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Original Research

Potential of Gibberellic Acid (GA3) and Uniconazole for Enhancing the Cd Absorption Efficiency of Maize (*Zea mays* L.)

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Abstract

In this study, gibberellic acid 3 (GA3) and uniconazole were applied as the means of improving of cadmium (Cd) polluted site phytoremediation using maize (*Zea mays* L.). The results showed that GA3 (40 mg/L), and uniconazole (800 mg/L) could increase the biomass, plant height, stem diameter, leaf area, and root parameters of maize plants under Cd stress (0.4 mg/L) by foliar spraying. GA3 (40 mg/L) could increase the SPAD value of maize leaves by 23.45% and the net photosynthetic rate by 645.93%. Both plant regulators could alleviate the oxidative damage of maize and increase the activity of SOD and POD up to 11.44~58.41% and 35.00~99.55%, respectively. The content of MDA and H_2O_2 could be reduced by GA3 to 31.91% and 24.77%, respectively. GA3 at 40 mg/L could significantly increase the accumulation of Cd ions in the shoots, roots and whole plants of maize by 222.01%, 603.13% and 341.70%, respectively, showing the best effect among tested treatments. It was found that the application of plant growth regulators (PGRs) could increase the biomass and Cd concentration in maize organs, ultimately achieving the purpose of improving the remediation efficiency of Cd by increasing the absolute content of Cd in maize biomass.

Keywords: facility soil, cadmium, phytoremediation, gibberellin, uniconazole

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Introduction

Cadmium (Cd) is a widely recognized toxic heavy metal that seriously harms soil environment and human health. It can enter agricultural soil through many ways, such as atmospheric settlement, mining, industrial waste, sewage irrigation and excessive application of fertilizers [1, 2]. Cd gradually accumulates in the soil but cannot be degraded by biological processes. Over time, this process negatively impacts soil biodiversity and microbial activity and reduces soil fertility [3]. Cd has certain biological availability in soil, and is easily absorbed and accumulated by different parts of plants (such as roots, stems, leaves, fruits, etc.), thus adversely affecting animal and human health through the food chain [4].

In recent years, facility agriculture has gradually become one of the main modes of vegetable production in China [5]. According to China's official bulletin, the total amount of heavy metal pollution in soil exceeds 16.1%, and soil Cd pollution ranks first, with a pollution percentage of 7.0% [6]. In 2016, Shandong province accounted for a quarter of China's total output of facility vegetables and was the main producing area of facility vegetables [7]. According to the latest research in 2019, through the statistical analysis of the heavy metal content in the soil of representative vegetable production areas in Shandong province, the geological accumulation index of Cd is between no pollution and moderate pollution, and up to 13.1% of the samples exceed the Cd limit value of 0.3 mg kg^{-1} stipulated in the agricultural industry standard (NY 5294-2004) [8]. Therefore, in order to ensure the production of vegetables of high quality, heavy metals must be removed from contaminated soil to prevent Cd from entering the human food supply.

Conventional methods, including physical and chemical methods, have been used for the recovery of heavy metal contaminated soils [9]. However, these methods are costly and labor-intensive, often damage the soil environment and lead to irreversible changes in soil properties. Compared with the physical and chemical methods, phytoremediation is a cost-effective and environmentally friendly method [10]. It has been used to purify various pollutants, including metals, nonmetals, petroleum and other organic pollutants [11]. However, plants under heavy metal polluted soil conditions are usually characterized by low biomass, slow growth and low heavy metal enrichment [12].

In order to achieve efficient heavy metal phytoremediation, plant biomass should be increased, and the ability to tolerate and accumulate higher concentrations of heavy metals should be improved. Plants with high heavy metal tolerance usually have low biomass, and many plants with high biomass cannot tolerate high concentrations of heavy metals. In order to realize the potential of increasing plant biomass and plant tolerance to heavy metals, several attempts have been made (applied to plants or added to soil/water), including agricultural strategies and chemical agents (such as plant growth regulators) to improve the efficiency of phytoremediation [13]. Gibberellins (GAs) are diterpenoid and phytohormone that has been widely used in agricultural production, which is capable of activating cell division and cell proliferation processes [14, 15]. Because GA can overcome apical dominance, it is easy to visually observe that it promotes stem growth [16]. Uniconazole is a potential plant growth regulator that can protect plants against high temperature stress, salinity stress, drought stress and water stress [17-20]. Studies have shown that the application of uniconazole can increase the activity of antioxidant enzymes and reduce the accumulation of malondialdehyde (MDA) in maize [21]. The application of uniconazole can improve photosynthetic efficiency, chlorophyll content and antioxidant enzyme activity of soybean, thus improving the yield [22].

Facility vegetables usually have a long planting time during the summer leisure season (from June to August). Therefore, it is necessary to select Cd restoration plants with a fast growth rate and high biomass, which are suitable for growing during this period. Maize belongs to thermophilic crops with a short growth cycle and much higher biomass than the general heavy metal enrichment plants. Based on the above analysis, this paper studied the effects of two PGRs (GA3 and uniconazole) on the growth status, tolerance and absorption efficiency of phytoremediation crops (maize) under Cd stress. It provides preliminary support for the next stage of phytoremediation of vegetable fields contaminated by Cd, and provides a feasible reference for the heavy metal phytoremediation scheme.

Materials and Methods

Plant Materials and Planting Methods

The tested maize variety was Haowei 556 (Gansu wuwei haowei garden planting co., LTD.). Selected seeds should be full and uniform in size. The method of planting in the plugs was adopted. The plugs (Taizhou Longji Plastic Industry Co., Ltd.) had a specification of 540×280×110 mm (L×W×H), and the volume was 190 cm^3 per hole. The number of holes was 32. The substrate was purchased from Jinan Yubeng Biotechnology Co., Ltd. Hoagland nutrient solution was prepared and $CdCl_2$ was added to reach a concentration of 0.4 mg/L. The nutrient solution was then applied to the substrate.

Test Arrangement

The experiment was conducted in the solar greenhouse of Shandong Academy of Agricultural Sciences from January to February 2019. The experimental treatments are shown in Table 1. The

Table 1. The types and concentrations of exogenous PGRs sprayed in each treatment.

Treatments	Exogenous plant growth regulator	Concentration
CK	Distilled water	—
A1	Uniconazole	200 mg/L
A2	Uniconazole	800 mg/L
A3	GA3	10 mg/L
A4	GA3	40 mg/L

In the table, different letters indicate a significant (p<0.05) difference between treatments in the same experiment.

treatments were arranged randomly with three replicates. During the experiment, PGRs (Uniconazole: 200 mg/L and 800 mg/L. GA3: 10 mg/L and 40 mg/L), purchased from Beijing Solarbio Technology Co., LTD, were prepared with distilled water and sprayed every 10 days and CK was distilled water. The net photosynthetic rate of maize was measured on the 20th day of maize growth. At the 30th day, the shoots and roots of maize were harvested separately and washed with distilled water. A portion of the samples was dried, crushed and stored in sealed bags for preservation. The other fresh samples were used for enzyme detection and stored at -80ºC after treatment with liquid nitrogen.

