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Contrasting effects of elevated CO_2 on Cu and Cd uptake by different rice varieties grown on contaminated soils with two levels of metals: Implication for phytoextraction and food safety

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ABSTRACT

A pot experiment in six open-top chambers with two levels of CO_2 and two multi-metal contaminated soils was conducted to investigate combined effects of elevated CO_2 levels and metals (Cu and Cd) on rice. Elevated CO_2 significantly increased the total dry weight biomass of six Chinese rice by 20–108 and 32–142% for low and high levels of contaminated soils, respectively. We observed dilution/little varied phenomena in grain Cu concentration in six rice varieties grown on both contaminated soils under elevated CO_2. We found significantly higher Cd concentrations in the parts of three rice varieties under elevated CO_2, but lower levels for the others. Two major conclusions can be drawn from our study: (1) rice varieties with significantly increased biomass and metal uptake under elevated CO_2 exhibit greater potential for phytoextraction and (2) given expected global increases in CO_2 concentration, CO_2-induced accumulation of metals in rice might be a component contributing to the potential health risk in the future, with Cd being a more important threat to human health than Cu.

1. Introduction

Mining, manufacturing, use of synthetic products, and land application of industrial or domestic sludge have contaminated a large proportion of agricultural land throughout the world, causing an increase in the soil concentrations of most metals. The situation is even worse in China where there are about 2.0×10^7 hm^2 of agricultural land contaminated with heavy metals [1]. Such widespread contamination of land in China poses a potential threat to crop quality as well as human health, especially for land contaminated with Cd and As. Therefore, more attention has been paid to the effect of toxic metals in the contaminated land upon crop growth development, and quality.

Rice (*Oryza sativa* L.) is one of the main staple crops that easily take up high concentrations of metals like Cd and As [2]. The accumulation of metals in rice is dependent upon many factors such as chemical characteristics of the metal, rhizosphere soil properties, and specific characteristics of plant species or cultivars [3–6]. Among all factors, plants may play a decisive role in uptake and translocation of metals. Previous studies have revealed considerable variation in the uptake and accumulation of Cu and Cd among rice varieties [1,7–10]. However, all those reported data were obtained from rice grown under the influence of either contaminated soils [1,5,11] or elevated CO_2 [12,13], and very little research has focused on the combined effects of elevated CO_2 and multi-metal contamination upon rice growth, development and uptake of metals.

The ongoing combustion of fossil fuels associated with industrialization is leading to increase in the atmospheric carbon dioxide concentration [14], and this process may affect crop growth, development, and quality. There is general acceptance that elevated CO_2 enhances plant growth in uncontaminated soils in terms of plant biomass, water and nutrient use efficiency, and the rate and intensity of photosynthesis [15–17]. However, there have been few studies of the effect of elevated carbon dioxide on plant uptake of metals in contaminated environments. In a previous study we showed that Indian mustard (*Brassica juncea* L. Czern.) and sunflower (*Helianthus annuus* L.) absorbs more Cu under elevated CO_2 levels [18]. We also showed that elevated CO_2 could trigger hyperaccumulation of Cs by *Sorghum vulgare* × *S. vulgare var. sudanense* and *Trifolium pretense* [19]. Guo et al. [20] and Jia et al. [21] reported decreasing accumulation of metals in rice grown on contaminated soil under free-air CO_2 enrichment (FACE) condition.

These studies highlight the need for a better understanding of the mechanisms by which CO_2 and heavy metals jointly affect crop

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growth, development, and uptake of metals, especially from the viewpoint of phytoextraction and food safety. Further, although positive responses to elevated CO_2 levels of plants grown on uncontaminated soil are known, the interactions between CO_2 levels, biomass, and crop uptake of metals can be more complicated when plants are grown on soils contaminated with heavy metals, and affected by many environmental factors such as water availability and climate conditions. Therefore, more research needs to be carried out to investigate crop responses when grown in soils contaminated with metals and under elevated CO_2 levels. Such work could help identify crops and varieties that have a strong biological ability to accumulate metals in their aboveground parts under elevated CO_2 conditions, and hence contribute to phytoextraction of contaminated soils. From a food safety perspective, it is also important to identify crops and varieties that accumulate little metals in their editable parts.

With atmospheric carbon dioxide concentration continuously increasing and farmland quality deteriorating due to metal contamination, the combined effects of elevated CO_2 and multi-metal contamination upon rice growth, development and uptake of metals are drawing more attention. The objective of this work was (1) to investigate the effect of elevated CO_2 on Cu and Cd uptake by the six Chinese rice varieties grown on contaminated soils with two levels (low and high) of copper and cadmium and (2) to assess their potential value for phytoextraction of contaminated paddy soils and possible risks to human health through grain consumption. To our knowledge, this is the first report on the effects of elevated CO_2 levels on multi-metal uptake by rice grown on multi-metal contaminated soils.

2. Materials and methods

2.1. Tested plant species

Seeds of six Chinese rice (*O. sativa* L.) varieties used for this study were provided by Guangdong Academy of Agricultural Science; these included Rong You 398 (RY), Shan You 428 (SY), Tian You 390 (TY), Yue Za 889 (YZ), Gui Nnong Zhan (GN), Yin Jing Ruan Zhan (YJ). These particular varieties were selected because they are widely cultivated in South China.

2.2. Soil preparation

The soils – soil-LC (low level of multi-metal contamination) and soil-HC (high level of multi-metal contamination) – used in this study were taken from the arable layers (0–25 cm) of agricultural land at two contaminated sites of Tongshan town, Nanjing City, Jiangsu Province, PR China where the Jiuhuashan Cu mine is located. This is largely a result of wastewater discharged from the mining operation. The physico-chemical characteristics of these two soils are listed in Table 1. The methods used for analysis of the soils follow Tang et al. [18]. Both soils were silty loams, with similar pH (though soil-HC was less alkaline) and organic matter (OM) content.

Fresh soil was passed through a 5-mm mild sieve and stored in the dark before use. Ten kilograms of dry soil were placed in each plastic pot (28 cm in diameter and 28 cm in height). Before planting, pots were fertilized with 0.20 g N, 0.12 g P and 0.26 g K per kilogram of soil in the form of $CO(NH_2)_2$, KH_2PO_4, K_2SO_4, respectively. The first 40% of N and 100% of P and K were applied as base fertilizers dissolved in deionized water and well mixed, submerged with 3 cm water above the soil surface for 1 week before the rice seedlings were transplanted into the soil. The other 60% of N was applied in three intervals (the 3rd day after transplanting the seedlings, at tillering stage and the 5th day before the appearance of panicles).

Table 1
Physical and chemical characteristics of soil used in this study.

