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Combined effects of elevated temperature and CO₂ concentration on Cd and Zn accumulation dynamics in *Triticum aestivum* L.

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ABSTRACT

A simulated climate warming experiment was conducted to evaluate the combined effects of elevated temperature and CO₂ concentration on the bioaccumulation, translocation and subcellular distributions of Cd and Zn in wheat seedlings (*Triticum aestivum* L. cv. Xihan 1.) at Dingxi, Gansu Province, China. The objective was to find evidence that global climate change is affecting the bioaccumulation of Cd and Zn in *T. aestivum* L. cv. Xihan 1. The results showed that compared to control A, elevated temperature and CO₂ increased Cd bioaccumulation in the shoots by 1.4–2.5 times, and increased that in the roots by 1.2–1.5 times, but decreased Zn levels in wheat shoots by 1.4–2.0 times, while decreased that in the roots by 1.6–1.9 times. Moreover, temperature and CO₂ concentration increase also led to increased Cd concentration, and decreased Zn concentration in subcellular compartments of wheat seedlings. The largest Cd concentration increase (174.4%) was observed in the cell wall and debris fractions of shoots after they were subjected to the highest CO₂ and temperature treatment (TC3). The largest Zn concentration decrease (53.1%) was observed in the soluble (F3) fractions of shoots after they were subjected to the medium CO₂ and temperature treatment (TC2). The temperature and CO₂ increase had no significant effect on the proportional distribution of Cd and Zn in the subcellular fractions. The root-to-shoot translocation of Cd increased with the increasing temperature and CO₂ concentration. However, the Zn distributions only fluctuated within a small range.

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Introduction

Global warming is regarded by many as an indisputable fact (IPCC, 2007, 2013). According to the IPCC AR5 scenario, at the end of the 21st century, global surface temperature change is likely to exceed 1.5–2°C to that in 1850 and 1900, and the CO₂

concentration is likely to reach 421 ppm, or even 936 ppm, by the year 2100 (IPCC, 2013). Multi-model results showed that the cumulative total emissions of CO₂ and the global mean surface temperature response are approximately linearly related (IPCC, 2013). In China, annual mean surface air temperature will rise by 2.7–2.9°C and the CO₂ concentration

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annual increase will be more than 5%–7% under the 2°C global warming scenario (Jiang and Fu, 2012; Cheng et al., 2013). Climate warming is expected to have direct effects on terrestrial ecosystems and associated ecosystem services through its effects on productivity, biodiversity and biogeochemistry (Rustad, 2008; Supit et al., 2012; Ding and Nunes, 2014). Moreover, agro-ecological systems, as major components of terrestrial ecosystems and the key provider of ecosystem services, are sensitive and vulnerable to climate warming (Lorencová et al., 2013). The effects of climate warming on agro-ecological systems have received increasing attention over the past few decades due to concerns about human subsistence security and sustainable development. Scientists believe that climate warming already has, and will continue to have a profound impact on agro-ecological systems in many ways (Srivastava et al., 2012; Meki et al., 2013). Temperature and atmospheric CO₂ concentration are two most important environmental variables that regulate physiological and phenological processes in plants (Kim et al., 2007). Thus, they are central to understand how climate warming influences agro-ecological systems. In general, if ambient temperature is below the optimum temperature for crops, warming is likely to have a positive impact on the growth and yield of crops. In contrast, if ambient temperatures are above the optimum temperature for crop growth, warming is likely to accelerate the phenological processes (Hu et al., 2005; Xu et al., 2008) and then to cause yield reductions of crops (Porter and Gawith, 1999). However, elevated CO₂ also positively influences the growth and yield of crops due to its carbon fertilization effect (Högy et al., 2010). Over the past several decades, studies on the separate effects of elevated CO₂ concentration and temperature rise on crop growth and yield have produced a wealth of valuable information. These studies have indicated that climate warming has begun to affect the agriculture ecological system (Tubiello et al., 2007). However, these studies did not concentrate on the effects that climate warming will have on trace element dynamic bioaccumulation and redistribution in crops, and little is known about the role of trace elements in this process, especially the combined effects of elevated temperature and CO₂ concentration on the dynamic bioaccumulation and redistribution of trace elements in crops (Tubiello et al., 2007; Li et al., 2014).

In recent decades, the contamination of agricultural soils with toxic heavy metals has become a serious problem throughout the world. At the same time, we are also faced with the problem of micronutrient malnutrition, so-called hidden hunger (Wang et al., 2011). Nutrient element deficiencies and toxic element stress in crops are still common problems in most agricultural land around the world (Lynch, 1995). To date, humans have not been able to effectively analyze and solve the problem. Moreover, the problems become more complex and urgent in all the global warming scenarios, and will affect ecological systems, agro-ecological systems and global food supply security on a larger scale than at present. Recently, scientists have begun to focus on the influences of climate change on the utilization of mineral elements, mineral stress and nutrient uptake in plants. Several available studies that suggest elevated temperature or CO₂ concentration can significantly influence the uptake, distribution and utilization

of trace elements in crops. Li et al. (2011b) observed that rising temperatures decreased the Cd and Cu trace element concentration in spring wheat grain, but increased the Fe and Zn concentration. A simulation experiment showed that warming affected the Cd, Pb, Fe, Zn and Cu distribution dynamics in *Solanum tuberosum* L. plants, and their soil dynamics and availability. A rise in temperature also increased Cu, Zn and Fe leaf concentrations, but decreased Cd, Pb, Fe, Zn and Cu tuber concentrations (Li et al., 2011a). CO₂ enrichment produced a decrease in macro and microelement concentrations in wheat, and this effect was greater in straw than in the grain (Manderscheid et al., 1995). Elevated CO₂ levels also led to lower Cu concentrations and higher Cd concentrations in the shoots and grain of both rice and wheat (Guo et al., 2011), and led to decrease the concentrations of N, K, S, Ca, Mg and Zn in nearly all of the wheat shoot tissues (Fangmeier et al., 1997).