Plant Growth Parameters

Plant height was measured with a centimeter ruler at harvest. The plants were collected separately according to shoots and roots, and the fresh weight was measured. The dry weight was measured by drying at 105ºC for 30 min and then at 70ºC until the weight became constant. The stem diameter was measured by an electronic caliper to determine the narrow width of the midpoint between the first nodes above the aerial root. To measure leaves area, root tips, root volume and total root length, the leaves or roots were arranged and floated on shallow water in a glass tray (30 cm×30 cm), scanned using a scanner (Epson Expression 1680 Scanner, Seiko Epson Corp., Tokyo, Japan), and then analyzed using WinRHIZO Analyzer System (Regent Instruments Inc., Quebec, Canada).

Relative Chlorophyll Content (SPAD) and Net Photosynthetic Rate

Leaves from the same parts of maize were selected and SPAD value was measured by a chlorophyll meter (SPAD-502 plus, China). The net photosynthetic rate was measured with a photosynthetic apparatus (Li-6400xt, USA).

Malondialdehyde (MDA) Content

The MDA content was measured with reference to Chaoui et al. [23]. A total of 0.2 g fresh maize leaves were mixed with 10% (w/v) TCA (10 mL) and centrifuged at 3000 rpm for 10 min. The supernatant (2 mL) was thoroughly mixed with 10% TCA (2 mL) containing 0.5% TBA and heated at 95ºC for 30 min, followed by rapid cooling in an ice bath. The content of MDA was determined at wavelengths of 532, 600 and 450 nm.

H_2O_2 Content

The H_2O_2 content was determined by the method of Velikova et al. [24]. Leaf tissue (0.2 g) was thoroughly ground and mixed with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath, followed by centrifugation at 12,000 rpm for 15 min. Phosphate buffer (0.5 ml, pH 7.0) and 1 mL KI (1 M) were added to the supernatant. The absorbance was read at 390 nm.

Antioxidant Enzyme Activity

The leaves were cut and placed in a bowl, which were homogenated in 50 mm Na_2HPO_4–NaH_2PO_4 buffer (pH 7.8) containing 0.2mm EDTA and 2% insoluble polyvinylpyrrolidone. The homogenate was centrifuged at 3500 rpm for 20 min at 4ºC. The supernatant was used to determine the enzyme activity using a spectrophotometer (Unico UV2100, USA). The SOD activity was measured by measuring the inhibitory effect of SOD on photochemical reduction of nitroblue tetrazolium [25]. The CAT activity was determined by measuring the absorbance at 240 nm [26]. The POD activity was determined by measuring the absorbance of guaiacol at 470 nm [27].

Proline Content

The determination of proline was performed using the method of Bates [28]. Fresh maize leaves (500 mg) were extracted with 3% 5-thiosalicylic acid solution and centrifuged at 5000 rpm. The absorbance of the supernatant was determined at 520 nm.

Vitamin E Content

The vitamin E content was determined using a commercial kit purchased from Nanjing Jiancheng Bioengineering Institute, China [29]. Accurately weigh the samples, added the extraction solution in the kit according to the weight (g): volume (mL) = 1: 9 ratio, fully homogenized, and centrifuged at 25ºC with a centrifuge (2500 rpm) for 10 Minutes, then taking the supernatant for testing. Added the reaction solution in the kit to the test solution, mixed thoroughly, and let stand for 2min, adjusted to zero with absolute ethanol, and measured the absorbance at 533 nm.

Table 2. Effects of uniconazole and GA3 on maize biomass under Cd stress.

Treatments	Roots		Shoots		Entire plant	
	Fresh biomass (g)±SD	Dry biomass (g) ±SD	Fresh biomass (g)±SD	Dry biomass (g) ±SD	Fresh biomass (g)±SD	Dry biomass (g) ±SD
CK	2.27±0.53c	0.22±0.06c	7.35±1.33c	0.66±0.20b	9.62±1.55c	0.87±0.25b
A1	2.81±0.11c	0.24±0.02c	10.15±0.44bc	0.88±0.19b	12.96±0.54b	1.13±0.20b
A2	3.85±0.48b	0.37±0.04b	12.44±2.62b	1.37±0.26a	16.29±3.09b	1.74±0.26a
A3	2.26±0.17c	0.21±0.02c	11.10±0.77b	0.95±0.16b	13.37±0.61b	1.16±0.14b
A4	5.07±0.38a	0.49±0.06a	18.53±1.73a	1.49±0.12a	23.60±2.01a	1.99±0.15a
F value at p<0.05	31.28	21.05	20.68	10.30	25.00	15.04

In the table, different letters indicate a significant (p<0.05) difference between treatments in the same experiment.

Cd content in Plants

For Cd analysis, the dried maize samples were digested in a mixture of HNO_3/$HClO_4$ (3/1, v/v), at 150ºC for 2 h and 210ºC for 1 h, and then dissolved in HCl (0.5 N). The concentration of Cd was determined by an atomic absorption spectrometer (Persee tas-986, China). Per plant (by roots and shoots) Cd accumulation was calculated as follows [30].

Total Cd accumulation by roots/shoots (μg/plant) = Dry biomass of roots/shoots (g) × Cd concentration (μg/g dry wt) by roots/shoots

Calculation of Translocation Factor (TF) and Concentration Index (CI) of Cd

The translocation factor was calculated according to the following equation:

$$\text{Translocation factor (TF)} = \frac{C \text{ aerial}}{C \text{ root}}$$

...where, C represents the concentration of metal in aerial or root (μg/g).

The concentration index (CI) was calculated by dividing the Cd concentration of the treated plants by the Cd concentration of the control plants as shown in the following equation [31]:

$$\text{Concentration index (CI)} = \frac{\text{Concentration of metal in treated plant}}{\text{Concentration of metal in control plant}}$$

Data Analysis

SPSS 21 software was used for one-way analysis of variance (ANOVA). The data were expressed as mean±standard error (SE) of three replicates. Duncan test was used to determine statistical significance when the probability level was P<0.05. Plotting was done by SigmaPlot 12.5.