Soil types	Soil-LC[a]	Soil-HC
Total N (g kg^{-1})	1.93	2.05
Total P (g kg^{-1})	0.96	0.98
Total K (g kg^{-1})	11.70	12.20
pH	7.86	7.78
OM (g kg^{-1})	27.40	28.10
Clay (%)	11.6	12.4
Silt (%)	45.0	46.8
Sand (%)	43.4	40.8
CEC (cmol kg^{-1})	12.22	13.38
Total Cd (mg kg^{-1})	1.65	3.31
Total Cu (mg kg^{-1})	82	607
Extractable Cd (mg kg^{-1})	0.05	0.08
Extractable Cu (mg kg^{-1})	6.40	63.40

[a] Soil-LC and soil-HC represent low contaminated soil and high contaminated soil, respectively.

2.3. CO_2 purity and structure of open-top chamber (OTC) for pot experiment

For a full description of the OTC system and performance, please refer to Wu et al. [19]; a brief introduction follows. The facility consists of six naturally lit chambers (aluminium frame and clear glass walls, 3 m diameter × 1.4 m wide × 3.3 m high). Three of them were used as control (ambient CO_2, 360 $\mu L\,L^{-1}$) while the three others had ambient plus 500 $\mu L\,L^{-1}$ CO_2 in air. The CO_2 enrichment took place from 9:30 a.m. to 5:30 p.m. on sunny days (i.e. no cloud cover). Automated measurements of CO_2 concentration, temperature, and humidity were taken every minute each day through the experiments (Table 2). Prior to the CO_2 treatment experiment, the growth chambers were tested for the gas homogeneity and for the CO_2 concentration–time relationship.

2.4. Plant materials and growth conditions

Rice seeds were surface sterilized by soaking in 1% NaClO for 10 min; rinsed in deionized water for five to seven times; and then germinated within two layers of moist gauze cloth at 32 °C for another 30 h. The germinated seeds were transplanted to a moistened mixture of perlite and vermiculite (1:1). After 15 days, the healthy and uniform-sized seedlings with four leaves were transplanted into the pots (three seedlings per pot). The pot soil was maintained under flooded conditions (with 3–4 cm of water above the soil surface) during the whole growth period (125 days). The pots were arranged in a randomized complete-block design with four replicates. To minimize pot position effects inside the chambers and chamber-to-chamber effects, pots were randomly switched among chambers every 20 days and placed in a new, randomly selected position within each chamber, at each move.

2.5. Sample preparation and analytical methods

After 125 days of growth, rice plants were harvested at maturity. Each rice plant was divided into its root, stem, leaf and grain parts, and each was washed carefully with deionized water and then oven-dried to constant weight at 70 °C for dry weight determination. Soils adhering to root (i.e. rhizosphere soil) were taken off with

Table 2
Environmental parameters in the six open-top chambers.

Environmental variable	Ambient	Elevated
CO_2 concentration (8 h mean, $\mu l\,l^{-1}$)	360 ± 15	860 ± 50
Air temperature (°C)	29.9 ± 2.2	30.4 ± 3.1
Light intensity ($\mu mol\,m^{-2}\,s^{-1}$)	530 ± 150	545 ± 168
Humidity (%)	40.1 ± 7.3	43.2 ± 8.8

a brush and then air-dried for the determination of pH value. Plant subsamples were digested in a microwave oven (Mars CEM 240/50) using HNO_3/H_2O_2 (7 and 1 ml, respectively). The extracts were analyzed for the metal concentrations using an Atomic Absorption Spectrometer (AAS) and a graphite tube equipped with an automatic sampler (ZEEnit 700, Analytikjena, Germany). Certified standard elemental samples (CRM Tea from IGGE, GBW10016) and metal standards (Fluka, Switzerland) for AAS were used to ensure the precision of the analytical procedures. The reliability of the digestion and analytical procedure was verified by including blanks and standard samples with every batch of sample digested, as part of the QA/QC protocol. Reagent blanks and at least two replicates of all samples were used to ensure accuracy and precision in the analysis.

2.6. Data analysis

2.6.1. Statistical analyses

Data were analyzed with the statistical package SPSS (SPSS Inc., Chicago, IL, Version 16.0) for Windows. The data were analyzed with a two-way analysis of variance (ANOVA) approach. If the hypothesis of equal means was rejected by the ANOVA test, the Duncan procedures were employed to distinguish among treatment means. The critical difference values between treatments were calculated at 5% probability levels.

2.6.2. Bio-accumlation factors (BFs)

The Cu or Cd bio-accumulation factor (BF) was calculated on the basis of dry weight using the equation below. It represents an index of the ability of rice to accumulate Cu or Cd with respect to its concentration in the soil substrate [22]:

$$\mathrm{BF}\ (\%) = \frac{\mathrm{Metal}_{\mathrm{shoot}}}{\mathrm{Metal}_{\mathrm{soil}}} \times 100 \qquad (1)$$

where $\mathrm{Metal}_{\mathrm{shoot}}$ ($\mathrm{mg\,kg^{-1}}$) and $\mathrm{Metal}_{\mathrm{soil}}$ ($\mathrm{mg\,kg^{-1}}$) represent Cu or Cd concentrations in shoots and soils, respectively.

2.6.3. Estimated daily intake (EDI) and target hazard quotient (THQ) of heavy metals

To appraise the health risk associated with heavy metal contamination of rice grown on soils contaminated with two levels of heavy metals under ambient and elevated CO_2, we calculated estimated dietary intake (EDI $\mu\mathrm{g\,kg^{-1}\,day^{-1}}$ Bw) and target hazard quotients (THQ) using the methods as suggested by Zheng et al. [23], and the Integrated Risk Information System (US-EPA, IRIS) [24].

The estimated daily intake (EDI) of Cu or Cd is dependent upon the metal concentrations in rice and the amount of rice consumed. The EDI of Cu or Cd for an adult was calculated using the following equation [23]:

$$\mathrm{EDI} = \frac{C \times \mathrm{Con} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{Bw} \times \mathrm{AT}} \qquad (2)$$

where C ($\mu\mathrm{g\,kg^{-1}}$, on fresh weight basis) is the concentration of heavy metals in the contaminated rice, Con represents the daily average consumption of rice (g $\mathrm{person^{-1}\,day^{-1}}$), EF is exposure frequency (365 $\mathrm{days\,year^{-1}}$), ED is exposure duration (70 years), Bw represents average adult body weight (65 kg $\mathrm{person^{-1}}$), and AT is the average time of exposure to noncarcinogens (365 $\mathrm{days\,year^{-1}}$ × number of exposure years, assuming 70 years in this study). Pan et al. [25] proposed that the average daily rice intakes of local inhabitants were 261.1 g $\mathrm{person^{-1}\,day^{-1}}$.

