low Cu concentrations. The *B. alboglabra* was insensitive to the soil Cu contaminations, which did not exceed the soil pollution limits.

decrease in the content of photosynthetic pigments. Cadmium (Cd²⁺) induces changes in the composition and structure of the light-harvesting complex II (Krupa *et al.* 1988). Cu²⁺ causes a slower Chl incorporation into PSI and PSII in greening barley seedlings (Capsi *et al.* 1999). The Chl biosynthesis is affected by heavy metals as it substitutes Mg²⁺ in Chl (Rai *et al.* 2016). In the present study, the results showed that soil Cu contamination resulted in the decline of photosynthetic pigments (Fig. 1). Our results are similar to the previous reports (Capsi *et al.* 1999, Rai *et al.* 2016, Vilas *et al.* 2016). Our results also showed that the ratio of Chl *a/b* of *B. alboglabra* and *C. coronarium* showed downward trends with the increasing Cu contamination.

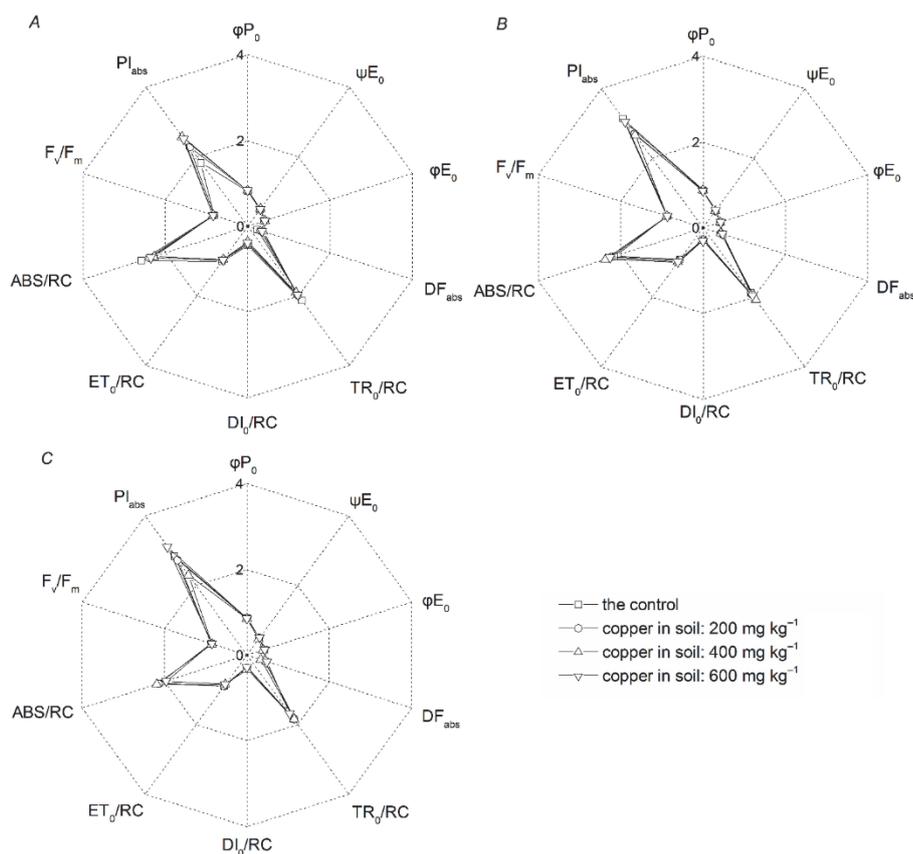


Fig. 2. Spider plot showing selected JIP-test parameters of *Brassica chinensis* (A), *Chrysanthemum coronarium* (B), and *Brassica alboglabra* (C) in different Cu-contaminated groups.

Photosynthesis: Cu contamination results in decreasing P_N in plants, e.g., in cucumber (Vinit-Dunand *et al.* 2002), *W. trilobata* (Lin *et al.* 2015), and *Oryza sativa* (Costa and Sharma 2016). Pollutants have direct effect on plants by affecting PSII and PSI and indirect effect on photosynthesis (Rai *et al.* 2016). In the present study, the results showed that all P_N at different Cu-contaminated groups were lower than that of the control, and overall, all P_N showed a downward trend with respect to the increasing of soil Cu contamination (Table 1). It is in line with the previous studies (Lin *et al.* 2015, Costa and Sharma 2016, Rai *et al.* 2016).