Results

Effects of PGRs (Uniconazole and GA3) on the Growth of Maize under Cd Stress

These two PGRs (GA3 and uniconazole) at different concentrations could significantly affect the biomass of roots, shoots and the entire plant (Table 2). In the roots section, the fresh and dry weight of uniconazole (800 mg/L) increased by 69.60% and 68.18%, respectively. Meanwhile, GA3 (40 mg/L) increased by 123.3% and 122.7%, respectively. Compared with CK, the changes above were significantly different (p<0.05). In the shoots section, GA3 (40 mg/L) increased the fresh and dry weight of by 152.11% and 125.76%, and uniconazole (800 mg/L) increased by

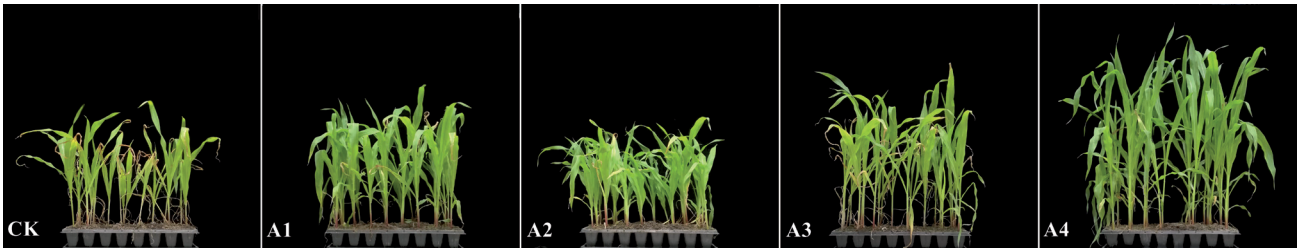

Fig. 1. Maize treated with GA3 and uniconazole under Cd stress.

Table 3. Effects of uniconazole and GA3 on main growth parameters (plant height, stem diameter, leaves area, root volume, root tips and total root length) of maize under Cd stress.

Treatments	Plant height (cm)	Stems diameter (cm)	Leaves area (cm^2)	Root Volume (cm^3)	Root tips	Total root length (cm)
CK	50.11±4.17b	0.81±1.61b	217.83±60.51bc	1.704±0.35b	5577.40±1730.57bc	618.35±244.71b
A1	41.56±2.60c	0.83±1.16b	286.82±58.99b	1.45±0.43bc	4187.00±1912.00cd	452.96±207.62bc
A2	42.11±3.30c	0.99±2.67a	252.09±40.14b	2.445±0.70a	6928.80±1511.95b	655.25±126.71b
A3	51.22±4.09b	0.77±1.01b	158.20±62.50c	0.949±0.13c	2644.40±981.69d	252.15±80.73c
A4	77.00±3.57a	1.00±0.94a	358.05±56.08a	2.433±0.36a	11422.40±1599.38a	1283.20±163.94a
F value at $p<0.05$	145.81	4.56	10.63	8.91	22.51	24.56

In the table, different letters indicate a significant ($p<0.05$) difference between treatments in the same experiment.

69.25% and 107.58%, all of which reached a significant level ($p<0.05$). The results showed that uniconazole (800 mg/L) and GA3 (40 mg/L) could significantly increase the maize plant biomass (shoots, roots system) under Cd stress.

Compared with CK (Fig. 1; Table 3), the plant height of the maize treated with GA3 (40 mg/L) increased by 53.66%, while uniconazole (200 mg/L and 800 mg/L) decreased by 17.06% and 15.96%, respectively. GA3 (40 mg/L) and uniconazole (800 mg/L) significantly

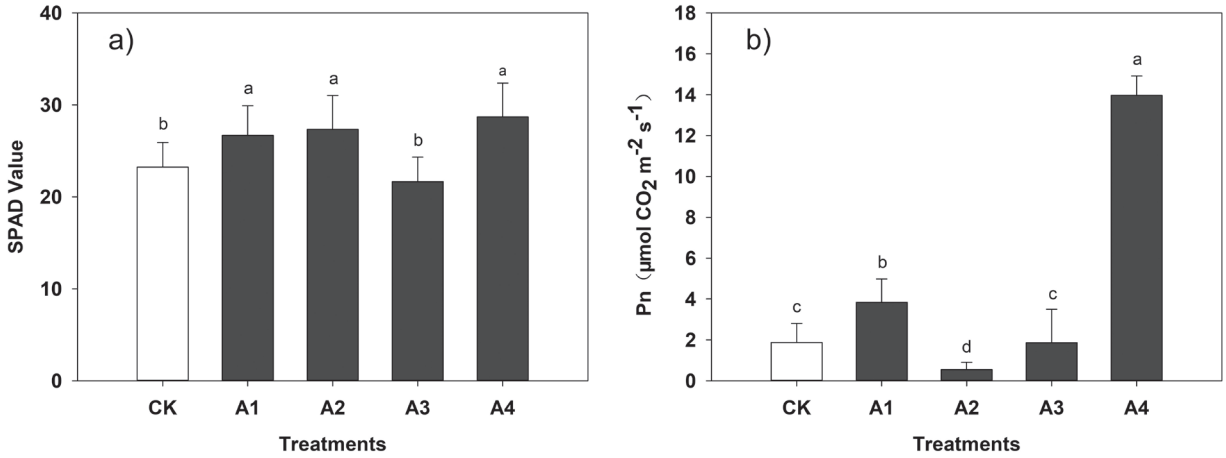

Fig. 2. Effects of uniconazole and GA3 on SPAD value a) and net photosynthetic rate b) of maize leaves under Cd stress.

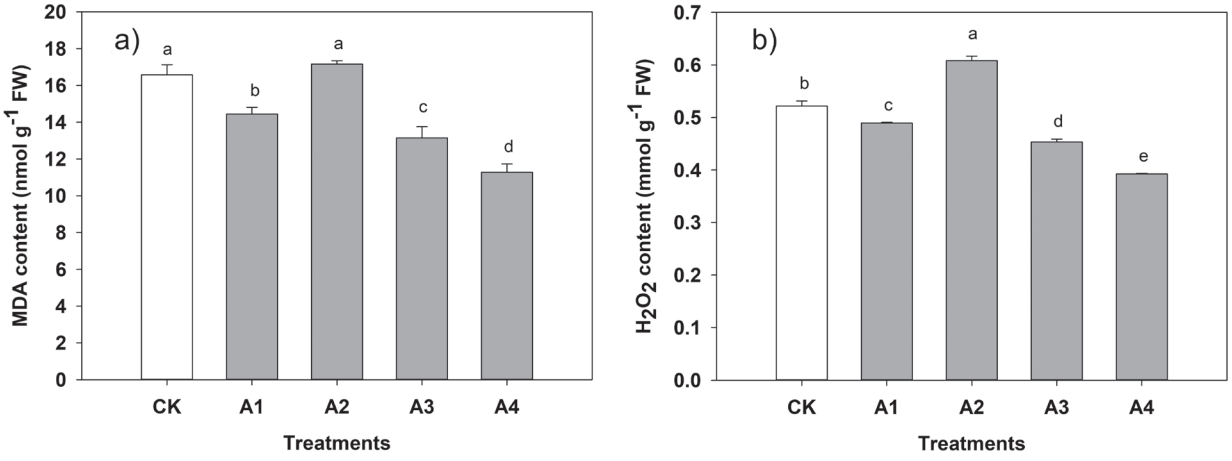

Fig. 3. Effects of uniconazole and GA3 on MDA a) and H_2O_2 b) of maize leaves under Cd stress.

