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Effects of elevated CO_2 and cadmium stress on vegetable quality and cadmium accumulation

Xiao Wang, Deyan Li, Xiaohui Song*

The Department is Agricultural College, Anshun University, Anshun, P. R. China

**Corresponding author: hopewangxiao@163.com*

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Abstract: The aim of this study was to determine the effects of elevated CO_2 (eCO_2) on the quality and cadmium (Cd) accumulation of vegetables grown in soil with a background Cd level or in Cd-contaminated soil. We used four types of vegetable: pak choi (*Brassica rapa* L.), water spinach (*Ipomoea aquatica* Forssk.), cherry radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin) and pepper (*Capsicum annuum* L.). Cd stress significantly reduced vegetable vitamin C and reducing sugar content; however, under eCO_2, vitamin C and reducing sugar content levels were significantly higher than they were under ambient CO_2 (aCO_2) levels. The nitrate content of pak choi, cherry radish and pepper was reduced under eCO_2, and the effect was more pronounced among plants grown under Cd stress. The Cd content of cherry radish and pepper roots grown under eCO_2 and Cd stress was significantly reduced (29.2% and 18.5%, respectively) compared with plants grown under aCO_2 conditions and Cd stress. The Cd transfer coefficient of pak choi and water spinach grown under eCO_2 and Cd stress was significantly lower (22.7% and 25.2%, respectively) than under a CO_2 conditions. Our data suggest that growing vegetables under e $CO_2$is beneficial, especially when grown in Cd-contaminated soil, because Cd accumulation is reduced and vegetable quality is improved.

Keywords: elevated CO_2; abiotic stress; vegetables; abiotic accumulation; heavy metal contamination

Elevated CO_2 (eCO_2) is one of the main factors of climate change and affects the physiological responses of plants in many ways (Abdelhakim et al. 2022). Not only does eCO_2 affect the growth of vegetables but also their quality, including their nutrient content. For example, under eCO_2 condition, the nitrate content of cucumber (*Cucumis sativus* L.) (Dong et al. 2018) and celery (*Apium graveolens* L.) (Jin et al. 2009) has been shown to decrease significantly, whereas the nitrate content of tomato (*Solanum lycopersicum* L.) (Wei et al. 2018), lettuce leaves (*Lactuca sativa* var. *ramosa* Hort.) (Pérez-López et al. 2015) and celtuce (*Lactuca sativa* var. *angustata*) (Jin et al. 2009) has been shown to increase significantly. Elevated CO_2 also affects the accumulation of ascorbic acid (vitamin C) in carrots (*Daucus carota* var. *sativa* Hoffm.) (Wu et al. 2017) and increases the concentration of total soluble sugars in tomato, which enhances their quality (Zhang et al. 2014). Sugar accumulation and subsequently phenol synthesis is also promoted in lettuce leaves under eCO_2 levels (Becker, Kläring 2016).

Cadmium (Cd) accumulation in vegetables is an important environmental issue that threatens

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human health globally (Khan et al. 2016). Studies of, for example, mustard (*Brassica juncea* L. Czern.) (Gill et al. 2011), mung bean (*Vigna radiata* L.) R. Wilczek) (Anjum et al. 2014), common bean (*Phaseolus vulgaris* L.) (Rady et al. 2019) and tomato (Altaf et al. 2022) grown under Cd stress have shown that not only is their quality affected by the accumulation of Cd but also other quality indicators. For example, Kaya et al. (2020) showed that Cd stress enhanced the leaf Cd^{2+} content of peppers (*Capsicum annuum* L.) and decreased the activities of monodehydroascorbate reductase and dehydroascorbate reductase.

The effect of Cd on plants grown under eCO_2 has been analysed in numerous studies. For example, the adverse effects of Cd stress on rice (*Oryza sativa* L.) can be alleviated under eCO_2 conditions (Yang et al. 2023). Likewise, eCO_2 has been shown to mitigate the effects of Cd stress on vegetable growth and antioxidant systems (Wang et al. 2023). To increase our understanding of how the growth physiology and quality of different vegetables could be affected by Cd-contaminated soils and eCO_2 levels under future climate change conditions, we analysed four different vegetables grown under Cd stress and eCO_2: pak choi (*Brassica rapa* L., a leafy vegetable); water spinach (*Ipomoea aquatica* Forssk., a stem vegetable); cherry radish (*Raphanus raphanistrum* subsp. *sativus* L.) Domin, a root vegetable); and pepper (*Capsicum annuum* L., a fruit vegetable).