The above findings suggested that the effects of elevated CO₂ concentration or temperature on the bioaccumulation and distribution of trace elements in crops were often not additive, which implied that the combined effects of these changes could not be predicted from knowledge of their individual effects. Furthermore, it seemed that the magnitude and direction of these effects were species- and cultivar-dependent, and element type dependent. This means that climate warming could have a profound impact on trace element stress in crops with complex mechanisms and processes. However, we still know very little about the combined effects of elevated CO₂ concentration and temperature on crops, and its mechanism and process. In addition, most of the previous studies were conducted in cultivated soil, which might introduce some uncertain factors into the experiment process and the result analysis. These previous results indicated that warming could affect soil organic matter, the soil pH and the activity of soil enzymes, and thus affected the availability of trace elements in the soil and plants (Sardans et al., 2008a; Li et al., 2011a). However, it is not clear whether these changes are the direct results of climate warming effect on plants themselves or indirect effects by changing the physicochemical and biological properties of the soil.

Our previous soil studies had explored the effects of warmer temperatures on the dynamics of trace element accumulation in some crop plants and gained some meaningful finds at the same study site (Li et al., 2011a, 2012b). Based on these finds and other previous data, we hypothesized that elevated temperature and CO₂ concentration would increase the accumulation of Cd and Zn and change the allocation of Cd and Zn in different plant organs, as a consequence of the observed increase in the enhancement of plant physiological activity and growth. To test these hypotheses a non-soil culture experiment was conducted in an artificial climate greenhouse to simulate the warming scenarios in the coming decades forecasted by multi-model (IPCC, 2001, 2007; Ding et al., 2006). The aim was to explore the combined effects of elevated temperature and CO₂ concentration on the accumulation, translocation and subcellular distribution of trace elements in the shoots and roots of wheat seedlings, and to fully appreciate the real impact of climate warming on trace element stress in crops, and to elucidate the possible mechanisms behind these changes. The results of this study could be used to predict crop responses to trace element stresses in future climate scenarios and the

influences of climate change on the ecological environment and food security over the coming decades.

1. Materials and methods

1.1. Study site

The study was carried out in the Dingxi Experimental Station, Institute of Arid Meteorology, China Meteorological Administration (CMA) on a west-facing in Dingxi City (35°35'N, 104°37'E), Gansu Province, China. The climate is characterized by cold and dry winters, and warm and wet summers. The annual average temperature is 7.7°C, and the average daily temperatures in September and October are 15.3 and 8.3°C, respectively. Major food crops and soil features were described by Li et al. (2011a).

1.2. Experimental design

The simulated warming experiment was carried out in the artificial climate greenhouse and the nature field near the greenhouse, at the Dingxi Experimental Station of Institute of Arid Meteorology, China (CMA). The artificial climate greenhouse was arch-ended, had an indoor area of 594 m² (28.8 m long, 20 m wide and 4.85 m high), and was divided into three independent rooms. The temperature and CO₂ treatments and the climate variable measurements were described in details by Li et al. (2011a, 2012b). Briefly, the artificial climate greenhouse was fitted with an intelligent system that controlled heating, force cooling, natural ventilation, solar shading and CO₂ levels. This system continuously provided automated controlling and monitoring of any climatic factors in the greenhouse, and regulated the temperature to 0–7°C lower than the outdoor temperature, or could increase the temperature to 0–3.5°C more than the outdoor temperature. The indoor CO₂ concentrations could be adjusted to 0–1000 µmol/mol more than the outdoor levels. In the past 7 years, the studies conducted in the greenhouse had shown that the performance parameters of the greenhouse had met the design requirements very well and have ensured operational stability of the climatic factors.

The treatments, TC1, TC2 and TC3 were carried out in the three independent rooms of the artificial climate greenhouse, respectively. The control experiments were conducted in the natural field near the artificial climate greenhouse. The simulated experiment consisted of four experimental plots: (1) an ambient temperature and CO₂ plot (control); (2) a plot with a 1°C temperature increase and 50 ppm CO₂ concentration increase (TC1); (3) a plot with a 2°C temperature increase

and 100 ppm CO₂ concentration increase (TC2); (4) a plot with a 3°C temperature increase and 150 ppm CO₂ concentration increase (TC3) (Table 1). In the experiment design, the treatments, TC1, TC2 and TC3 represented respectively, low, medium and high elevated temperature and CO₂ concentrations, compared to the control (A). The trace elements, Cd and Zn, were selected as experiment factors. The Cd (CdCl₂) concentration used in the experiments was 10 µg/L, which was the level used in some other studies (Florijn and Beusichem, 1993; Wan et al., 2003). The Zn (Zn(SO₄)₂) concentration used in the experiments was also 10 µg/L. The wheat seeds were sown on 15 September, 2013.