The health risks from consumption of rice by local inhabitants were assessed according to the target hazard quotient (THQ) determined by the following equation [23]:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}} \qquad (3)$$

where RfD represents the reference oral dose ($\mu\mathrm{g\,kg^{-1}\,day^{-1}}$), and it can be obtained from the Integrated Risk Information System (US-EPA, IRIS, 2008). For Cu and Cd, the values are 40 and 1.0 $\mu\mathrm{g\,kg^{-1}\,day^{-1}}$, respectively [24].

THQs of less than 1 imply a low risk of noncarcinogenic effects whereas those probability of noncarcinogenic effects increases with THQ for THQ values greater than 1 [22].

3. Results

3.1. Metal concentrations in soils taken from the contaminated sites

The concentrations of metals in soils are dependent upon parent rock and the presence of mineralised terrains, and anthropogenic emissions. The main differences between soil-LC and soil-HC were the total and available Cu and Cd levels. The total Cu and Cd contents were estimated by digesting dry soil samples in a mixture of HNO_3/HF (5 and 3 ml, respectively) with a microwave (Mars CEM 240/50). The available Cu and Cd contents were determined by extraction of the soils with 0.005 M DTPA ($\mathrm{pH} = 7.3$). The total Cu content of soil-LC and soil-HC was 82 and 607 $\mathrm{mg\,kg^{-1}}$, respectively while the total Cd content of soil-LC and soil-HC was 1.65 and 3.31 $\mathrm{mg\,kg^{-1}}$, respectively. Both soils Cu and Cd levels approach or exceed the national guidelines (i.e. 100 mg Cu $\mathrm{kg^{-1}}$ and 0.6 mg Cd $\mathrm{kg^{-1}}$, $\mathrm{pH} \geq 7.5$ for farmland) set by the National Standards (GB 15618-1995) (Table 1). Other metals, such as Pb and Zn, are lower than those set by the National Standards (GB 15618-1995) (300 mg Pb $\mathrm{kg^{-1}}$, $\mathrm{pH} \geq 6.5$–7.5; 300 mg Zn $\mathrm{kg^{-1}}$, $\mathrm{pH} \geq 6.5$–7.5). Therefore, this land is a typical multi-metal (Cu and Cd) contaminated land. It poses a serious threat to the food safety, and needs to be remediated.

3.2. Plant biomass

The total dry weights of the six rice varieties grown with two levels of soils at harvest are shown in Fig. 1(a). There were significant differences in the total dry weight biomass among various rice genotypes for both soils ($P < 0.001$). All rice varieties exhibited higher dry weight biomass in soil-LC than soil-HC regardless CO_2 treatment. The total dry weights of all six rice varieties grown in both soils under elevated CO_2 were significantly greater than those grown under ambient CO_2 ($P < 0.01$), with increases ranging from 21 to 108% and 32 to 142% in soil-LC and soil-HC, respectively ($P < 0.001$). The GN variety grown on soil-HC under elevated CO_2 had the largest biomass increase (142% higher than the CO_2 control). It should be noted that most of the six rice varieties had significantly higher dry weights of roots, stems, leaves and grains with elevated CO_2 for both soil treatments (Fig. 1(b)–(e)). The root, stem, leaf and grain dry weight biomass of the six different rice varieties grown under elevated CO_2 increased by 16–198, 11–225, 5–88 and 13–138%, respectively, compared to the ambient CO_2 control.

4. Metal concentrations in rice

Significant differences in the Cu and Cd concentrations were found in the four parts (roots, stems, leaves, and grains) of the six rice varieties, depending upon soil type, CO_2 level, and genotype ($P < 0.001$) (Figs. 2(a)–(d) and 3(a)–(d); Table 3). The Cu concentrations of the six rice varieties grown in soil-HC were larger than for those grown in soil-LC. For both soils, the order of distribution of Cu concentrations by part in the six rice varieties grown was as follows: root stem leaf grain. In nearly all cases, an elevated CO_2 level led to significant reduction in the Cu concentrations