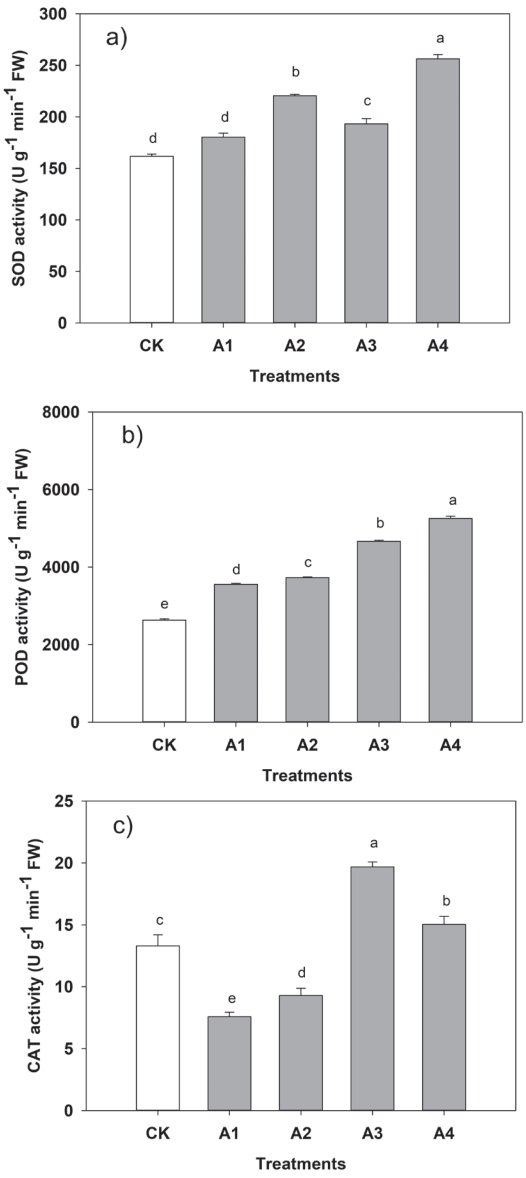

Fig. 4. Effects of uniconazole and GA3 on SOD a), POD b) and CAT c) in maize leaves.

increased the stem diameter by 23.46% and 22.22% (p<0.05), respectively. GA3 (40 mg/L) increased the leaf area significantly by 64.37% (p<0.05). In terms of roots, uniconazole (800 mg/L) significantly increased the root volume of the maize to 43.49% (p<0.05). GA3 (40 mg/L) significantly (p<0.05) increased the root volume, root tip number and total root length of maize by 42.78%, 104.80% and 107.52%, respectively.

Effects of PGRs (Uniconazole and GA3) on SPAD Value and Photosynthesis of Maize Leaves under Cd Stress

Compared with CK (Fig. 2a), the SPAD value of maize leaves under uniconazole (200 mg/L), uniconazole (800 mg/L) and GA3 (40 mg/L) was significantly increased (p<0.05) by 14.82%, 17.639% and 23.45%, respectively. However, the difference between uniconazole (800 mg/L) and CK did not reach a significant level (p>0.05). Compared with CK (Fig. 2b), the net photosynthetic rate of plants under uniconazole (200 mg/L) and GA3 (40 mg/L) increased by 104.99% and 645.93% (p<0.05), respectively. There was no significant difference between GA3 (10 mg/L) and CK (p>0.05), and uniconazole (800 mg/L) was significantly lower than CK (p<0.05).

Effects of PGRs (Uniconazole and GA3) on Oxidative Damages and Antioxidant Activities of Maize under Cd Stress

Compared with CK (Fig. 3a), MDA of uniconazole (200 mg/L), GA3 (10 mg/L) and GA3 (40 mg/L) decreased by 12.81%, 20.69% and 31.91% (p<0.05), respectively, while uniconazole (800 mg/L) and CK showed no significant differences (p>0.05). The changing trend of the H_2O_2 content in maize leaves of each treatment was similar to that of MDA (Fig. 3b). Uniconazole (800 mg/L) increased the H_2O_2 content,

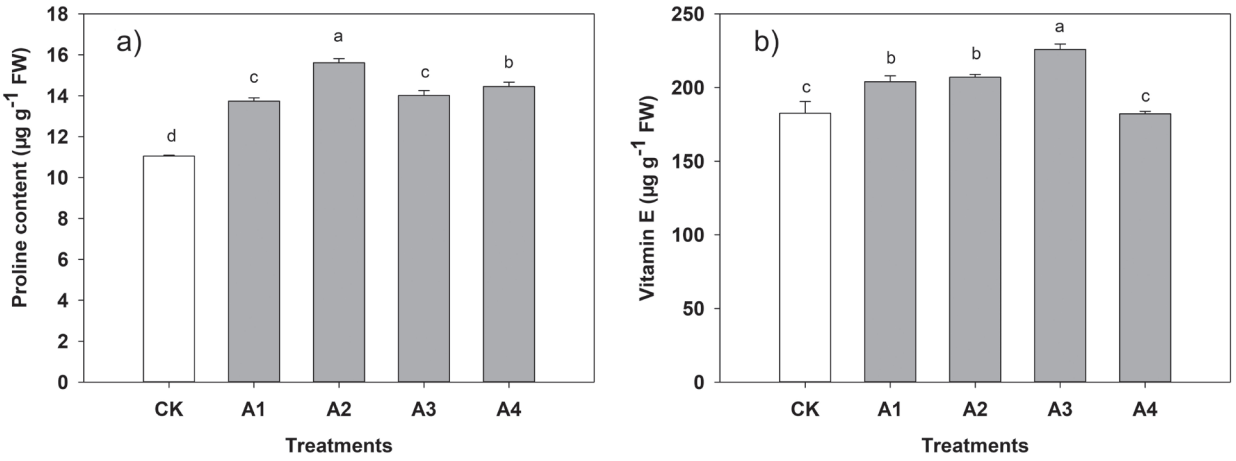

Fig. 5. Effects of uniconazole and GA3 on proline a) and vitamin E b) in maize leaves.