1.3. Plant culture

Wheat seeds from *Triticum aestivum* L cv. Xihan 1 were disinfected in 30% H₂O₂. They were germinated on moist quartz sands and then uniform seedlings were transferred 5 days after germination to plastic flat-bottomed pots containing 2.5 L Hoagland's nutrient solution. The pH was adjusted to 6.0. There were eight seedlings per pot. Each experimental plot was put into 6 uniform pots. After 3 days, the six pots were randomly divided into two groups, which were labeled A and B respectively. Each group had three pots, which represented three replications. The three replications of group A were labeled A₁, A₂ and A₃, respectively. The three replications of group B were labeled B₁, B₂ and B₃, respectively. Then, the CdCl₂ was added to the nutrient solution in the three replications of group A (A₁, A₂ and A₃) to make a final concentration of 10 µg/g, respectively. The Zn(SO₄)₂ was added to the nutrient solution in the three replications of group B (B₁, B₂ and B₃) to also make a final concentration of 10 µg/g, respectively. The nutrient solutions were replaced every 3 days. EDTA in the nutrient solution affects the absorption of Cd in wheat, so the Fe-EDTA in the nutrient solution was replaced with FeSO₄ (FeSO₄·7H₂O) when the CdCl₂ was supplied (Wolterbeek et al., 1988). After 27 days, all the wheat seedlings were harvested and washed for 10 min with deionized H₂O and placed in Na₂-EDTA solution (pH 6.0, 10 mmol/L) for 30 min. Then they were again washed several times with deionized H₂O and blotted between paper towels. The roots and shoots of the wheat seedlings were separated (the stem had not yet formed) and stored in a freezer at –70°C until needed.

1.4. Separation of subcellular fractions and chemical analyses

Five grams each of fresh roots and shoots were separately homogenized using a mortar and pestle in 5 mL of a medium containing 0.25 mol/L sucrose, 50 mmol/L Tris–HCl (pH 7.5)

Table 1 – Details about the three simulated climate treatments (TC1, TC2, and TC3) and the untreated control condition (A) for wheat seedlings.

A		TC1		TC2		TC3	
Elevated temp. (°C)	Elevated CO ₂ (ppm)	Elevated temp. (°C)	Elevated CO ₂ (ppm)	Elevated temp. (°C)	Elevated CO ₂ (ppm)	Elevated temp. (°C)	Elevated CO ₂ (ppm)
0	0	1	50	2	100	3	150

and 1 mmol/L dithio-erythritol ($C_4H_{10}O_2S_2$). All the steps were performed at 4°C. Following Hans and Hans (1980), the homogenates were separated by using differential centrifugation into a cell wall and debris fraction (F1), an organelle fraction (F2) and a soluble fraction (F3). Chemical analyses of the Cd and Zn in each fraction were conducted at the Instrument Analysis Research Center, Lanzhou University, as described by Li et al. (2011a).

1.5. Statistics and calculations

SPSS 16.0 for Windows (SPSS Inc., USA) was used for the statistical analyses. One-way analysis of variance (ANOVA) with Bonferroni/Dunn's post-hoc test ($p < 0.05$) was performed to identify statistical differences between individual treatments. Origin 9.0 (OriginLab Corporation, USA) was used to generate the graphs.

The bioaccumulation factor (BF) was used to evaluate metal accumulation effectiveness in the plants and was calculated as follows:

$$BF = \frac{C_{shoot}}{C_{medium}}$$

where, C_{shoot} ($\mu\text{g/g}$ FW) is the selected trace element concentration (Cd, Zn) in shoots, and C_{medium} ($\mu\text{g/g}$) is the total selected trace element concentration (Cd, Zn) in the nutrient solution. The translocation factor (TF) was used to evaluate the transport capacity of crops to translocate selected trace elements (Cd, Zn) from root to shoot, and was calculated as follows:

$$TF = \frac{C_{shoot}}{C_{root}}$$

where, C_{shoot} ($\mu\text{g/g}$ FW) and C_{root} ($\mu\text{g/g}$ FW) were the selected trace element (Cd, Zn) concentrations in shoots and roots (Qiu et al., 2011).

2. Results

2.1. Cd and Zn bioaccumulation factor (BF) values for the shoots and roots

The combined elevated temperature and CO_2 concentration treatments had striking effects on Cd accumulation in *T. aestivum* shoots and roots. The BF values of Cd in the shoots and roots significantly rose with increasing temperature and CO_2 concentration (Fig. 1, $p < 0.05$), except for the highest elevated temperature and CO_2 concentration treatment (TC3). It seemed that a very high temperature and CO_2 concentration tended to slow down the increase in Cd accumulation. The BF values of Cd in the shoots of plants subjected to the TC1, TC2 and TC3 treatments increased 1.4 times, 2.4 times and 2.5 times, respectively, compared to the control (A). The BF values of Cd in the roots increased 1.2 times, 1.5 times and 1.4 times, respectively, compared to the control. It was obvious that the effects of elevated temperature and CO_2 concentrations on the accumulation of Cd in the wheat shoots were stronger than that in the roots. However, under the same treatment

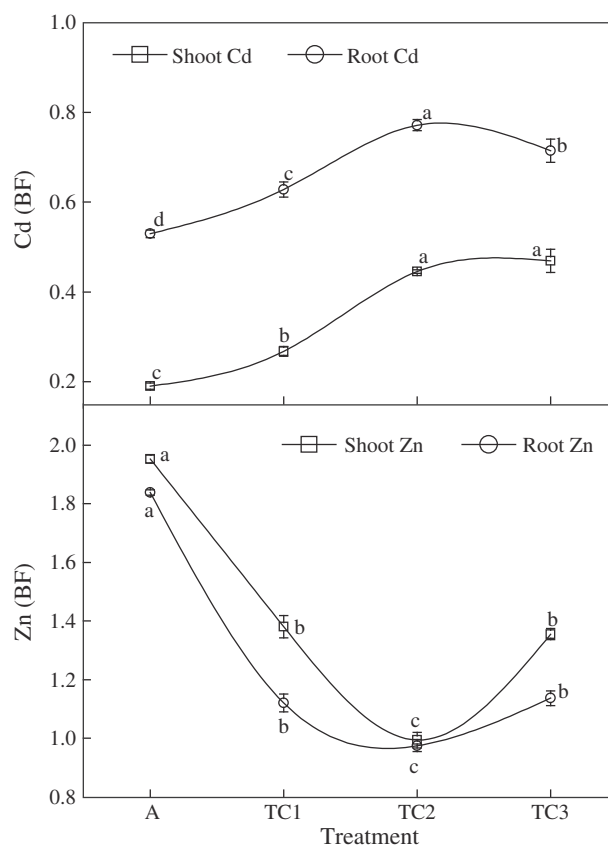


Fig. 1 – The combined effects of elevated temperature and CO_2 concentrations on the BF values for Cd and Zn in wheat shoots and roots. Different letters indicate significant statistical differences between treatments ($p < 0.05$) according to the Bonferroni–Dunn test. BF: bioaccumulation factor.

conditions, the root BF values of Cd were 2.8 times, 2.3 times, 1.7 times and 1.5 times more than that of the shoots, respectively, which means Cd mainly accumulated in the roots of wheat seedlings.