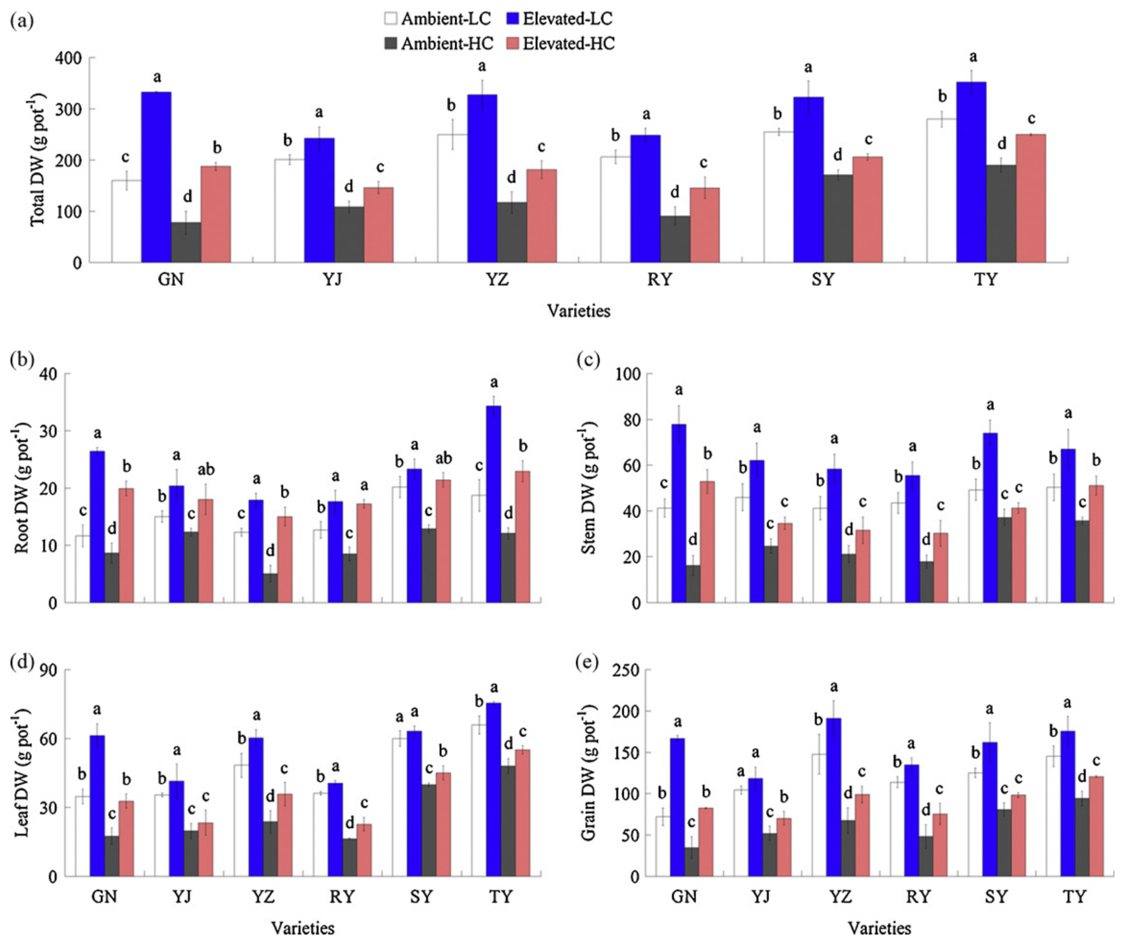

Fig. 1. Effects of elevated CO_2 on dry weight (DW) of the six rice varieties grown on two contamination soils. Different letters within the same variety indicate significant differences between CO_2 treatments ($P < 0.05$), by analysis of variance [ANOVA].

in the roots, stems, and leaves. Exceptions arose in the YJ and YZ roots, SY stems and the YZ leaves on soil-HC (Fig. 2(a)–(c)). The effect of elevated CO_2 on Cu concentrations in some parts of the six tested rice varieties grown on two contaminated soils was noted as insignificant in comparison with the CO_2 control rice.

Unlike the results for Cu, the distribution patterns of Cd by plant parts is significantly affected by soil type, genotype, and CO_2 level

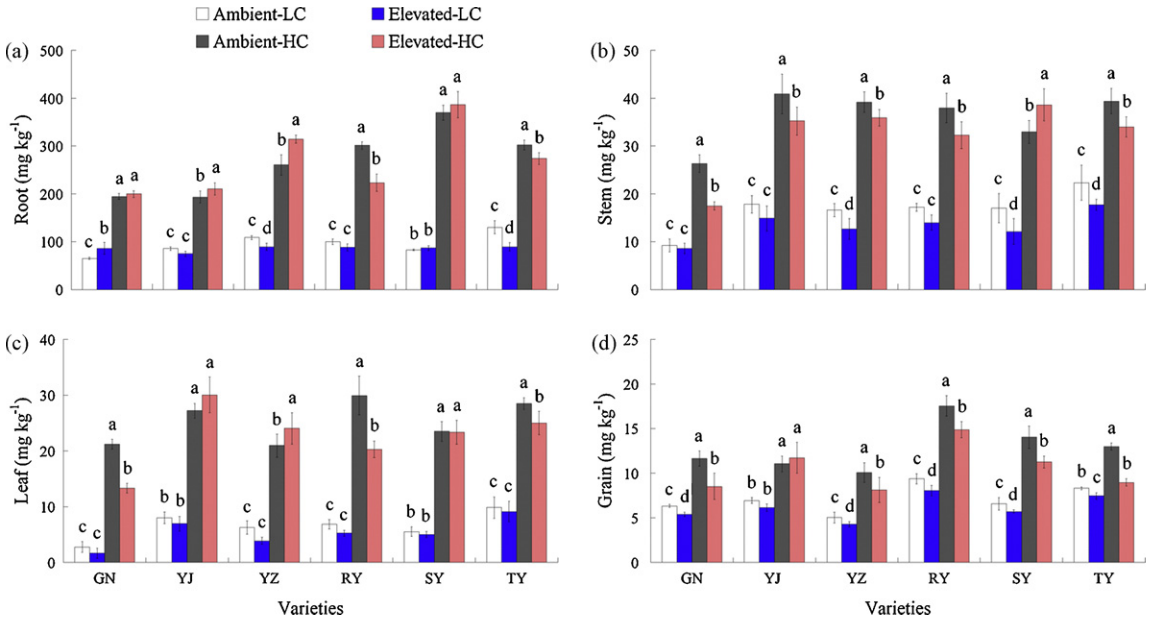

Fig. 2. Effects of elevated CO_2 on Cu concentrations in different parts of the six rice varieties grown on two contamination soils. Different letters within the same variety indicate significant differences between CO_2 treatments ($P < 0.05$), by analysis of variance [ANOVA].

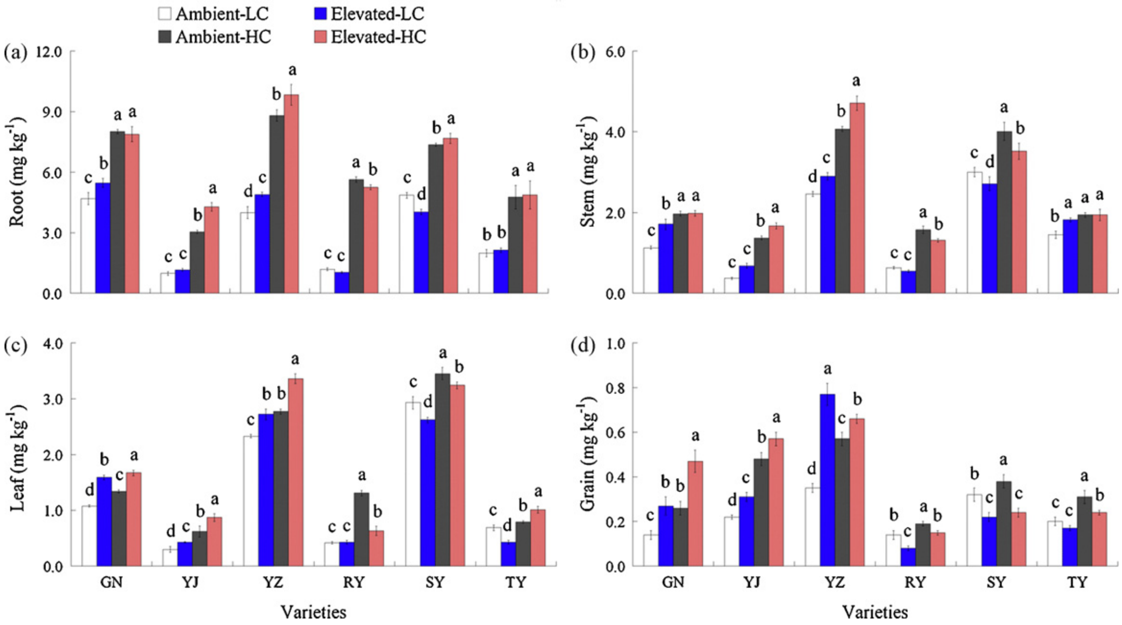

Fig. 3. Effects of elevated CO_2 on Cd concentrations in different parts of the six rice varieties grown on two contamination soils. Different letters within the same variety indicate significant differences between CO_2 treatments ($P < 0.05$), by analysis of variance [ANOVA].