MATERIAL AND METHODS

Material. Plant materials and fertilizer applications used in this study are described in detail in Wang et al. (2023). In brief, the potting soil was a yellow-brown soil containing available nitrogen at 67.58 mg/kg, available phosphorus at 63.49 mg/kg, available potassium at 169.27 mg/kg and total Cd content at 0.196 mg/kg. We used four different vegetables: pak choi (a leafy vegetable), water spinach (a stem vegetable), cherry radish (a root vegetable) and pepper (a fruit vegetable).

Experimental design. The open-top gas chamber was used to simulate eCO_2 conditions. The ambient CO_2 gas chamber was ventilated with natural air, the eCO_2 gas chamber was ventilated with CO_2 gas to maintain the elevated CO_2 level. The four different vegetables were grown under either ambient CO_2 conditions (aCO_2, 350 ± 70 μmol/mol) or elevated CO_2 conditions (eCO_2, 700 ± 10 μmol/mol) in an open-top gas chamber, in soil that either had a background Cd level (Cd0) or that had been sprayed with an exogenous Cd solution (Cd3; 3.0 mg/kg $CdCl_2$-2.5 H_2O) on 24 March 2022 and that had then been homogenized for 2 months (i.e., 16 treatments in total). During the test period, the maximum and minimum temperatures were 31 °C and 7 °C. To ensure normal growth of vegetables, black shade net with 60% light transmission is used for shading. The edible part of each plant was sampled to determine the vitamin C content, the reducing sugar content and the nitrate content during the growing period (20 days after germination for pak choi, 25 days for water spinach, 35 days for cherry radish and 80 days for pepper). When the vegetables reached maturity, the Cd content of roots and shoots was measured.

Indicator measurement

Vitamin C content determination. Each fresh sample (1.0 g) was homogenized in a mortar with 20 mL of 1% oxalic acid. After filtering through gauze, the sample was centrifuged. 10 mL of the filtrate was sucked into a 50 mL triangular flask and titrated with 0.1% 2,6-dichlorophenol indophenol sodium solution until a clear pink colour appeared and did not disappear within 15 second. The volume of titrant used was recorded and the vitamin C content was calculated from the titrant volume.

Reducing sugar content determination. Each sample (1.0 g) was ground in a mortar in a small amount of distilled water, transferred to a test tube, homogenized, and then placed in a water bath at 80 °C for 30 minutes. After removing and cooling, the extract was filtered, and the filtrate was collected in a volumetric flask. The extract (2.0 mL) was placed in a test tube with 1.5-mL 3,5-dinitrosalicylic acid reagent. The absorbance at 540 nm was measured to calculate the reducing sugar content of the sample.

Nitrate content determination. Each sample (2 g) was placed in a graduated test tube with 10 mL of non-ionized water, and then placed in a boiling water bath. After 30 minutes, the extract was filtered into volumetric flasks, and the volume was made up to 25 mL. The extract (0.1 mL) was pipetted into a test tube, mixed well with 5% salicylic acid-sulfuric acid solution (0.4 mL) and then left at room temperature for 20 minutes. Next, 9.5 mL of 8% NaOH solution was added slowly. The absorbance at 410 nm was measured to calculate the nitrate content of the sample.

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Determination of Cd content. The Cd content of each sample was determined using a VARIAN AA240 graphite furnace atomic absorption spectrometer (Palo Alto, CA, USA). Determination method used in this study are described in detail in Wang et al. (2023).

Transfer coefficient. To determine the Cd transfer coefficient, the Cd concentration in plant tissues aboveground was divided by the Cd concentration in plant tissues belowground.

Data analysis and statistics. Data analyses and graphing were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Microsoft Office 2016 (Redmond, USA), respectively.

RESULTS

Effect of different treatments on the vitamin C content of different types of vegetables. Under aCO_2, the vitamin C content of the edible part of pak choi, water spinach, cherry radish and pepper grown in Cd3 soil was 0.32, 0.16, 0.30, 0.51 mg/kg, respective ly, which was significantly lower (31.9%, 30.4%, 37.5%, and 24.2%, respectively) than that of plants grown in Cd0 soil (Figure 1). Under eCO_2, the vitamin C content of the edible part of water spinach, cherry radish and pepper grown in Cd3 soil was significantly lower (36.4%, 29.0% and 16.4%, respectively) than that of plants grown in Cd0 soil.