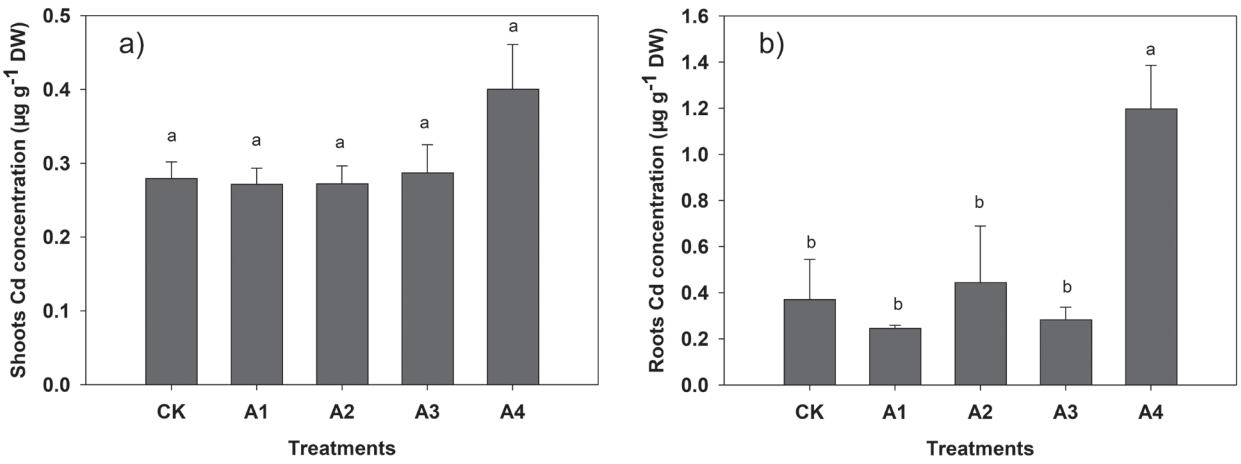

Fig. 6. Effects of uniconazole and GA3 on Cd concentrations in the upper a) and subterranean b) parts of maize.

while A4 showed the largest decrease among all treatments, reaching 24.77%.

Compared with CK (Fig. 4a), SOD of each treatment increased to 11.44~58.41%, and the difference between uniconazole (800 mg/L), GA3 (10 mg/L) and GA3 (40 mg/L) and CK was significant (p<0.05) with GA3 (40 mg/L) being the highest. The order of the POD activity in each treatment was GA3 (40 mg/L)>GA3 (10 mg/L)>uniconazole (800 mg/L)> uniconazole (200 mg/L)>CK with an increase of 35.00~99.55%, and the difference was significant (p<0.05) (Fig. 4b). In terms of the CAT activity (Fig. 4c), the difference between GA3 (10 mg/L), GA3 (40 mg/L) and CK was significant (p<0.05), which was 47.95% and 13.03% higher than CK, respectively, while uniconazole (200 mg/L) and uniconazole (800 mg/L) were smaller than CK.

Both of uniconazole and GA3 could significantly increase the proline content in maize leaves (Fig. 5a). Compared with CK, uniconazole (200 mg/L), uniconazole (800 mg/L), GA3 (10 mg/L) and GA3 (40 mg/L) increased the proline content by 24.38%, 41.35%, 26.89% and 30.78%, respectively, and the difference reached a significant level (p<0.05). Compared with CK, the vitamin E content in leaves of uniconazole (200 mg/L), uniconazole (800 mg/L) and GA3 (10 mg/L) maize increased by 11.80%, 13.43% and 23.80% respectively (Fig. 5b), all of which were significantly different from CK (p<0.05). , The proline content under GA3 (40 mg/L) decreased slightly with no significant difference from CK (p>0.05).

Effects of PGRs (Uniconazole and GA3) on Cd Enrichment in Maize under Cd Stress

As shown in Fig. 6, the Cd concentration in the shoots of the maize field treated with GA3 (10 mg/L) and GA3 (40 mg/L) increased by 2.83% and 43.31%, respectively (Fig. 6a). However, the difference did not reach a significant level (p>0.05). Compared with CK, uniconazole (800 mg/L) and GA3 (40 mg/L) could increase the concentration of maize roots Cd to 19.77% and 222.49% respectively (Fig. 6b), among which the field under GA3 (40 mg/L) increased to a significant level (p<0.05), while the other treatments did not show any significant difference (p>0.05).

Table 4. Effects of uniconazole and GA3 on the Cd Concentration Index (CI) and Translocation Factor (TF) of maize.

Treatments	Concentration index		Translocation factor
	Roots	Shoots	
CK	—	—	0.873±0.393 a
A1	0.665±0.032 b	0.973±0.076 a	1.100±0.519 a
A2	1.198±0.656 b	0.975±0.085 a	0.743±0.365 ab
A3	0.763±0.143 b	1.028±0.135 a	1.026±0.155 a
A4	3.225±0.504 a	1.433±0.718 a	0.337±0.166 b
F value at p<0.05	24.394	1.083	3.982

In the table, different letters indicate a significant (p<0.05) difference between treatments in the same experiment.

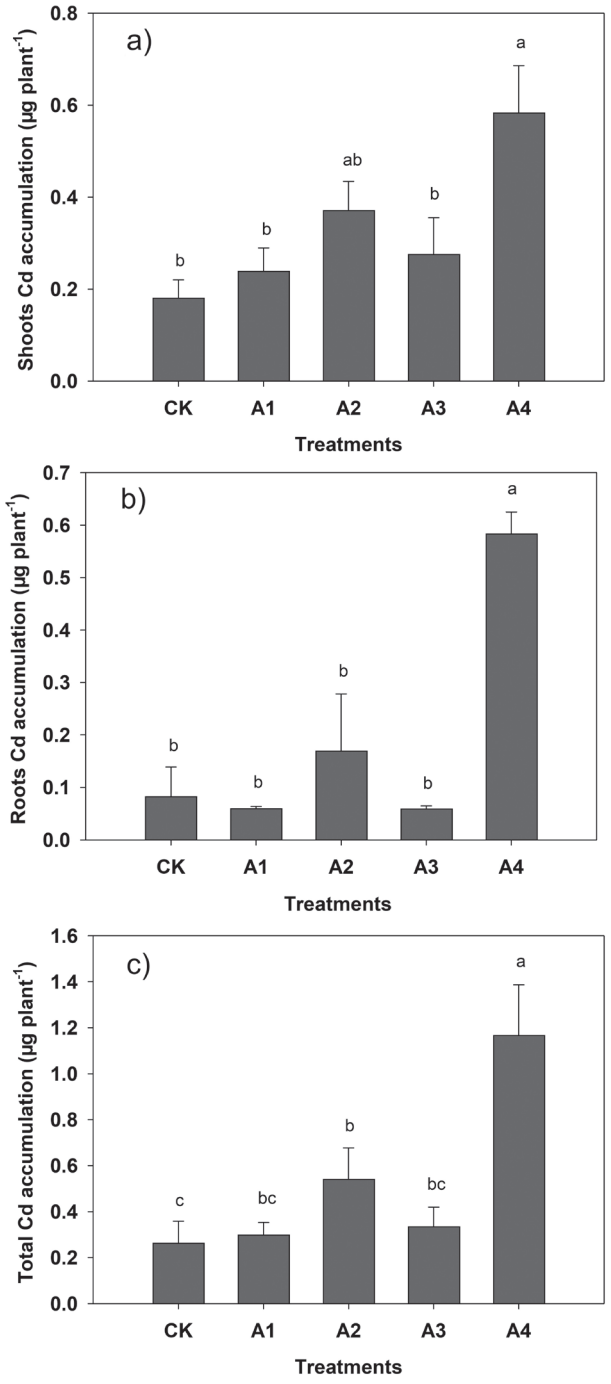

Fig. 7. Effects of uniconazole and GA3 on Cd accumulation in shoots a), roots b) and total c) of maize.