($P < 0.001$) (Fig. 3(a)–(d) and Table 3). The Cd concentrations of the six rice varieties grown in soil-HC were larger than for those grown in soil-LC regardless CO_2 treatment. For both soils, the order of Cd concentration by part for the six rice varieties was as follows: root stem leaf grain (Fig. 3(a)–(d)). Cd concentration varied greatly among rice genotypes. The grain Cd concentration of YZ variety was the highest among the six varieties grown in soil-LC, and that of RY cultivar was the lowest in soil-LC. The grain Cd concentrations of RY rice were much lower than those of the other varieties grown in both soils.

The Cd concentrations in the stems, leaves, and grains of the three rice varieties (GN, YJ and YZ) grown under elevated CO_2 increased on average by 32, 32, and 62%, respectively, whereas those of the other three rice varieties (RY, TY, and SY) decreased on average by 4, 13 and 28%, respectively. The maximum increase of Cd concentration in roots arose with YJ variety, up to 41% with an elevated CO_2 level in soil-HC (Fig. 3(a)). There were significantly higher Cd concentrations in the grains of the GN, YJ, and YZ rice varieties grown under elevated rather than ambient CO_2 levels. By contrast, the concentrations of Cd in the grains of RY, SY, and TY varieties grown under elevated CO_2 were significantly lower than those under ambient CO_2.

4.1. Soil pH variations

The pH value in the rhizosphere soils of the six rice varieties exhibited a slightly decreasing trend under elevated CO_2 compared to the ambient CO_2 control, but significant differences were found (Table 4). The decrease in pH values in the rhizosphere soil across the rice varieties due to elevated CO_2 was within the range of 0.04–0.15 (0.08 on average), and 0.04–0.12 (0.07 on average) units for soil-LC and soil-HC, respectively, compared to the ambient CO_2 control. There is no big difference in pH between the first three rice varieties (GN, YJ, and YZ) and the other three rice varieties (RY, SY, and TY) for both soils under either ambient or elevated CO_2.

Table 3
Analysis of variance of interactive effects of CO_2, soil type and genotype on metal concentration and uptake in different parts of the six rice varieties.

		CO_2	Soil type	Genotype	CO_2 × soiltype	CO_2 × genotype	Soil type × genotype
Cu concentration	Root	0.015	<0.001	<0.001	n.s.[a]	<0.001	<0.001
	Stem	<0.001	<0.001	<0.001	n.s.	0.020	<0.001
	Leaf	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001
	Grain	<0.001	<0.001	<0.001	<0.001	0.003	<0.001
Cd concentration	Root	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001
	Stem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Leaf	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001
	Grain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total Cu uptake	Root	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Stem	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001
	Leaf	0.005	<0.001	<0.001	0.001	n.s.	<0.001
	Grain	<0.001	n.s.	<0.001	n.s.	0.002	0.001
Total Cd uptake	Root	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Stem	<0.001	<0.001	<0.001	0.008	<0.001	<0.001
	Leaf	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001
	Grain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] n.s., not significant.

Table 4
Rhizosphere soil pH values of the six rice varieties grown under either ambient or elevated CO_2.

Rice varieties	Ambient-LC	Elevated-LC	Ambient-HC	Elevated-HC
GN	7.55 ± 0.04 a	7.40 ± 0.02 c	7.53 ± 0.04 a	7.46 ± 0.02 b
YJ	7.61 ± 0.02 a	7.57 ± 0.02 b	7.61 ± 0.03 a	7.57 ± 0.01 b
YZ	7.54 ± 0.04 a	7.46 ± 0.03 bc	7.51 ± 0.02 ab	7.43 ± 0.02 c
RY	7.54 ± 0.03 a	7.48 ± 0.03 b	7.53 ± 0.01 a	7.46 ± 0.04 b
SY	7.50 ± 0.03 a	7.37 ± 0.03 b	7.48 ± 0.04 a	7.36 ± 0.02 b
TY	7.56 ± 0.04 a	7.43 ± 0.02 b	7.52 ± 0.02 a	7.47 ± 0.03 b

Note: Different letters within the same row indicate significant differences between CO_2 treatments ($P < 0.05$).

4.2. Total uptake of metals by rice and its implication for phytoextraction

We calculated total uptake of Cu and Cd removed through harvesting each part by multiplying the biomass per pot by the average metal concentrations in different plant parts of the six rice varieties (Figs. 4(a)–(d) and 5(a)–(d)). Total Cu and Cd uptake were shown to be dependent upon rice genotypes, soil type, and levels of CO_2 ($P < 0.001$) (Figs. 4 and 5 and Table 3). There were significant differences in total uptake of Cu among different rice genotypes, with greater variation in roots, stems, and leaves and less variation in grains. In most cases, the roots, stems, and leaves of the rice varieties exhibited higher total uptake of copper in soil-HC than soil-LC regardless CO_2 treatment. On average, elevated CO_2 significantly increased the total Cu uptake of rice crops by 80, 33, 8 and 24% for the roots, stems, leaves and grains of the six rice varieties, respectively (Fig. 4(a)–(d)).

The total Cd uptake by different parts of the six rice varieties was also dependent upon rice genotype, and soil type, and CO_2 levels ($P < 0.001$) (Fig. 5(a)–(d) and Table 3). Significant differences were noted in total uptake of Cd among different rice genotypes, with greater variation in the roots, stems, leaves, and grains. For GN, YJ, and YZ varieties growing under elevated CO_2, the average increase in total Cd uptake by the roots, stems, leaves, and grains were 128, 129, 92, 178%, respectively, compared to the ambient CO_2 control. However, the RY, TY and SY varieties showed a more complicated variation trend in responses to elevated CO_2, with some rice parts having an increased total uptake of Cd, and others having an opposite trend.

Table 5 shows metal BFs for different rice varieties. Most of the rice varieties grown on soil-LC had higher BFs than when grown on soil-HC. The TY cultivar had the highest Cu BF while SY had the highest Cd BF.

4.3. Health risk analysis on the basis of dietary intake of metals and target hazard quotients

The THQ values of the six rice varieties varied from 0.434 to 1.514 for Cu and 0.276 to 2.660 for Cd (Fig. 6(a)–(d)). The Cu values of THQs were higher for all six rice varieties grown on soil-HC than on soil-LC regardless CO_2 treatment (Fig. 6(a) and (b)). The THQs of Cu via rice consumption were less than 1 for all the six varieties grown on soil-LC regardless of CO_2 concentrations (Fig. 6(a)). When grown on soil-HC, the GN, RY, SY and TY varieties under ambient CO_2, and the YJ and RY varieties under elevated CO_2 had THQs of Cu via rice consumption greater than 1, showing risk of noncarcinogenic effects (Fig. 6(b)).