Effect of different treatments on the reducing sugar content of different types of vegetables. Under a CO_2, the reducing sugar content of the edible part of pak choi, water spinach, cherry radish and pepper grown in Cd3 soil was significantly lower (20.4%, 22.5%, 28.2% and 39.6%, respectively) compared with that of plants grown in Cd0 soil (Figure 2). Under eCO_2, the reducing sugar content of pak choi, water spinach, cherry radish and pepper grown in Cd3 soil was significantly lower (24.1%, 12.7%, 21.9% and 33.0%, respectively) than that of plants grown in Cd0 soil treatment.

Effect of different treatments on the nitrate content of different types of vegetables. Under aCO_2, the nitrate content of pak choi, water spinach, cherry radish and pepper grown in Cd3 soil was 354.16, 106.27, 281.12, 211.30 μg/g, respectively. Under eCO_2, the nitrate content of cherry radish and pepper grown in Cd0 soil was significantly lower (18.1% and 16.3%, respectively) than that of plants grown under aCO_2 (Figure 3). Under eCO_2, the nitrate content of pak choi, water spin-

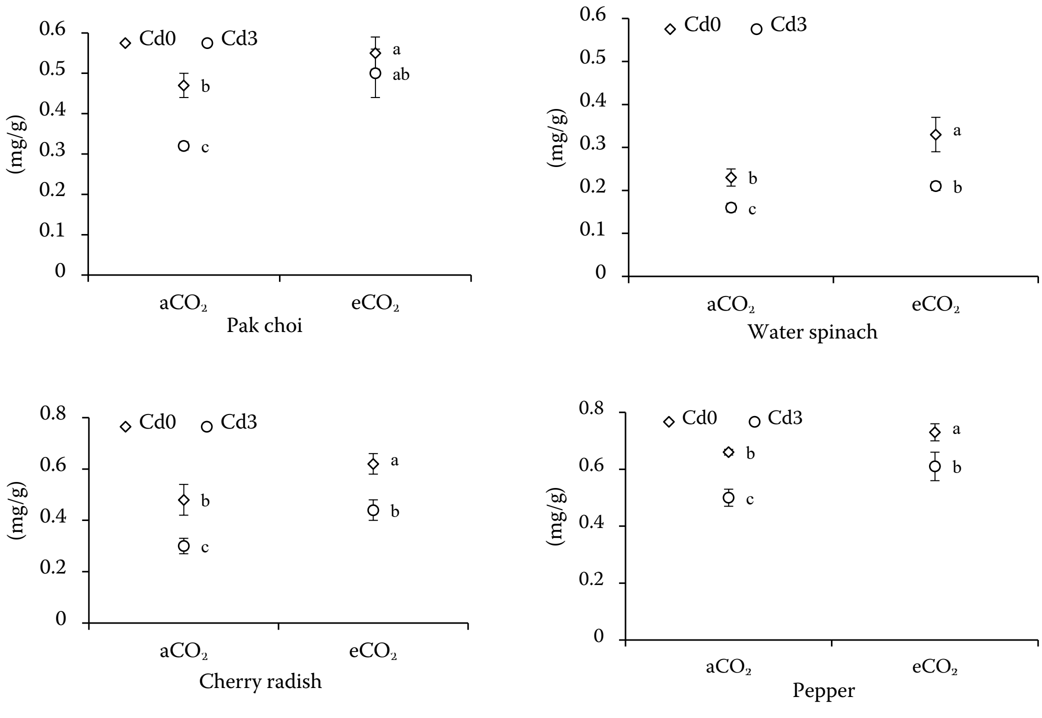

Figure 1. Effects of the different treatments on vitamin C content of different vegetables

Data points represent mean values (n = 5) ± SD; data points with different letters show significant differences (P < 0.05) according to Tukey's test