The degree of photosynthesis often declines due to stomatal factors. The decline of photosynthesis usually occurs in plants under low water stress or high-temperature stress, with respect to the downregulation of g_s for reducing moisture loss in leaves. Stomatal closure usually occurs in plants under other abiotic stresses, e.g., organic or inorganic pollution. However, Vinit-Dunand *et al.* (2002) found that the P_N of cucumber plants decline at a nearly constant C_i which indicates that stomatal closure does not account for the inhibition of photosynthesis under Cu contamination. In the present study, the results showed that generally the C_i of *C. coronarium* and *B. alboglabra* declined due to the soil Cu contamination and the g_s of the

three vegetables also showed a downward trend due to the soil Cu contamination except for the *B. alboglabra* assessed on 4 November 2015 (Table 1). It suggests that the decreasing P_N of the three vegetables due to stomatal factor resulted from the soil Cu contamination. Pollutants also lead to a remarkable decrease in total free amino acids (Chen *et al.* 2001). Soil Cu contamination might lead to metabolic limitations and result in accumulation of signaling molecules, e.g., abscisic acid (Chen *et al.* 2001), thus inducing the stomatal closure (Mishra *et al.* 2006), because the hormone, abscisic acid, regulates the opening and closing of stomata (Mishra *et al.* 2006).

Chl *a* fluorescence: Pollutants usually negatively impact on the PSII. Heavy metals (e.g., Cu and Pb) are directly involved in electron transport and disturb electron transport in light reactions and affect various enzymes in dark reactions (Rai *et al.* 2016). Cu contamination may inactivate the PSII reaction centers (RC) in *Chlorella pyrenoidosa* and inhibit the electron transport on the acceptor side (Xia and Tian 2009). The ABS/RC increases with increasing Cu contamination, but the ET_0/RC decreases, and excess Cu has an insignificant effect on the TR_0/RC (Xia and Tian 2009). F_0 , F_m , F_v/F_m , ϕP_0 , ϕE_0 , ψE_0 , ABS/RC, TR_0/RC , ET_0/RC , DI_0/RC , and PI_{abs} are affected

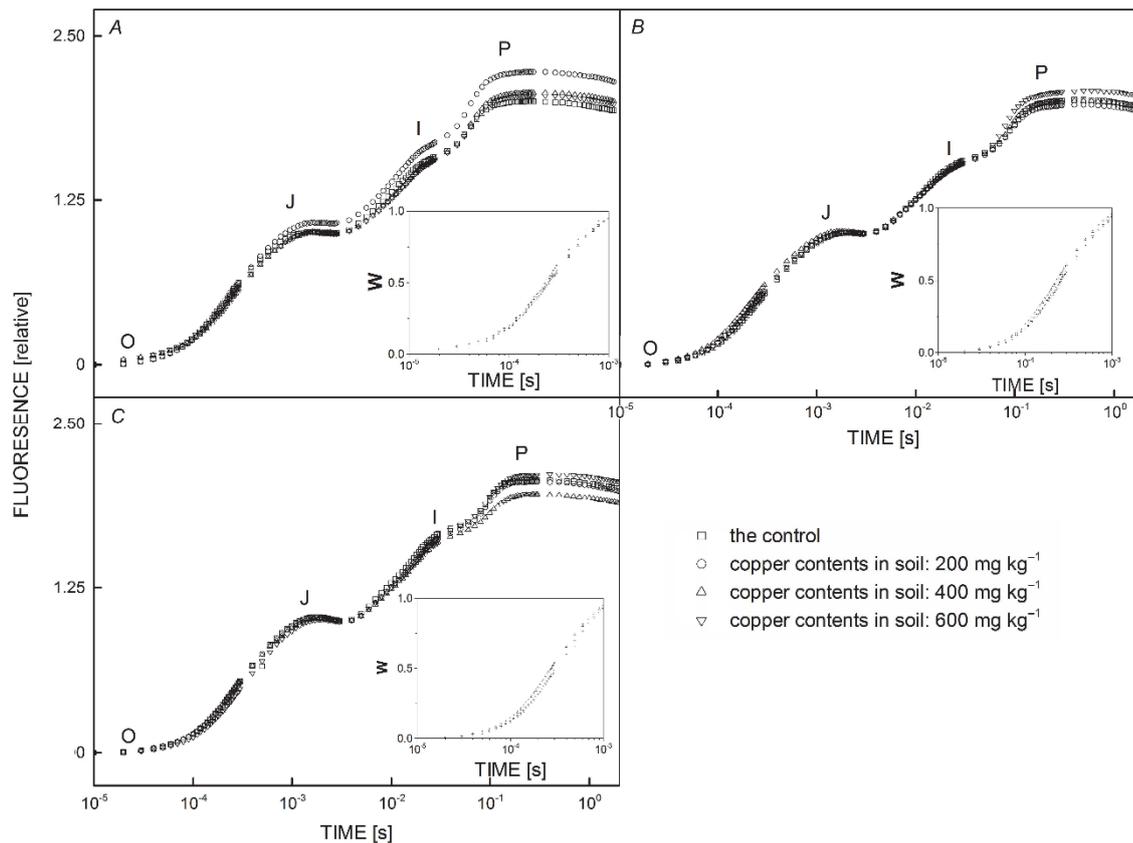


Fig. 3. Effects of soil Cu contamination on the OIJs of *Brassica chinensis* (A), *Chrysanthemum coronarium* (B), and *Brassica alboglabra* (C).