The distribution patterns of the THQs of Cd via rice consumption were obviously different from those of Cu. According to the effect of elevated CO_2 on the THQs of Cd, the six rice varieties can be classified into two groups: Group One, comprising GN, YJ and YZ, and Group Two, comprising RY, SY and TY (Fig. 6(c) and (d)). With elevated CO_2, the THQs of Cd for GN, YJ and YZ varieties increased by 90, 41 and 120%, respectively, with the highest value being up to 2.660 for YZ variety grown on soil-LC under elevated CO_2. This implies that Group One had higher risk of noncarcinogenic effects when grown under elevated CO_2 rather than ambient CO_2. By contrast, RY, SY and TY varieties showed a decreasing trend for the

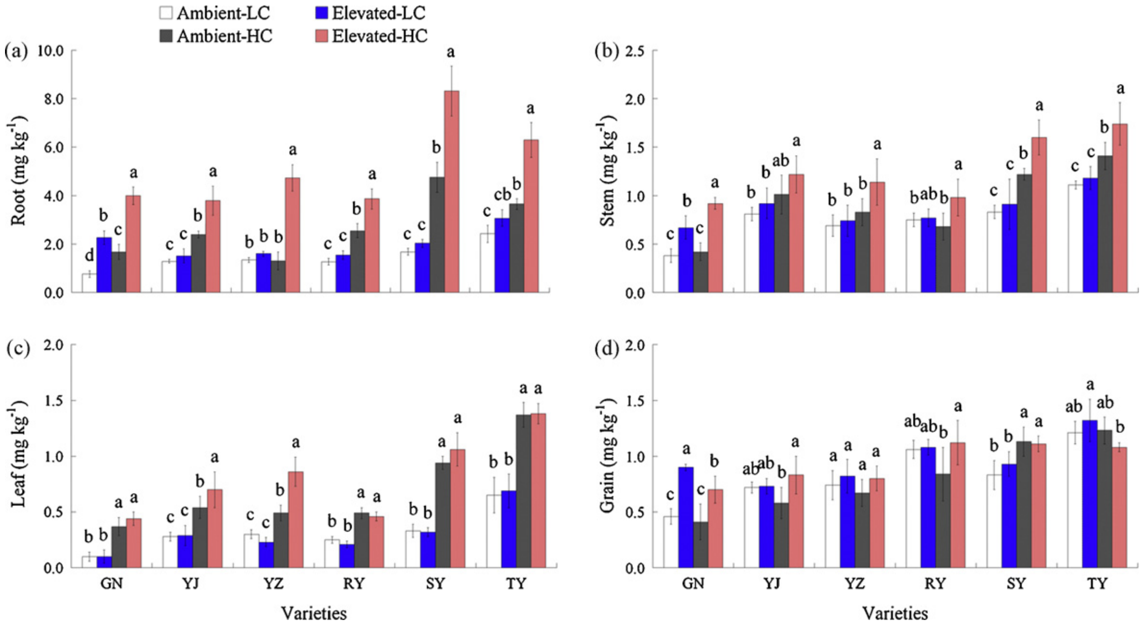

Fig. 4. Effects of elevated CO_2 on Cu uptake in the six rice varieties grown on two contamination soils. Different letters within the same variety indicate significant differences between CO_2 treatments ($P < 0.05$), by analysis of variance [ANOVA].

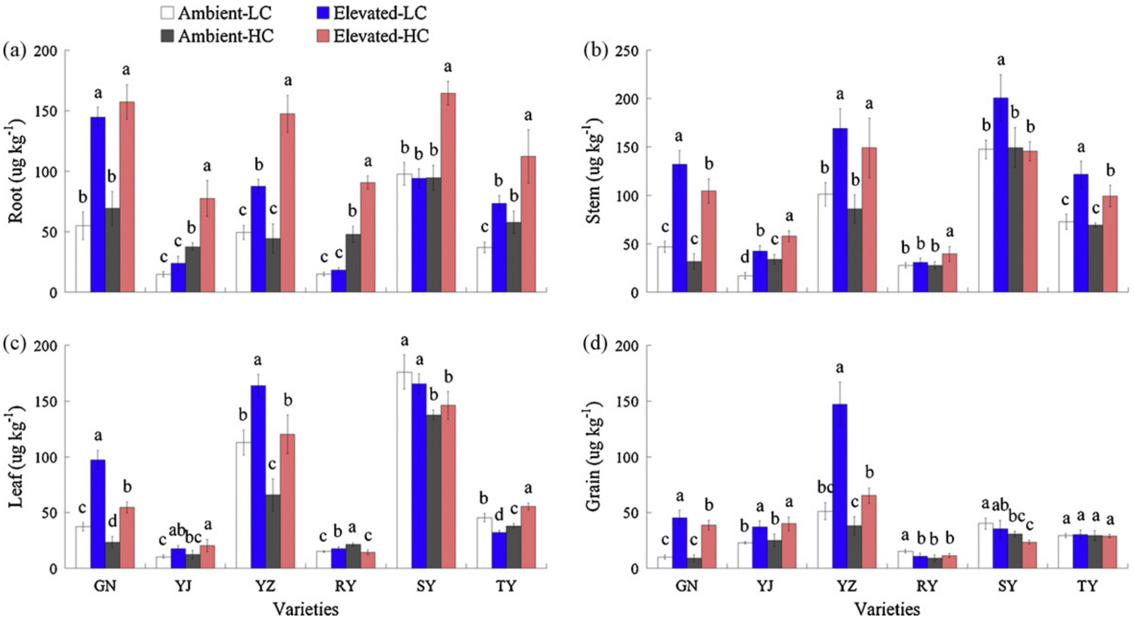

Fig. 5. Effects of elevated CO_2 on Cd uptake in the six rice varieties grown on two contamination soils. Different letters within the same variety indicate significant differences between CO_2 treatments ($P < 0.05$), by analysis of variance [ANOVA].

Table 5
Cu and Cd BFs (bioconcentration factors: shoot metal concentration/soil metal concentration) for the six rice varieties growing under either ambient or elevated CO_2.