The BF values of Zn in the shoots and roots significantly decreased in all treatments with the rising of temperature and CO_2 concentrations ($p < 0.05$, Fig. 1), except for the highest elevated temperature and CO_2 concentration treatments (TC3). It seemed that a high temperature and CO_2 concentration (TC3) tended to slow down the decrease in Zn accumulation in both the shoots and roots, and even led to a slight increase compared to TC2. The BF values of Zn in the shoots were significantly different to the control and were 1.4 times, 2.0 times and 1.4 times lower in TC1, TC2 and TC3, than the control respectively. The BF values of Zn in the roots were 1.6 times, 1.9 times and 1.6 times lower, than the control, respectively. It was clear that the effects of elevated temperature and CO_2 concentration on the accumulation of Zn in the shoots were very similar to the roots. Under the same treatment conditions, the root BF values of Zn were 1.1 times, 1.2 times, 1.0 times and 1.2 times less than that in the shoots for the control, TC1, TC2 and TC3, respectively, which meant that Zn accumulation in the shoots was similar to that in the roots.

2.2. Cd and Zn in subcellular compartments

The elevated temperature and CO₂ concentration treatments significantly affected the Cd and Zn subcellular compartment concentrations, and there were statistical differences between most treatments ($p < 0.05$, Fig. 2). The Cd concentrations in shoot subcellular compartments rose with temperature and CO₂ increasing. The TC1, TC2 and TC3 treatments increased the Cd concentrations in F1 by 43.1%, 143.5% and 174.4%, in F2 by 39.9%, 94.4% and 97.0%, and in F3 by 41.1%, 149.7% and 155.7%, respectively, compared to the control. The treatments increased the root Cd concentrations in F1 by 34.5%, 61.4% and 39.9%, in F2 by 19.0%, 57.5% and 38.4%, and in F3 by 13.9%, 38.1% and 32.6%, respectively, compared to the control. This suggested that the Cd concentrations in the subcellular compartments of roots increased rapidly with increasing temperature and CO₂ concentration under the TC1 and TC2 treatments, but TC3 tended to slow down the increase rate. The soluble fraction (F3) represented the main Cd enrichment sites in wheat seedlings. In the shoots the Cd in F3 ranged from 47.4% to 50.5% and in the roots Cd in F3 ranged from 60.6% to 63.9%.

The Zn concentrations in the shoot and root subcellular compartments decreased in TC1 and TC2 (Fig. 2), but the decrease rate tended to slow down in the TC3 treatment and some Zn concentration even increased compared to TC1 and TC2. In the shoots, TC1, TC2 and TC3 decreased the Zn concentrations in F1 by 30.2%, 52.4% and 44.1%, in F2 by 24.9%, 36.1% and 26.4%, and in F3 by 30.7%, 53.1% and 23.4%, respectively, compared to the control. In the roots, the

treatments decreased the Zn concentrations in F1 by 29.3%, 45.2% and 37.9%, respectively, in F2 by 40.2%, 45.8% and 29.4%, respectively, and in F3 by 44.1%, 48.6% and 42.7%, respectively, compared to the control. The soluble fraction (F3) represented the main Zn enrichment sites in wheat seedlings. In the shoots the Zn in F3 ranged from 42.8% to 51.2% and in the roots Zn in F3 ranged from 44.1% to 48.1%.

2.3. Proportional distribution of Cd and Zn in the subcellular fractions

Elevated temperature and CO₂ concentration had few effects on the Cd proportional distribution between the subcellular fractions in the shoots or roots (Fig. 3). The Cd proportions in the shoots F1 were 29.4%, 29.8%, 30.5% and 32.6% in the control, TC1, TC2 and TC3 treatments, respectively, 23.0%, 22.8%, 19.1% and 18.3% in F2, and 47.5%, 47.4%, 50.5% and 49.1% in F3. The Cd proportions in roots F1 were 18.7%, 21.2%, 20.7% and 19.3% in the control, TC1, TC2 and TC3 treatments, respectively, 17.4%, 17.4%, 18.8% and 17.8% in F2, and 63.9%, 61.4%, 60.6% and 62.8% in F3. Statistically significant differences were only observed between the control and TC2, and the control and TC3 in shoot F2. The Cd proportion order in the subcellular fractions was F3 > F1 > F2.