The accumulation of shoot Cd in each treatment is shown in Fig. 7a). The Cd in the plants treated by GA3 (40 mg/L) increased by 222.01% compared with that of CK (p<0.05), while uniconazole (200 mg/L), uniconazole (800 mg/L) and GA3 (10 mg/L) had no significant difference with CK (p>0.05). In the roots (Fig. 7b), Cd accumulation was shown as the order of GA3 (40 mg/L)>uniconazole (800 mg/L)>CK> uniconazole (200 mg/L)>GA3 (10 mg/L). The Cd in the plants treated by GA3 (40 mg/L) increased by 603.13% compared with CK (p<0.05). The Cd level in the plants treated by uniconazole (800 mg/L) increased by 105.06%, but the difference did not reach a significant level (p>0.05). The Cd level in the plants treated by uniconazole (200 mg/L) and GA3 (10 mg/L) were lower than that of CK. In terms of the total Cd accumulation of the whole maize plant (Fig. 7c), the trend was similar to that of the shoot Cd accumulation, and increased in each treatment compared with CK. The Cd level in the plants treated by GA3 (40 mg/L), uniconazole (800 mg/L), GA3 (10 mg/L) and uniconazole (200 mg/L) increased by 341.70%, 105.07%, 27.17% and 13.51%, respectively, among which GA3 (40 mg/L) and uniconazole (800 mg/L) were significantly different from CK (p<0.05). It shows that these two plant regulators could promote the absorption of Cd in maize at high concentrations, but not at low concentrations. The Cd concentration index (CI) of uniconazole (800 mg/L) and GA3 (40 mg/L) in roots was larger than 1 (Table 4), indicating that the Cd concentration in roots was increased compared with CK. However, the CI of uniconazole (200 mg/L) and GA3 (10 mg/L) was less than 1, indicating that the Cd concentration of both was lower than CK. The CI of GA3 (40 mg/L) was the highest, and the difference from other treatments was significant (p<0.05). The GA3 (10 mg/L and 40 mg/L) treatments increased the shoot Cd concentration of maize compared with CK, while a decreasing trend was observed in plants treated by uniconazole (200 mg/L) and uniconazole (800 mg/L). However, the difference did not reach a significant level (p>0.05). The translocation factor (TF) of GA3 (40 mg/L) was significantly lower than that of CK (p<0.05), indicating that the Cd concentration in maize roots was higher in this treatment.

Discussion

Plant Growth and Biomass

Due to the toxicity of heavy metal elements, their accumulation usually inhibits the growth of plants. Plant growth and biomass are negatively influenced by Cd [32]. In this study, PGRs (GA3 and uniconazole) were selected to effectively increase maize biomass (both aboveground and underground parts), while the dry weight and fresh weight of shoots and roots of the control were at the minimum. Zheng et al. also reported a similar reduction of fresh biomass under Cd stress in *Glycyrrhiza uralensis* plants [33]. Hadi et al. [34] reported that the application of GA3 in Cd-polluted soil significantly increases plant growth and biomass. The phenotype of maize in this study, high concentration of GA3 (40 mg/L) proved significantly increase the plant height, stem diameter, leaf area, root volume, root tip number and total root length of maize, while low concentration of GA3 (10 mg/L) and uniconazole (200 mg/L) could not significantly increase the above parameters, part of which even showed a decreasing

trend. Ji et al. [14] showed that under the stress of Cd, the application of GA3 with concentrations of 10, 100 and 1000 mg l^{-1} has no significant effect on the root length of *Solanum nigrum* L, but significantly increases the biomass accumulation. Our study showed that uniconazole was not conducive to the improvement of crop phenotype and had a limited effect on the increase of crop biomass. The suitable amount of GA3 can increase the biomass of crops under Cd stress.

SPAD Value and Photosynthesis

Cd at high concentrations in plants can inhibit the photosynthase activity, chlorophyll synthesis, and photosynthetic efficiency [35, 36]. High levels of Cd ions can reduce plant leaf SPAD values and suppress crop photosynthesis [37]. This study showed that uniconazole (200 mg/L) and GA3 (40 mg/L) could increase the SPAD value and the net photosynthetic rate of maize leaves. Previous studies have shown that application of GA3 to soybeans under Cd stress can increase the contents of chlorophyll a and chlorophyll b in *Glycine* Max leaves [38]. Application of GA3 has a significant effect on improving the net photosynthetic efficiency of rice (*Oryza sativa* L.) leaves under Cd stress [39]. In this study, the application of the appropriate concentration of uniconazole and GA3 also showed similar effects on increasing the SPAD value and net photosynthetic rate of maize leaves.

Antioxidant Response

In this study, malondialdehyde (MDA) and H_2O_2 in the control had a higher level than those in the other treatments. However, the application of uniconazole and GA3 could reduce the content of MDA and H_2O_2 in maize leaves, and the best effect was achieved by GA3 (40 mg/L), indicating that it could promote free radical scavenging system in leaves of studied plants. In order to prevent ROS from harming cells, efficiently functioning antioxidant defense systems composed of enzymes and non-enzymatic is needed prerequisite [40]. Cd stress changes antioxidant enzyme activities in plants. Superoxide dismutase (SOD) can decompose O_2^- into O_2 and H_2O_2. Its activity change is an important indicator of O_2^- yield change. In this study, it was found that the activities of SOD and POD in the control plants were lower than those planted treated with uniconazole and GA3 under Cd stress. GA3 treatment enhanced the activities of SOD, POD and CAT in maize leaves, which may be beneficial to reduce the H_2O_2 content. Proline is an organic osmotic substance. Environmental stress (such as heavy metals, drought, soil acidification, etc.) can promote the accumulation of proline in plants, which has the function of scavenging reactive oxygen species (ROS) in cells and stabilizing protein structures [1,41]. Our study showed that the application of uniconazole and GA3 could increase the content of proline between in maize leaves by 24.38%-41.35%, indicating that both could promote the synthesis of proline in maize leaves under Cd stress. Vitamin E is a fat-soluble chain breaking antioxidant that controls peroxidation and protects plants from oxidative damage by removing or deactivating free radicals [42]. Our results showed that uniconazole and GA3 could promote the increase of vitamin E in maize leaves, indicating that both had good antioxidant effects on maize under Cd stress. Gong et al. found that application of exogenous calcium and speramine increases the vitamin E content in ramie [29]. It shows that it is feasible to regulate Cd tolerance of crops by exogenous application of PGRs.