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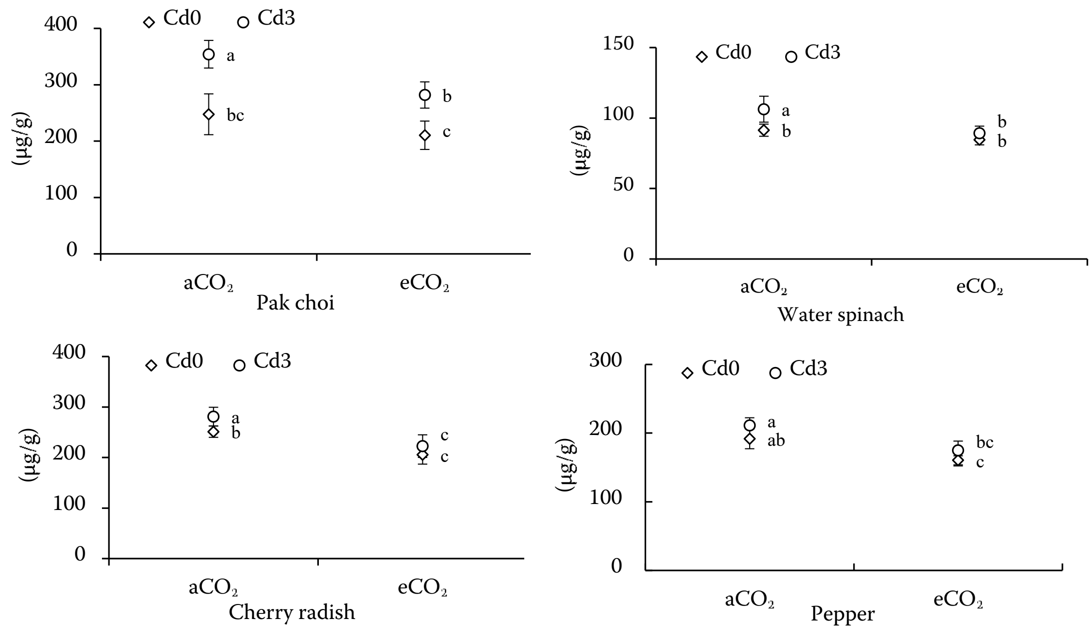

Figure 2. Effects of the different treatments on reducing sugar content of different vegetables

Data points represent mean values (n = 5) ± SD; Data points with different letters show significant differences (P < 0.05) according to Tukey's test

ach, cherry radish and pepper grown in Cd3 soil was significantly lower (20.4%, 16.1%, 20.8% and 17.4%, respectively) than that of plants grown under aCO_2.

Effect of different treatments on the cadmium accumulation of different types of vegetables. Under eCO_2, the Cd content of shoots of pak choi and water spinach grown in Cd0 or Cd3 soil

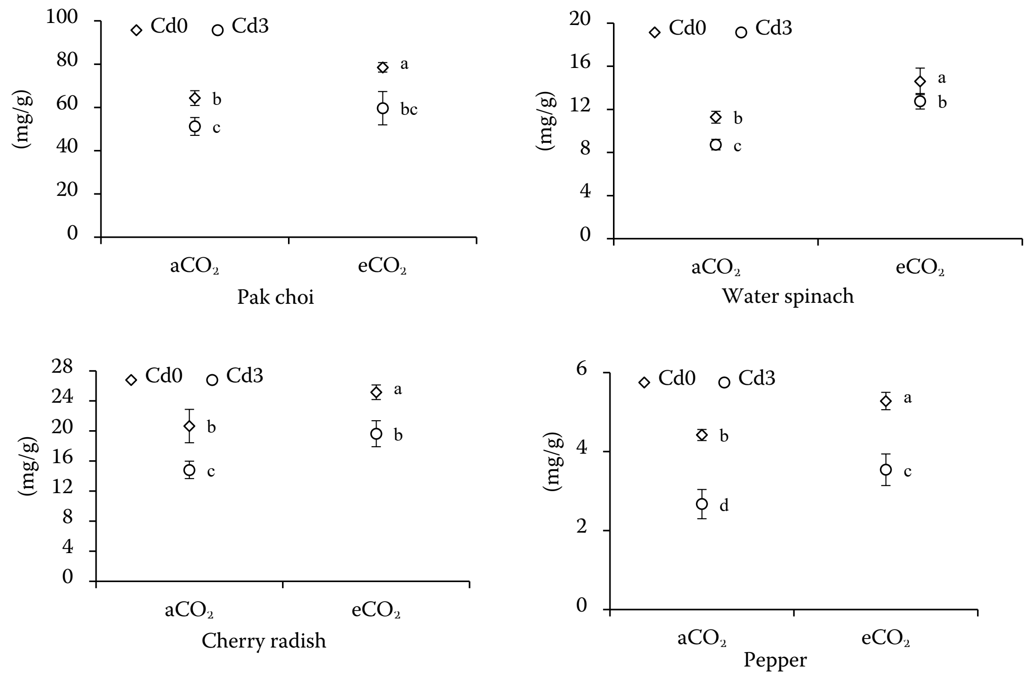

Figure 3. Effects of the different treatments on nitrate content of different vegetables