The Zn proportional distribution in both the shoot and root subcellular fractions showed no consistent trends (Fig. 3). The Zn proportions in shoots F1 were 31.5%, 31%, 29.4% and 25.3% in the control, TC1, TC2 and TC3, respectively, 22.1%, 23.5%, 27.8% and 23.5% in F2, and 46.4%, 45.5%, 42.8% and 51.2% in F3. The proportions of Zn in roots F1 were 27.9%, 32.3%, 28.8% and

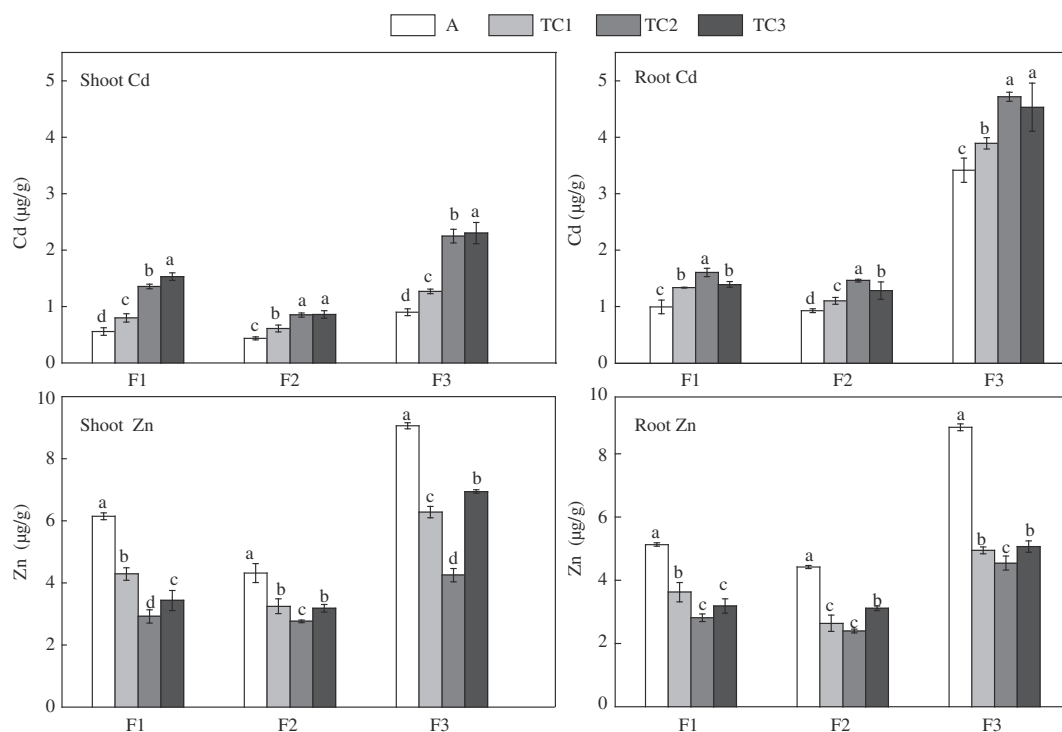


Fig. 2 – The combined effects of elevated temperature and CO₂ concentrations on the subcellular distribution of Cd and Zn in wheat shoots and roots. Different letters indicate significant statistical differences between treatments ($p < 0.05$) according to the Bonferroni-Dunn test.

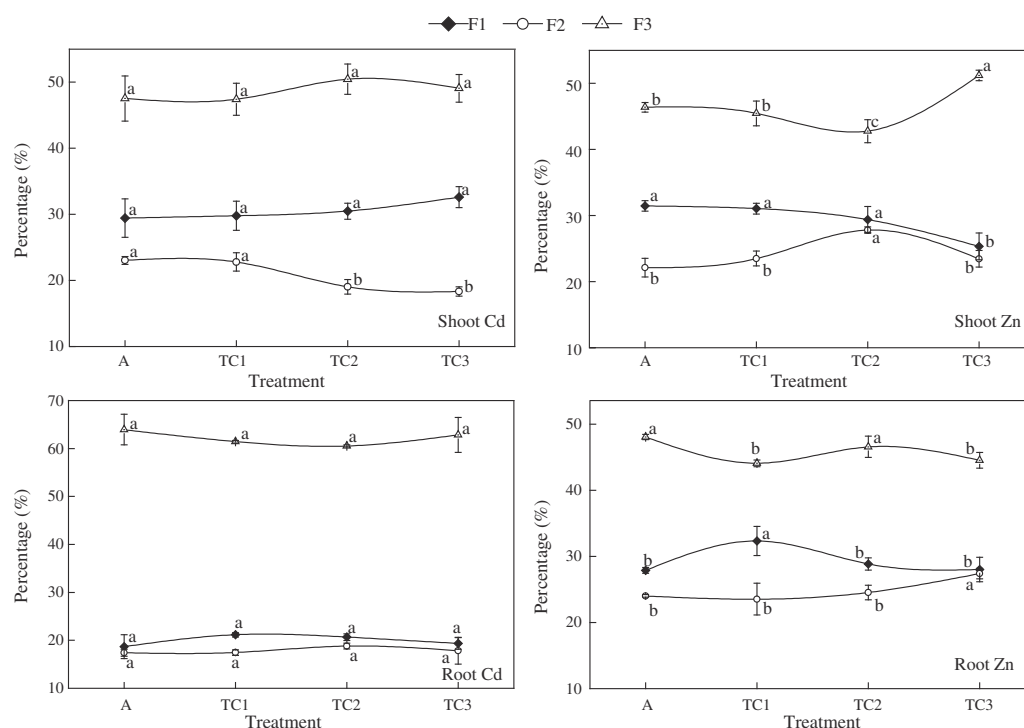


Fig. 3 – The combined effects of elevated temperature and CO₂ concentrations on the proportional distribution of Cd and Zn in the subcellular fractions of wheat roots and shoots. Different letters indicate significant statistical differences between treatments ($p < 0.05$) according to the Bonferroni–Dunn test.

28.0% in the control, TC1, TC2 and TC3, respectively, 24.0%, 23.6%, 24.5% and 27.4%, in F2, and 48.1%, 44.1%, 46.6% and 44.6% in F3. Generally, elevated temperature and CO₂ concentration led to slight changes in the proportional distribution of Zn in the subcellular fractions. The Zn proportional distribution order in the subcellular fractions was F3 > F1 > F2, which was the same as Cd.