Cd Uptake in Plants

The results of this study showed that GA3 (40 mg/L) had no significant effect on the Cd concentration in the shoots of the maize, but could significantly increase the Cd concentration in maize root system. This may be because the root system is in contact with Cd in the cultivated soil, leading to its more accumulation in the roots [43]. In addition, the Cd accumulation in the shoots and roots of maize treated with GA3 (40mg/L) was significantly higher than that of other treatments. This result was supported by Ahmad et al. [44] who found that application of GA3 significantly increases the concentration and accumulation of Cd in leaves, stems and roots of *Veronica anagallis aquatica* and *Epilobium laxum*. Ji also found that applying GA3 with a concentration of 1000 mg/L could significantly increase the biomass of *S. nigrum* by 56% [14]. Translocation factor (TF) is an important indicator for plants to transfer heavy metals from contaminated soil to aerial parts. Generally speaking, a higher TF value indicates a higher phytoremediation efficiency [45]. The results of this experiment showed that TF of the plants treated with uniconazole (200 mg/L) and GA3 (10mg/L) was greater than 1. However, in terms of the total Cd absorption, GA3 (40 mg/L) had the best effect by increasing the Cd concentration in the upper and underground parts of maize under Cd stress. The fresh weight of the plant biomass increased, similar to the report by Ji et al. [14]. Cd enriched plants have high Cd concentrations in the body, but often have low biomass, slow growth and low repair efficiency [46]. Considering the short restoration time of soil Cd in facilities, the restoration plants should have the characteristics of heat resistance, high humidity resistance and so on. Plant growth regulators can be used to improve the tolerance of Cd in maize, thereby increasing the biomass and Cd concentration of maize, which is one of the phytoremediation approaches of Cd-contaminated soil in facilities.

Conclusions

Maize has the characteristics of fast growth, biomass increase and high temperature resistance,

which is suitable for planting in the leisure season of the facility vegetable field in summer. In this study, it was found that the adverse effects of Cd stress on maize growth could be alleviated by applying PGRs (GA3 and uniconazole). GA3(40 mg/L) showed the best performance. It could significantly improve the maize growth, enhance the ability to resist oxidative damage, and significantly increase the biomass and Cd concentration in the shoots and roots of maize. Finally, the absolute amount of Cd in maize increased significantly. The above results indicate that maize is a potential remediation plant suitable for Cd contaminated vegetable fields of facilities. Meanwhile, we suggest that in the actual production, before the removal of maize, appropriate wetting soil and deep turning should be adopted to ensure the integrity of the whole plant (roots and shoots) of maize as much as possible, so as to achieve the optimization of the absorption efficiency. This study provides a preliminary basis for improving the phytoremediation efficiency of Cd-contaminated vegetable fields in facilities.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. ALI N., HADI F. Phytoremediation of cadmium improved with the high production of endogenous phenolics and free proline contents in Parthenium hysterophorus plant treated exogenously with plant growth regulator and chelating agent. Environmental Science and Pollution Research, **22** (17), 13305, **2015**.
2. FAGERBERG B., BARREGARD L., SALLSTEN G., FORSGARD N., ÖSTLING G., PERSSON M., BORNé Y., ENGSTRöM G., HEDBLAD B. Cadmium exposure and atherosclerotic carotid plaques – results from the Malmö diet and Cancer study. Environmental Research, **136**, 67, **2015**.
3. OKEDEYI O.O., DUBE S., AWOFOLU O.R., NINDI M.N. Assessing the enrichment of heavy metals in surface soil and plant (Digitaria eriantha) around coal-fired power plants in South Africa. Environmental Science and Pollution Research, **21** (6), 4686, **2014**.
4. ANSARI M.M., NEHA., KHAN H.A. Effect of cadmium chloride exposure during the induction of collagen induced arthritis. Chemico-Biological Interactions, **238**, 55, **2015**.
5. ZHANG H.D., HUANG B., DONG L.L., HU W.Y., AKHTAR M.S., QU M.K. Accumulation, sources and health risks of trace metals in elevated geochemical background soils used for greenhouse vegetable production in southwestern China. Ecotoxicology and Environmental Safety, **137**, 233, **2017**.
6. SUN Y.B., LI Y., XU Y.M., LIANG X.F., WANG L. In situ stabilization remediation of cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite. Applied Clay Science, **105**, 200, **2015**.
7. DONG J., ZHAO Z.W., LIANG B., LI J.L. The development status of China's facilities and vegetable industry. Chinese Horticulture Abstract*s*, **33** (01), 75, **2017**.
8. SUN K.N., WEN D., YANG N., WANG K.A., LI X.H., YU L. Heavy metal and soil nutrient accumulation and ecological risk assessment of vegetable fields in representative facilities in Shandong Province, China. Environmental Monitoring and Assessment, **191** (4), **2019**.
9. ZHANG J., ZHANG G., CAI D., WU Z. Immediate remediation of heavy metal (Cr (VI)) contaminated soil by high energy electron beam irradiation. Journal of Hazardous Material*s*, **285**, 208, **2015**.
10. VITHANAGE M., DABROWSKA B.B., MUKHERJEE A.B., SANDHI A., BHATTACHARYA P. Arsenic uptake by plants and possible phytoremediation applications: a brief overview. Environmental Chemistry Letters, **10** (3), 217, **2012**.
11. MEMON A.R., SCHRöDER P. Implications of metal accumulation mechanisms to phytoremediation. Environmental Science and Pollution Research, **16** (2), 162, **2009**.
12. PERSANS M.W., SALT D.E. Possible molecular mechanisms involved in nickel, zinc and selenium hyperaccumulation in plants. Biotechnology Genetic Engineering Reviews, **17** (1), 389, **2000**.
13. HADI F., BANO A. Utilization of Parthenium hysterophorus for theremediation of lead-contaminated soil. Weed Biology and Management, **9** (4), 307, **2009**.
14. JI P., TANG X., JIANG Y., TONG Y., GAO P., HAN W. Potential of Gibberellic Acid 3 (GA3) for Enhancing the Phytoremediation Efficiency of Solanum nigrum L. Bulletin Environmental Contamination and Toxicolog*y*, **95** (6), 810, **2015**.
15. MAHOUACHI J., IGLESIAS D.J., AGYSTI M., TALON M. Delay of early fruitlet abscission by branch girdling in citrus coincides with previous increases in carbohydrate and gibberellin concentrations. Plant Growth Regulation, **58** (1), 15, **2009**.
16. NISHIJIMA T., KOSHIOKA M., YAMAZAKI H., MIURA H., MANDER L.N. Endogenous gibberellins and bolting in cultivars of Japanese radish. Acta Horticulturae, **394**, 199, **1995**.
17. UPADHYAYA A., DAVIS T.D., LARSEN M.H., WALSER R.H., SANKHLA N. Uniconazole-induced thermotolerance in soybean seedling root tissue. Physiologia Plantarum, **79** (1), 78, **1990**.
18. HUSSEIN M.M., BAKHETA M.A., ZAKI S.N. Influence of uniconazole on growth characters, photosynthetic pigments, total carbohydrates and total soluble sugars of hordium vulgare l plants grown under salinity stress.