Data points represent mean values (n = 5) ± SD. Data points with different letters show significant differences (P < 0.05) according to Tukey's test was significantly lower (pak choi, 32.1% and 29.8%; water spinach, 36.3% and 37.5%, respectively) than that of plants grown under aCO_2 (Figure 4). Under eCO_2, the Cd content of roots of cherry radish and pepper grown in Cd0 soil was not significantly different to that of plants grown under aCO_2; however, the Cd content of roots of cherry radish and pepper plants grown in Cd3 soil and under eCO_2 was 0.17, 1.37mg/kg, respectively, which was significantly lower than that of plants grown under aCO_2.

Effect of different treatments on the cadmium transfer coefficient of different types of vegetables. Under eCO_2, the Cd transfer coefficient of pak choi, water spinach and cherry radish grown in Cd0 soil was significantly lower (16.1%, 28.7% and 12.0% respectively) than that of plants grown under aCO_2 (Figure 5). Under eCO_2, the Cd transfer coefficient of pak choi and water spinach grown in Cd3 soil was significantly lower (22.7% and 25.2%, respectively) than that of plants rown under aCO_2. The Cd transfer coefficients of pepper grown in Cd0 or Cd3 soil were not significantly affected by the CO_2 concentration.

Correlation analysis of vegetable quality and cadmium accumulation indicators. There was a highly significant positive correlation ($P < 0.01$) between the Cd content of roots and shoots (Table 1). There was a significant positive correlation ($P < 0.05$) between the Cd transfer coefficient and nitrate content. There was a highly significant negative correlation ($P < 0.01$) between the reducing sugar content of different vegetables and the root Cd content as well as significant positive correlations between reducing sugar content and nitrate content and vitamin C content ($P < 0.05$).

DISCUSSION

Vitamin C and reducing sugar content are two important nutritional quality indicators of vegetables. The content of reducing sugars reflects not only the quality but also the photosynthetic efficiency and growth of vegetables. Our analyses revealed significant increases in vitamin C and reducing sugar content in different vegetables under eCO_2 conditions, which is consistent with the findings of Pimenta et al. (2023). Transcriptional profiling of carrot genes revealed that eCO_2 can affect antioxidant (i.e., vitamin C) accumulation via a complex process involving the synthesis, recycling and degradation of vitamin C (Wu et al. 2017). A study by Becker and Kläring (2016) concluded that an increase in the content of soluble sugars enhances antioxidant synthesis