2.4. Translocation factor values for Cd and Zn transport from the roots to the shoots

The translocation factors (TF) for Cd and Zn from roots to shoots are shown in Fig. 4. The TF values of Cd significantly rose with temperature and CO₂ concentration increasing ($p < 0.05$, Fig. 4). The TF values of Cd increased by 1.2 times, 1.6 times and 1.8 times in TC1, TC2 and TC3, respectively compared to the control A. However, the fluctuation in TF values for Zn was just 1.02–1.23.

3. Discussion

3.1. Combined effects of elevated temperature and CO₂ concentration on the bioaccumulation factor (BF) values for Cd and Zn in shoots and roots

Crops respond directly to climate warming through a series of complex physiological and biochemical responses, and these are accompanied by a variety of physical and chemical

changes (Vu et al., 2001; Kim et al., 2007; Lee, 2011). These responses and changes can considerably influence and regulate the absorption and distribution of nutritional and non-nutritional elements in crops (DaMatta et al., 2010; Li et al., 2011a, 2011b). Cd is a non-essential element for plants

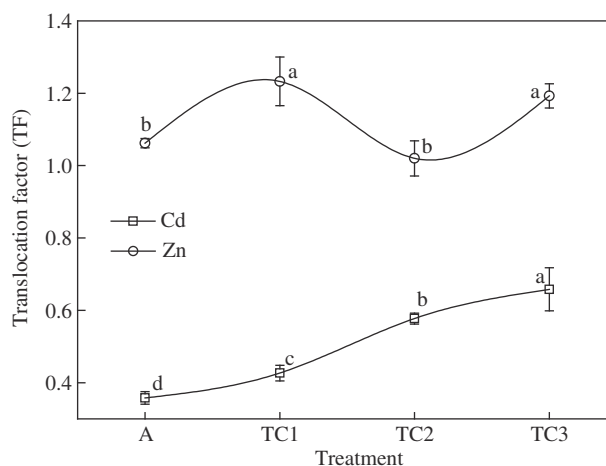


Fig. 4 – The combined effects of elevated temperature and CO₂ concentrations on the translocation factor (TF) values for Cd and Zn translocation from wheat roots to shoots. Different letters indicate significant statistical differences between treatments ($p < 0.05$) according to the Bonferroni–Dunn test.

and can be toxic to plants, animals and humans. It is readily absorbed by plant roots and is actively transported to the shoots where it exerts its toxic effects (Gallego et al., 2012; Xu et al., 2012). Absorption by the roots is considered a key process in overall plant Cd accumulation (Uraguchi et al., 2009). In this study, the BF values of Cd in wheat roots rose with the temperature and CO₂ concentration increasing (Fig. 1). This may be caused by a variety of reasons. (1) Higher temperature can improve both metabolism and protein synthesis, and this may result in an increase in the number of active sites on the root surface or an increased release of molecules that facilitate metal uptake, thereby increase metal uptake (Gonzalez Davila et al., 1995; Nilsen and Orcutt, 1996; Li et al., 2011a); (2) higher temperatures and the toxic effects of Cd itself can increase the fluidity of the plasma membrane (Uemura et al., 2006; Markovska et al., 2009), which may facilitate both passive and active metal flux through the membrane (Fritioff et al., 2005); (3) elevated temperatures and CO₂ concentrations may improve resource capture by the root through the enhancement of photosynthesis, transpiration and plant growth (Sardans et al., 2008b; DaMatta et al., 2010). In C₃ plants, enhanced growth and photosynthesis are generally observed in response to elevated CO₂ concentrations. Moreover, the optimum temperature for photosynthesis rises 5–10°C as the CO₂ concentration increases (Conroy et al., 1994; Drake et al., 1997; Kim et al., 2007). However, plant responses to elevated temperature are relatively complex. In general, plant photosynthesis and growth is enhanced by increasing temperature when the treatment temperature is below the optimum temperature, but they are inhibited when the treatment temperature is higher than the optimum temperature. In this study, the highest treatment temperature (24°C) was not yet over the optimal temperature for wheat (about 30°C) (Reddy et al., 1995; Fang et al., 2010). Therefore, we suggest that the combined effects of elevated temperature and CO₂ concentration will further enhance photosynthesis and plant growth, which will lead to an increase in Cd uptake. However, it is difficult to ascertain the interactive effects of elevated temperature and CO₂ concentration on plant transpiration in this study because of the opposite effects of elevated temperature and CO₂ concentrations alone on transpiration (Reddy et al., 1995). (4) If the root metal uptake rate exceeds their growth rate, then this may induce metal accumulation. As mentioned above, elevated temperatures and CO₂ concentrations may enhance plant growth, but higher temperatures can also increase root sensitivity to Cd and enhance Cd toxicity, which leads to root growth inhibition (Li et al., 2011a, 2011b). This may offset the positive impacts of elevated temperatures and CO₂ concentrations on growth, and this may mean that the root metal uptake rate exceeds their growth rate. (5) Elevated temperature and CO₂ may increase Cd uptake by increasing enzyme activity and protein synthesis. It is well known that a considerable number of proteins and enzymes, such as ZIP transporters, Nramp family proteins and Ca²⁺ transporters, are involved in Cd²⁺ uptake processes in the roots (Clemens, 2006; Kramer et al., 2007). Within a certain range, elevated temperatures and CO₂ concentrations can increase enzyme activity and protein synthesis (Nilsen and Orcutt, 1996; Högy et al., 2009), which results in higher metal uptake. (6) Thermodynamic factors must be taken into account. Studies have indicated that Cd²⁺ sorption by *Spirulina platensis* was an

endothermic process, and higher temperatures led to greater sorption (Seker et al., 2008). The TC3 treatment tended to slow down the increase of Cd accumulation in the roots, which was most likely due to the increase in Cd translocation from the roots to the shoots observed in this study (Fig. 4). In addition, higher temperatures and CO₂ concentrations can accelerate plant growth and may accelerate root growth, which can dilute tissue Cd concentrations to a certain extent.