by pollutant (Susplugas *et al.* 2000, Appenroth *et al.* 2001). Strasser *et al.* (2000) and others (Susplugas *et al.* 2000, Appenroth *et al.* 2001, Meng *et al.* 2016) have defined the biological significance of these fluorescence parameters. The decrease of F_v/F_m and PI_{abs} may be considered as a potential indicator for environmental stress effects before appearance of visible physiological disturbances. Electron transport and energy fluxes are influenced due to the damage of PSII RCs under environmental stress (Strasser *et al.* 2000, Susplugas *et al.* 2000, Appenroth *et al.* 2001, Meng *et al.* 2016). The variation in an initial slope of the OJIP curve, ϕE_0 and ψE_0 , which are induced by inhibition of electron transport of Q_A-Q_B and Q_A accumulation, may explain why the proportion of opened PSII RCs and the number of electrons used to carbon fixation (*i.e.*, P_N) is reduced and may also explain why the energy dissipated (Meng *et al.* 2016). In the present study, our results showed that all parameters of Chl *a* fluorescence were not significantly affected by the soil Cu contamination, *i.e.*, the PSII function in all three vegetables were not significantly influenced. Our results are not similar to the results of Susplugas *et al.* (2000) or Appenroth *et al.* (2001), but partly similar to the results of Xia and Tian (2009).

All oxygenic photosynthetic plants investigated so far show a fluorescence rise consisting of a sequence of

phases, labeled as O, J, I, and P, and this O-J-I-P polyphasic transient has been found to change its shape according to changes in environmental conditions (Susplugas *et al.* 2000, Appenroth *et al.* 2001). The K peak can appear in the OJIP under stressful conditions, *e.g.*, high temperature, Cr contamination (Susplugas *et al.* 2000, Appenroth *et al.* 2001). In the present study, the results showed that all Chl *a* fluorescence OJIPs produced normal OJIPs; no K peak appeared (Fig. 3). It indicates that the oxygen-evolving complex (OECs), or the organisations of the light-harvesting antenna (Susplugas *et al.* 2000) of the three vegetables were not damaged by soil Cu contamination. Our results are in disagreement to the results of Susplugas *et al.* (2000) or Appenroth *et al.* (2001).

Why was not the PSII function in all three vegetables significantly influenced by soil Cu contamination? It might be owing to a lower toxicity of Cu to plants in comparison to other pollutants. At low concentration, Cu is an essential element for plant growth and development. It plays an important role in many physiological processes, such as photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein metabolism, *etc.* (Xu *et al.* 2006, Rai *et al.* 2016). Cu and Zn, which either serve as cofactor or activators of enzyme reactions, *e.g.*, in forming enzymes/substrate metal complex, are considered