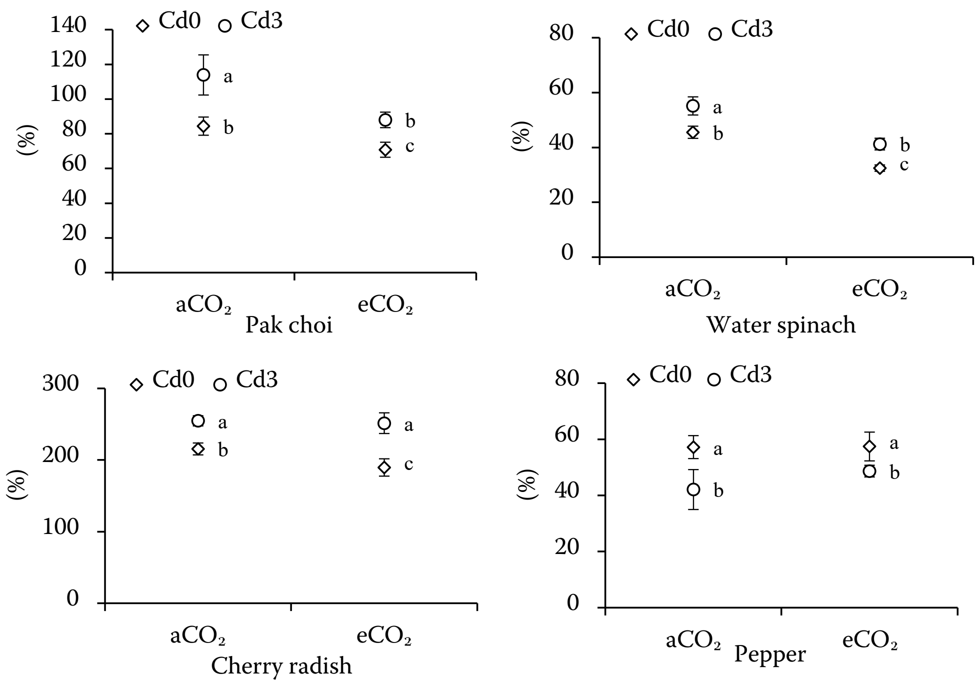

Figure 4. Effects of the different treatments on Cd content of different vegetables
Data points represent mean values (n = 5) ± SD. Data points with different letters show significant differences ($P < 0.05$) according to Tukey's test

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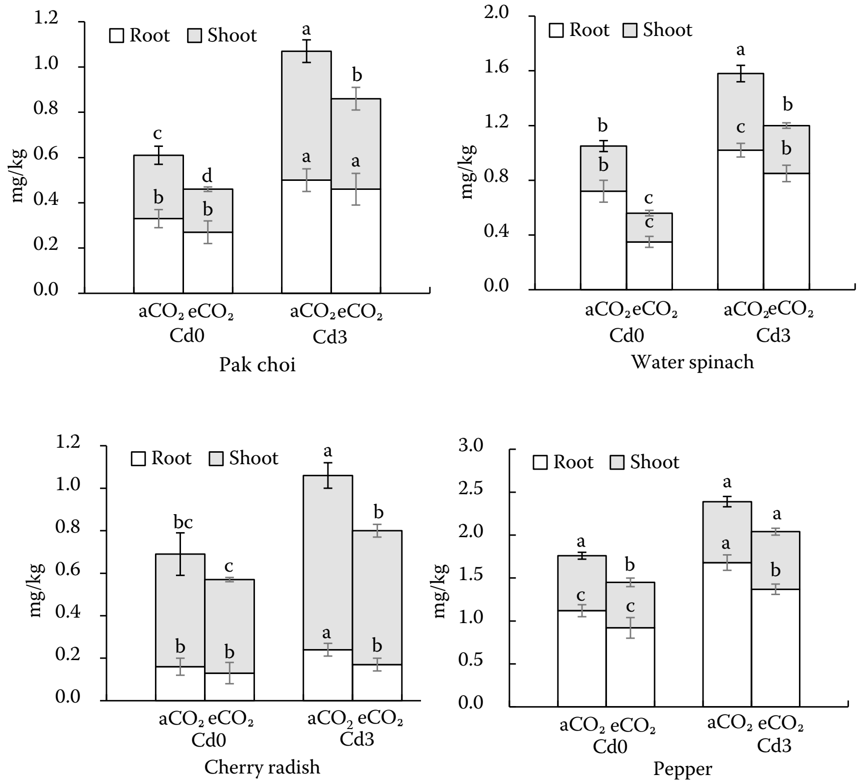

Figure 5. Effects of the different treatments on Cd transfer coefficient of different vegetables
Data points represent mean values (n = 5) ± SD; data points with different letters show significant differences (P < 0.05) according to Tukey's test

and accumulation. The eCO_2 increases the photosynthetic substrates of different vegetables to make more carbohydrates, which in turn promotes antioxidant synthesis. Therefore, our study found that elevated CO_2 concentration increased not only the reducing sugar content but also the VC content of different vegetables. Our analyses also showed a significant positive correlation between the reducing sugar content and vitamin C content of vegetables. Soil contaminated with Cd has a negative effect on vegetable growth and quality, including vitamin C and reducing sugar content (Hou et al. 2018). Our analyses showed that although the vitamin C and reducing sugar content of pak choi, water spinach, cherry radish and pepper was reduced when grown under Cd stress, this was reduced in magnitude when grown under eCO_2 conditions rather than under aCO_2. This indicates that the effect of Cd stress on the accumulation of vitamin C and reducing sugar in different vegetables could be mitigated to a certain extent by eCO_2. This may be because CO_2, as a raw material for carbon metabolism, can promote carbon metabolism and vitamin C synthesis and accumulation under eCO_2 concentrations. The findings are consis-