In this study, the BF values of Cd in the shoots increased when the temperature and CO₂ concentrations were elevated, and the shape of the fitted curves were similar to those for the roots (Fig. 1). This suggested that the shoot Cd concentrations were strongly correlated with the root Cd concentrations. Earlier investigations suggested that shoot Cd concentrations were determined largely by Cd entry into the root, sequestration within root vacuoles and translocation into the xylem and phloem (Lux et al., 2011). Xylem loading is an energy-dependent active process mediated by P-type ATPase AtHMA4 (Mori et al., 2009). Increasing temperatures, within a certain range, may enhance Cd loading into the xylem and promote the translocation of Cd from the roots to the shoots. The TF values of Cd increased when the temperature and CO₂ concentrations were elevated (Fig. 4), which suggested that enhanced Cd xylem loading, combined with increased Cd absorption by the roots might be responsible for the increase in the shoot Cd BF values. Under the same treatment conditions, the Cd concentration in the roots was higher than in the shoots, which was consistent with the results of other studies (Stritsis and Claassen, 2012). Previous research (Larsson et al., 2002; Wójcik and Tukiendorf, 2004) had suggested that most plant species retained most of the Cd they accumulated in their roots, which could be due to complexation of the metals with the sulfhydryl groups. Those complexes are less easily translocated to the upper parts of the plant (Rodriguez et al., 2011). The higher Cd content in the roots, compared to other tissues, is considered to be an important tolerance and protective mechanism in plants.

Zn is an essential trace element, and is known to act as an enzyme cofactor for >200 enzymes that have important biological functions and regulate many physiological processes, including DNA synthesis, behavioral responses and reproduction (Vallee and Auld, 1990). Zn is a plant micronutrient and is usually presented in low concentrations, but it becomes toxic at elevated concentrations. Most plant species are damaged when the Zn concentration reaches 200 to 300 µg/g dry weight of leaves (Påhlsson, 1989). In this study, the tissue Zn concentration ranged between 9.94 and 19.52 µg/g fresh weight (moisture content was less than 80%), which would have no toxic effect on wheat seedlings and would in fact facilitate growth. Studies had shown that treatment with 30 mg/L Zn significantly promoted the growth of wheat roots and shoots (Jiang et al., 2008). Increased temperatures and CO₂ concentrations would further enhance plant growth. Thus, the dilution effect due to accelerated plant growth is probably responsible for the decline of Zn BF values when the shoots and roots were subject to the elevated temperature and CO₂ concentration treatments. As observed in other studies, elevated CO₂ concentrations also reduced the content of Zn in wheat plants as a result of dilution caused by tissue carbohydrate accumulation (Fangmeier et al., 1997). Furthermore, plant Zn²⁺ sorption is an exothermic process, and an increase in temperature would cause a decrease

in the sorption capacity for Zn (Rakhshaei et al., 2006). This may also contribute to the decrease in Zn concentration.

The shape of the fitted curves for Zn and Cd BF values in the shoots was very similar to the fitted curves for the roots (Fig. 1). It seems that the Zn concentrations in the shoots were mainly determined by Zn entry into the roots. Under the same treatment conditions, Zn accumulation in the shoots was slightly higher than that in the roots, which was consistent with previous findings (Hu et al., 2009). Vacuolar sequestration and cell wall binding may play a major role in increasing Zn accumulation in the shoots (Hu et al., 2009).

3.2. Combined effects of elevated temperature and CO₂ concentration on the subcellular distributions of Cd and Zn

The subcellular distribution of metals may provide a mechanistic approach to investigate metal toxicity and tolerance (Wallace et al., 2003; Wang and Rainbow, 2006). In this study, the Cd concentrations in the F1, F2 and F3 subcellular shoot and root fractions all increased significantly with the elevated temperature and CO₂ concentration treatments, but the opposite change trends were observed for the subcellular distribution of Zn (Fig. 2). This result showed that elevated temperature and CO₂ concentration may enhance Cd sensitivity and toxicity to wheat seedlings by increasing its physical accumulation and cellular damage in cells (Wang and Wang, 2011), which would depress the development of crops. Moreover, it seems that elevated temperature and CO₂ concentration may exacerbate Zn deficiency in crops due to the Zn concentration decrease in the subcellular fractions. In the long run, the effects of climate warming on the subcellular distribution of trace elements may induce more negative impacts on crops. These negative impacts may degrade grain quality, which could be a potential threat to human health. The Cd and Zn concentration change trends in the F1, F2 and F3 subcellular fractions were highly consistent with the change trend of total Cd and Zn concentrations in the shoots and roots. This suggested that Cd and Zn concentrations in the root subcellular fractions were determined largely by their uptake into roots, and in shoots the subcellular fractions were determined largely by root-to-shoot translocation.

In general, the elevated temperature and CO₂ concentrations had no significant effects on the proportional distribution of Cd and Zn in the subcellular fractions, although statistically significant differences were observed between some of the treatments. It seems that the elevated temperature and CO₂ concentrations may have balancing effects on the distribution of Cd and Zn in the F1, F2 and F3 subcellular fractions. Li et al. (2011a, 2011b) found that increased temperature tended to decrease the relative Cd distribution in the HDP (heat-denatured proteins) fraction but increase the HSP (heat-stable proteins) fraction in wheat roots, the relative Cd distribution in cell debris remained the same. Weng et al. (2011) found that the distributions of Cd, Cu, Fe, Mn and Zn increased in the soluble fraction but decreased in the cell wall fraction in wheat seedling roots as the temperature rose. These studies suggested that increased temperature could change the proportional distribution of trace elements in the subcellular fractions. However, now still little data available about the effects of elevated CO₂ concentrations on the

subcellular distribution of trace elements. One possible interpretation is that elevated temperature and CO₂ concentrations may induce opposite effects on the subcellular distributions of trace elements, and their effects tend to offset each other, which meant that the subcellular distributions are relatively stable, but this needs to be confirmed by further research.