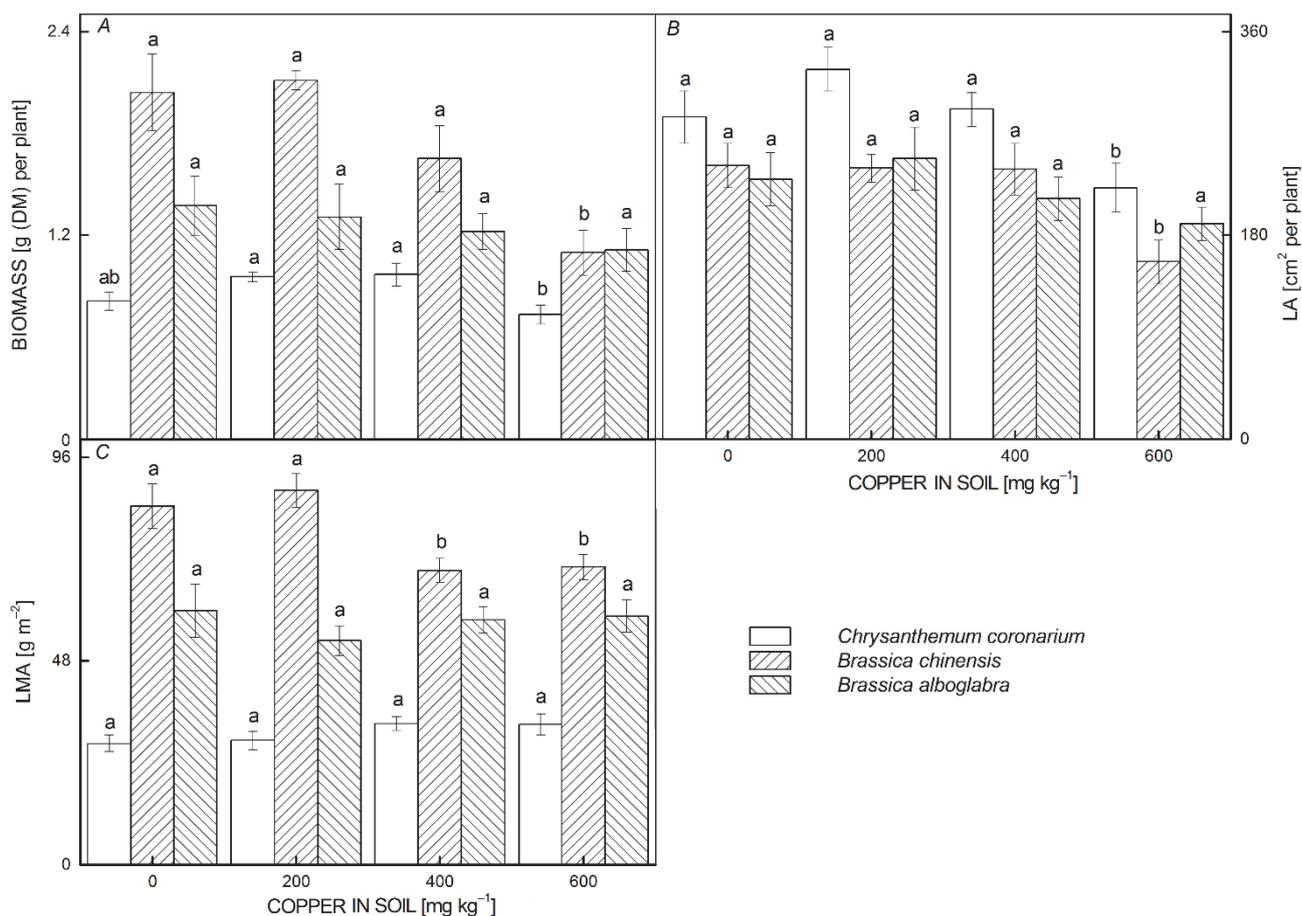


Fig. 4. Effects of soil Cu contamination on the biomass (A), leaf area (B), and leaf mass per area (C) of three vegetables. Different lowercase letters show significant difference between different Cu-contaminated groups at $P < 0.05$, and, error bars show standard error (SE), $n = 7$.

to be essential for plant growth. Therefore, heavy metal toxicity depends on plant species, specific metal, concentration, its chemical form, soil composition, and pH (Rai *et al.* 2016).

Plant growth: Soil pollution may inhibit the growth of plants. LA and LMA are often closely related to light conditions. Under low light, the LMA of *Euonymus fortunei* shows a decline, but the LA of *E. fortunei* shows an increase (Song and Li 2016). Compared with control, LA of *T. platyphyllos* was reduced by 29–60% which was associated with a level of pollution and irradiance as well, while the LMA exceeded the control by 4–25% at the most contaminated plots, but it diminished with an increasing distance from the pollution source (Lykholat *et al.* 2016).

In the present study, we showed that soil Cu contamination significantly decreased the biomass of *B. chinensis* and *C. coronarium* at the high Cu concentration, and significantly increased the biomass of *C. coronarium* at

the low Cu concentration (Fig. 4A). The *B. alboglabra* might be less sensitive to the soil Cu contamination. The LMA of *C. coronarium* and *B. alboglabra* showed an upward trend with the increasing Cu contamination, while the LMA of *B. chinensis* showed a downward trend with the increasing Cu, while the LA of the three vegetables showed a downward trend with the increasing Cu contamination (Fig. 4B,C). As mentioned above, variation in heavy metal toxicity depends on plant species and specific metal (Rai *et al.* 2016); thus, soil Cu contamination inhibited the growth of the three vegetables at different degrees.

Conclusions: The soil Cu contamination for the three plant types (*Brassica chinensis*, *Chrysanthemum coronarium*, *Brassica alboglabra*) showed the lower content of photosynthetic pigments, affected negatively photosynthesis, decreased the growth of vegetables. No significant influence on the PSII functions was observed due to the toxic performance of Cu.

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