Table 1. Correlation analysis of different vegetable quality and cadmium accumulation indexes

	RCd	SCd	VC	RS	NO_3^-	TC
RCd	1	0.768**	0.083	–0.759**	–0.295	–0.354
SCd		1	0.141	–0.361	–0.017	–0.375
VC			1	0.555*	0.256	0.019
RS				1	0.524*	0.067
NO_3^-					1	0.543*
TC						1

Values represent the means ± the SE; n = 5; *P < 0.05; **P < 0.01; RCd – root Cd content; SCd – shoot; Cd content; VC – vitamin C content; RS – reducing sugar content; NO_3^- – nitrate content; TC – Cd transfer coefficient

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tent with those of Hu et al. (2020) who have previously shown that increased photosynthetic efficiency under eCO_2 concentrations can increase the vitamin C content of *Zea mays* L.

Nitrate accumulated in vegetables can be converted into nitrite, which is harmful to human health when ingested by humans (Colla et al. 2018). Our analyses showed that the nitrate content of the edible portion of different vegetables was lower in plants grown under eCO_2 than under aCO_2. There are two hypotheses that could explain the mechanism by which eCO_2 reduces the nitrate content of crops: one is that eCO_2 promotes plant growth and the increase in plant dry matter mass has a dilution effect on the nitrate content; the other is that CO_2 promotes nitrogen assimilation and metabolism due to the enhancement of photosynthesis, which results in more nitrate being converted to amino acids (Weerakoon et al. 1999). Vegetables grown under Cd stress accumulated more nitrate than those grown in Cd0 soil, which may be because Cd stress is detrimental to the growth of vegetables, which reduces their biomass and, hence, increases their nitrate concentration. The Cd transfer coefficient of pak choi and water spinach grown in Cd0 and Cd3 soil under eCO_2 was significantly lower than when grown under aCO_2. However, the Cd transfer coefficient showed a significant positive correlation with nitrate content. The co-accumulation of Cd and nitrate in vegetables may explain why vegetables grown under Cd stress conditions have a higher nitrate content than those grown in uncontaminated soil (Tang et al. 2019).

Our analyses showed that Cd stress increased Cd accumulation in the roots and / or shoots of the four different vegetables, and that eCO_2 reduced Cd accumulation in different parts of pak choi and water spinach. Although eCO_2 had no significant effect on the Cd content of cherry radish or pepper roots grown in Cd0 soil, the Cd content of plants grown in Cd3 soil was significantly lower than that of those grown under aCO_2. Furthermore, the Cd transfer coefficients of peppers grown in Cd0 or Cd3 soil were not significantly affected by eCO_2, suggesting that the effects of eCO_2 on plant Cd accumulation varied according to vegetable species. The study by Blanco et al. (2023) concluded that no regular patterns were observed in relation to climatic influence on heavy metal uptake. Feng et al. (2023) have shown that eCO_2 increases the resistance of plants to Cd stress. By contrast, a study involving *Sedum alfredii* showed that the total Cd uptake per plant pot under eCO_2 was significantly higher than that under aCO_2, and that the plant Cd extraction efficiency was significantly higher under eCO_2 after 60 days of growth (Li et al. 2012). Lower copper concentrations and higher Cd concentrations have been reported in shoots and grains of both rice and wheat grown under eCO_2 levels in soils contaminated with either copper or Cd (Guo et al. 2011). The uptake of Cd by different vegetables under eCO_2 may vary because eCO_2 affects the exudation of low molecular weight organic compounds by the plant root system, which have different binding strengths to different heavy metals (Andrews et al. 2001), and the release of heavy metals from the soil (Cheng et al. 2010). A study by Yang et al. (2023) showed that eCO_2 reduced Cd uptake by plant roots because under eCO_2 conditions, more low-crystalline Fe oxides in iron plaques were deposited on the surface of the root system along with an increase in dissolved Fe2+ due to the lower redox state of the soil. Overall, our data suggest that growing vegetables under eCO_2 is beneficial, particularly when vegetables are grown in Cd-contaminated soil, because Cd accumulation is reduced and vegetable quality is improved.

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