We found that most of the Cd and Zn in the shoots and roots accumulated in the soluble fraction (shoot Cd: 47.4%–50.5%, root Cd: 60.6%–63.9%; shoot Zn: 42.8%–51.2%, root Zn: 44.1%–48.1%), whereas only a small amount was found in the cell wall fractions (shoot Cd: 29.4%–32.6%, root Cd: 18.4%–21.2%; shoot Zn: 25.3%–31.5%, root Zn: 27.9%–32.3%) and organelle fractions (shoot Cd: 18.3%–23.0%, root Cd: 17.4%–18.8%; shoot Zn: 22.1%–27.8%; root Zn: 23.6%–27.4%). This result partially agrees with other investigations (Wu et al., 2005; Li et al., 2006), which showed that over 65% of the total Cd and Zn were bound to cell walls or transported into the vacuole (major constituent of the soluble fraction). The largest amount was found in the soluble fraction (mainly in vacuoles), which further confirmed the claim that the vacuole could be the main storage site for metals in plant cells (Yang et al., 2005). Regionalization through cell wall deposition and vacuolar compartmentalization plays a major role in heavy metal detoxification, tolerance and hyper-accumulation in plants (Zhang et al., 2014). In general, heavy metal stress may be alleviated or avoided through sequestration by the cell wall and the vacuole (Wu et al., 2005).

3.3. Combined effects of elevated temperature and CO₂ concentration on the translocation of Cd and Zn from the roots to the shoots

The Cd and Zn concentrations in the roots and shoots dynamically changed when the plants were subjected to elevated temperature and CO₂ concentration, and the TF was used to evaluate the capacity of crops to translocate Cd and Zn from the roots to the shoots. As shown in Fig. 4, the TF values of Cd significantly increased when the plants were subjected to elevated temperature and CO₂ concentration. However, the TF values of Zn only fluctuated between 1.02 and 1.23. This result suggested that the elevated temperature and CO₂ concentrations may induce non-equivalent effects on Cd and Zn transport capacity from the roots to the shoots. Generally, the translocation of trace elements from the roots to the shoots are dependent on several steps (Clemens et al., 2002): (1) symplastic absorption in the roots; (2) root sequestration; (3) xylem loading and transport and (4) unloading and tissue distribution. Symplastic absorption and xylem loading are both energy-dependent processes (Mori et al., 2009), and are mediated by several families of plasma membrane transporters, such as P1B-type ATPases, ZIPs, Nramps, CDFs and CAXs (Kramer et al., 2007). Generally, most transport proteins are sensitive to temperature, and increasing temperature within a certain range may enhance their activity and improve the transport efficiency of trace elements. It is not known whether elevated CO₂ concentrations are involved in the processes. Trace elements that are only partly sequestered by the root can also be transported into the xylem by remobilization and this reduces root sequestration (Clemens

et al., 2002). Interestingly, this study found that both the Cd TF values and the Zn TF values had a highly negative correlation with their proportions in the root F3 fraction, which was the fraction most affected by root sequestration, especially for the Zn TF values. It seemed that there was high negative correlation between the TF values and the root sequestration of Cd and Zn. Some studies indicated that Zn and Cd partially shared the same transport pathway and similar transport systems (Clemens et al., 2002), so an increase in Zn root-to-shoot translocation should be expected when temperature and CO₂ concentration rose. Unfortunately, this was not observed in our study. This may be due to the higher dilution effect caused by the accelerated shoot growth. Wang et al. (2003) found that zinc application promoted the growth of maize shoots more than that of roots. It is possible that increased temperature and CO₂ concentration may widen the difference by further enhancing the growth of shoots, which may offset the effect caused by the increased transport capacity of the transporters. Taken together, we suggest that the elevated temperature and CO₂ concentrations may increase the root-to-shoot translocation of Cd by enhancing the remobilization and the transport capacity of the transporters. The fluctuation in Zn TF values is probably due to the changes in root sequestration.

4. Conclusions

In a conclusion, the increasing of temperature and CO₂ concentration will change the dynamics of Cd and Zn in wheat seedlings. The elevated temperature and CO₂ concentrations increase the bioaccumulation of Cd in shoots and roots by enhancing Cd adsorption and uptake in the roots and increasing its root-to-shoot translocation. This inhibits root and shoot growth. However, Zn accumulation in the shoots and roots decrease due to the dilution effect of accelerated seedling growth in a warmer environment with an increased CO₂ concentration. The elevated temperature and CO₂ concentration will enhance Cd sensitivity and toxicity to wheat seedlings by increasing its physical accumulation in subcellular compartments. Furthermore, elevated temperature and CO₂ concentration may exacerbate Zn deficiency in crops by decreasing its subcellular compartmental distribution. Most of the Cd and Zn in shoots and roots accumulate in the soluble fractions and cell wall fractions, which may be an important mechanism for detoxifying heavy metals and improving tolerance in crops. Elevated temperature and CO₂ concentration can enhance the transport capacity of Cd and Zn transporters and promote their root-to-shoot translocation. In addition, our results indicate that temperature and CO₂ concentration increasing do not enhance the root-to-shoot translocation of Zn, due to the dilution effect of accelerated shoot growth.

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