International Journal of Science and Research, **3** (12), 2208, **2014**.
19. KIM H.Y., CHOI B.J., SANG C.K. Effects of uniconazole on the drought resistance of Pilea cadierei-(I)-Morphological changes and water loss in leaves. Journal of the Korean Society for Horticultural Science, **35**, 493, **1994**.
20. AHMAD I., KAMRAN M., ALI S., CAI T., BILEGJARGAL B., LIU T.N., HAN Q.F. Seed filling in maize and hormones crosstalk regulated by exogenous application of uniconazole in semiarid regions. Environmental Science and Pollution Research, **25** (33), 33225, **2018**.
21. LI L., VANSTADEN J. Effects of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. Plant Growth Regulation, **24** (1), 55, **1998**.
22. YAN Y.H., YAN W., LIU W.G., WANG X.C., YONG T.W., YANG W.Y., ZHAO L.L. Influence of seed treatment with uniconazole powder on soybean growth, photosynthesis, dry matter accumulation after flowering and yield in relay strip intercropping system. Plant Production Science, **18** (3), 295, **2015**.
23. CHAOUI A., MAZHOUDI S., GHORBAL M.H., FERJANI E.E. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzymes activities in bean (*Phaseolus vulgaris* L.). Plant Science, **127** (2), 139, **1997**.
24. VELIKOVA V., YORDANOV I., EDREVA A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science, **151** (1), 59, **2000**.
25. STEWART R.R., BEWLEY J.D. Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiology, **65** (2), 245, **1980**.
26. PATRA H.K., KAR M., MISHRA D. Catalase activity in leaves ad cotyledons during plant development and senescence. Biochemical Pharmacology, **172** (4), 385, **1978**.
27. NICKEL K.S, CUNNINGHAM B.A. Improved peroxidase assay method using Ieuco 2, 3', 6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. Analytical Biochemistry, **27** (2), 292, **1969**.
28. BATES L.S., WALDREN R.P., TEARE I.D. Rapid determination of free proline for water stress studies. Plant and Soil, **39** (1), 205, 1**973**.
29. GONG X.M., LIU Y.G., HUANG D.L., ZENG G.M., LIU S.B., TANG H., ZHOU L., HU X., ZHOU Y.Y., TAN X.F. Effects of exogenous calcium and spermidine on cadmium stress moderation and metal accumulation in *Boehmeria nivea* (L.) Gaudich. Environmental Science and Pollution Research, **23** (9), 8699, **2016**.
30. BAUDDH K., SINGH R.P. Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* (L.) and *Brassica juncea* (L.) in salinity and drought affected cadmium contaminated soil. Ecotoxicology and Environmental Safety, **85**, 13, **2012**.
31. KIEKENS L., CAMERLYNCK R. Transfer characteristics for uptake of heavy metals by plants. Landwirtsch Forsch, **39**, 255, **1982**.
32. HADI F., BANO A., FULLER M.P. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere, **80** (4), 457, **2010**.
33. ZHENG G., LV H.P., GAO S., WANG S.R. Effects of cadmium on growth and antioxidant responses in Glycyrrhiza uralensis seedlings. Plant Soil and Environment. **56** (11), 508, **2010**.
34. HADI F., ALI N., AHMAD A. Enhanced phytoremediation of cadmium-contaminated soil by Parthenium hysterophorus plant: Effect of gibberellic acid (GA3) and synthetic chelator, alone and in combinations. Bioremediation Journal, **18** (1), 46, **2014**.
35. THAPAR R., SRIVASTAVA A.K., BHARGAVA P., MISHRA Y., RAI L.C. Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated Anabaena doliolum. Journal of Plant Physiology, **165** (3), 306, **2008**.
36. VAJPAYEE P., TRIPATHI R.D., RAI U.N., ALI M.B., SINGH S.N. Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. Chemosphere, **41** (7), 1075, **2000**.
37. DONG J., WU F.B., ZHANG G.P. Effect of cadmium on growth and photosynthesis of tomato seedlings. Journal of Zhejiang University Sciences, **6B** (10), 974, **2005**.
38. GHORBANLI M., SEPEHR M.F., KAVEH S.H. Effects of cadmium and gibberellin on growth and photosynthesis of Glycine max. Photosythetica, **37** (4), 627, **2000**.
39. MOYA J.L., ROS R., PICAZO I. Heavy metal-hormone interactions in rice plants: Effects on growth, net photosynthesis, and carbohydrate distribution. Journal of Plant Growth Regulation, **14** (2), 61, **1995**.
40. ZHENG M., TAO Y., HUSSAIN S., JIANG Q., PENG S., HUANG J., CUI K., NIE L. Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. Plant Growth Regulation, 7**8** (2), 167, **2015**.
41. VERBRUGGEN N., HERMANS C. Proline accumulation in plants: a review. Amino Acids, **35**, 753, **2008**.
42. CARETTO S., PARADISO A., D'AMICO L., DE GARA. L. Ascorbate and glutathione metabolism in two sunflower cell lines of differing α- tocopherol biosynthetic capability. Plant Physiology and Biochemistry, **40** (6-8), 509, **2002**.
43. ANJUM S.A., TANVEER M., HUSSAIN S., BAO M., WANG L.C., KHAN I., ULLAH E., TUNG S.A., SAMAD R.A., SHAHZAD B. Cadmium toxicity in maize (*Zea mays* L.): consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. Environmental Science and Pollution Research, **22** (21), 17022, **2015**.
44. AHMAD A., HADI F., ALI N., JAN A.U. Enhanced phytoremediation of cadmium polluted water through two aquatic plants *Veronica anagallis-aquatica* and *Epilobium laxum*. Environmental Science and Pollution Research, **23** (17), 17715, **2016**.
45. DOTY S.L. Enhancing phytoremediation through the use of transgenics and endophytes. New Phytologist, **179** (2), 318, **2008**.
46. WANG W.Z., XU W.H., ZHOU K., XIONG Z.T. Research progressing of present contamination of Cd in soil and restoration method. Wuhan University Journal of Natural Science, **20** (5), 430, **2015**.