Metal	Treatments	BF					
		Rice varieties					
		GN	YJ	YZ	RY	SY	TY
Cu	Ambient-LC	0.08	0.16	0.13	0.15	0.13	0.18
	Elevated-LC	0.07	0.14	0.10	0.12	0.11	0.16
	Ambient-HC	0.04	0.06	0.05	0.06	0.05	0.05
	Elevated-HC	0.03	0.05	0.05	0.04	0.05	0.05
Cd	Ambient-LC	0.67	0.20	1.45	0.32	1.79	0.62
	Elevated-LC	1.00	0.35	1.70	0.31	1.62	0.65
	Ambient-HC	0.50	0.31	1.02	0.44	1.12	0.39
	Elevated-HC	0.56	0.41	1.21	0.31	1.02	0.44

THQs of Cd under elevated CO_2 compared to the ambient CO_2 control, exhibiting lower risk of noncarcinogenic effects when grown under elevated CO_2.

5. Discussion

5.1. Effects of elevated CO_2 on biomass increase and Cu and Cd uptake: implication for phytoextraction

We showed that the dry weights of the rice plants are significantly affected by rice genotypes ($P < 0.001$), and soil type ($P < 0.001$). The total dry weight biomass of the six rice varieties was significantly higher when grown with elevated CO_2, by 20–108% and 32–42% in soil-LC and soil-HC, respectively ($P < 0.001$). These

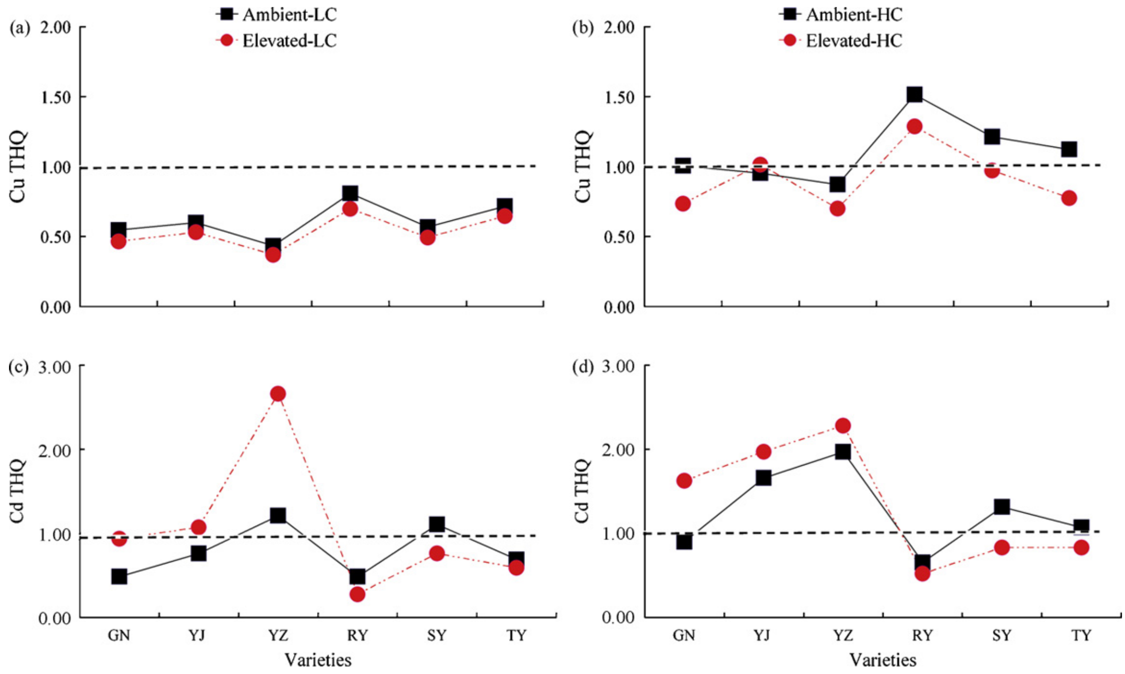

Fig. 6. Effects of elevated CO_2 on Cu and Cd THQ values via consumption of the six rice varieties grown on two metal contaminated soils.

increases are consistent with the results documented in the literature [21,26,27]. It is now realized that the yield responses of rice to a near doubling of CO_2 levels has varied greatly [27]. Cheng et al. [28] reported that the effects of elevated CO_2 on different parts of rice varied. Moya et al. [29] suggested an increase of 5–60% in rice yield with elevated CO_2, varying much among rice genotypes in responses to elevated CO_2. Horie et al. [26] reported the average increase in rice yield with a doubling in CO_2 was about 30%. Ziska et al. [30] showed that increase in grain yield under elevated CO_2 among the 17 different rice varieties was genotype-dependent, varying between 30 and 400%. From the viewpoint of phytoextraction, our finding that CO_2 elevation increases the biomass of all six different rice varieties grown on contaminated soils has favorable implications for using CO_2-induced rice varieties for phytoextraction purposes.

Our present study revealed a different accumulation pattern in paddy rice grown on contaminated soils under elevated CO_2 for Cd and Cu. Cd mainly showed accumulation and dilution phenomena whileas Cu exhibited dilution phenomena, depending upon rice varieties. Those responses to elevated CO_2 can be assessed in links with phytoextraction and food safety. A survey of literature indicates that there might be three responses to elevated CO_2 in terms of uptake of metals by plants: dilution, little variation, and accumulation.

Dilution phenomena are quite often detected in the pot studies where nutrient supplies are generally limited. Högy and Fangmeier [17] showed that elevated CO_2 resulted in decreased concentrations of all micro-elements in wheat by 3.7–18.3% in the different exposure systems. Yang et al. [31] reported a significant 20% decrease in grain Cu concentration under elevated CO_2. Guo et al. [20] showed that elevated CO_2 decreased Cd accumulation in leaves, stems, roots and grains of rice. Seneweera and Conroy [32] found that elevated CO_2 diluted grain Cu concentration. Jia et al. [21] reported a Cu reduction in the leaves of rice grown on Cu contaminated soils under free-air CO_2 enrichment (FACE) with $570\,\mu L\,L^{-1}$ of CO_2. A similar trend was found by Zheng et al. [33] for ferns *Pteridium revolutum* and *Pteridium aquilinum* grown on Cu contaminated soils. In view of potential benefits of metal dilution and the very limited available data on the subject, we proposed that this might have important positive implications for the food quality from the contaminated soil when crops are harvested.

Fangmeier et al. [34] noted little variation in the concentrations of P, K, Zn, and Mn in spring wheat crops under elevated CO_2, but more data need to be collected from wider ranges of plant species or cultivars. Our present study suggested little change in Cu concentration in some rice varieties grown under elevated CO_2 (Fig. 2(a)–d)).

Tang et al. [18,19] reported accumulation or hyperaccumulation phenomena, showing that elevated CO_2 triggered hyperaccumulation of Cu by *B. juncea* and *H. annuus* when grown on Cu contaminated soil [18], and hyperaccumulation of Cs by *S. vulgare var. sudanense* and *T. pretense* on Cs contaminated soil [19]. Lieffering et al. [12] reported that metals like Fe, Zn and Mn showed a strong tendency to increase in concentration with elevated CO_2 in the rice grains. However, the limited data derived from plants growing in pots placed in environmental enclosures like OTC are not broad enough to understand the mechanisms involved and more work is needed on the physiological or even molecular responses to elevated CO_2 as well as differences among plant varieties.

Phytoextraction technology for treatment of heavy mental contaminated soils has many advantages over conventional engineering technologies [35]. The most important challenge to using this technology is knowing how to improve its efficiency by increasing the accumulation of metals in plants, or by improving key plant biological traits that would in turn enhance metal uptake. Most hyperaccumulating plants are not suitable for this purpose due to their low biomass. Therefore, the identification of plants with optimal biological traits and strong ability to accumulate metals in aboveground plant parts is considered a priority for phytoextraction. Special attention has recently been devoted to metal-tolerant crops that have fast growth rate and high biomass. Their greater biomass can compensate for a relatively low capacity for metal accumulation in tissues, which results in the overall greater accumulation of heavy metals [36,37].

Our previous studies have considered possible implications of increased biomass and uptake of metals by plants grown under elevated CO_2 for phytoextraction [18,19,33], but all the data were obtained from investigation of special plant species like Indian mustard, sunflower, ferns, and Sudangrasse. The present study showed that CO_2-induced increase in Cd uptake by some rice varieties can become a potential supplementary method in phytoextraction since the total uptake of the metal by those rice varieties increases to a greater extent as shown by higher Cd BFs of the GN, YJ, and YZ varieties under elevated CO_2 than under ambient CO_2. Use of elevated CO_2 to assist phytoextraction of metal contaminated soils may shorten the remediation time needed in comparison with no CO_2-assisted phytoextraction technology.

Taking into account our present results and previously obtained results, we proposed that the GN, YJ, and YZ varieties can be candidates for phytoextraction under assistance of elevated CO_2. This would widen considerably the range of species and varieties suitable for metal phytoextraction as shown in Murakami and Ae [37].

5.2. Effect of rice genetype and soil pH on metal uptake under elevated CO_2 and multi-metal contamination

Genetic variation in uptake of Cu and Cd under the conditions of either contaminated soils or elevated CO_2 has been recognized for many years [1,7,9,10,38,39] and there appears to be possible for us to develop the species or cultivars with special (extremely high or remarkably low) ability to absorb Cd from contaminated soil for phytoextraction purposes or food safety. This strategy has been successfully applied in sunflower (*H. annuus* L.) and durum wheat (*Triticum turgidum* cultivar group durum) [40–42]. Under the combined effect of elevated CO_2 and multi-metal contamination, we recorded genetic variation in Cd uptake by rice as discussed above. These genetic differences can be exploited to develop crop cultivars with improved ability to accumulate metals for phytoextraction, or to improve food safety through reduced uptake.

Elevated CO_2 might lower pH on rhizosphere soils, favor the release of metals into soil solution, and as a result, help the plant to take up more metals [18,19]. In the present study, the pH values in the rhizosphere soil across rice varieties exhibited a slightly decreasing trend in links with some secondary effect of CO_2 (e.g. increased root exudation might lead to changes in rhizosphere pH and metal availability) (Table 4), but significant differences were found. Therefore, it can link elevated CO_2 levels to increasing solubility and phytoavailability of Cd under elevated CO_2 for explanation of the higher Cd concentrations in the different parts of the GN, YJ and YZ rice varieties. The lower Cd concentrations in the different parts of the RY, SY and TY rice varieties grown under elevated CO_2 might be due to genetic differences. More research needs to be done to investigate the relationship between elevated CO_2 and the increased solubility and phytoavailability of Cd under elevated CO_2.

5.3. Target hazard quotient (THQ) of heavy metals and implication for food safety

The THQs are widely used for evaluation of the health risk via consumption of metal contaminated food crops [43]. The THQs of Cd for the GN, YJ and YZ varieties grown under elevated CO_2

approached or exceeded 1 for both soils (Fig. 6(c) and (d)). This study shows that there will be greater potential health risk in the Jiuhuashan mine area from consuming contaminated rice grown under elevated rather than ambient CO_2 levels, but this risk is lower for the RY, SY, and TY rice varieties than the GN, YJ, and YZ rice varieties. Given expected global increases in CO_2 concentration, there is need for more in-depth research to cure heavy metal contamination in the region's soil and to screen rice varieties with weak metal translocation from soil to rice grains. Our results also suggest that Cd is a more important threat to human health in the region than Cu.

6. Conclusions

The pot experiment results presented here show contrasting responses of the six rice genotypes grown on low and high levels of multi-metal contaminated soils to elevated CO_2. We believe this to be the first such report for rice crops from the viewpoint of phytoextraction and food safety since all other previously reported data were obtained from single factor controlled experiments, i.e., contaminated soils or elevated CO_2, not both. At least for our experiment, with crops of rice growing in Cu and Cd combined contaminated soils, Cu showed dilution or little varied phenomena in all the six rice varieties grown on the two contaminated soils under elevated CO_2 compared to the ambient CO_2 control, but Cd exhibited accumulation or dilution patterns in terms of CO_2 effect, depending upon rice varieties. This conclusion is partially contrary to the general conclusions of Loladze [13] who agued that in pot experiments nutrient dilution is likely to be common phenomena. Combining metal concentrations in the different rice parts with corresponding increase in biomass under elevated CO_2, we proposed that the GN, YJ, and YZ varieties have greatest potential for phytoextraction as indicated by their metal BFs if they are grown for phytoextraction purposes under assistance of elevated CO_2. By contrast, the RY, TY and SY varieties exhibited a more complicated variation trend in responses to elevated CO_2. The local inhabitants will be exposed to a greater potential health risk if they consume contaminated GN, YJ, and YZ rice varieties rather than contaminated RY, SY, and TY varieties grown under elevated CO_2. Since this risk increases with CO_2 levels, additional screening of appropriate rice varieties for the region that have weak metal translocation from soil to rice grains is a needed response to increases in global CO_2 concentration. However, because the above mentioned conclusions were based on data obtained from rice grown in multi-metal contaminated soil under OTC and pot growth conditions, we think that the extent of accumulation or dilution was probably exaggerated and the potential for elevated CO_2 to trigger changes in Cd concentrations might be overestimated. Therefore, more research should be conducted on crops of rice grown under realistic field conditions under elevated CO_2 in order to make reasonable predictions on combined effects of elevated CO_2 and multi-metal contaminated soils on metal uptake by rice, and to screen appropriate rice varieties with weak metal translocation from soil to rice grains as alternative ways against global CO_2 increasing concentration.

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