# EFFECT OF DIFFERENT NITROGEN FORMS IN ALLEVIATING CADMIUM-INDUCED CHANGES ON PLANT GROWTH, METAL ACCUMULATION, OXIDATIVE AND ANTIOXIDATIVE ENZYMES IN SOLANUM AETHIOPICUM PLANT

**Alieu Mohamed Bah[1] & Chen Xion Hong[1] & Edwin J.J. Momoh[2] &Guoping Zhang[1] & Feibo Wu[1]**

1. Department of Agronomy, College of Agriculture and Biotechnology, Huajiachi Campus, Zhejiang University, Hangzhou 310029, People's Republic of China

2. Department of Crop Science, School of Agriculture, Njala University, Njala Campus, Sierra Leone West Africa

## ABSTRACT

*Investigation was carried out to study the effect of different nitrogen forms in alleviating cadmium-induced changes on plant height, Leaf number, and Leaf dry weight (DW).The study also include Cd concentration in plant tissues and oxidative stress under two Cd levels (0 and 100mg Cd kg−1 soil).in African egg plant (Solanum aethiopicum) at the first and second growth stages. African egg plants were transplanted into soil that was spiked with Cd which had been pre-incubated for two weeks, after which it was followed by the addition of 3mM of different nitrogen forms,$(NH_4)_2SO_4$, $CO(NH_2)_2$, $NH_4NO_3$, and $Ca(NO_3)_2$ respectively. Cadmium addition had a distinct effect on SPAD value, and the effect was also dependent on Cd treatment and growth stage. Cd addition into the soil significantly increased Cd concentrations in plant tissues with the roots in both stages accounting for the highest. The result showed that Cd toxicity also causes oxidative stress, changing the activities of various antioxidant enzymes.*

**Keywords: Antioxidative enzyme. Heavy metal, Nutrition. Alleviating, African Egg Plant**

## Introduction

Over recent decades, the annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (1). Sources of cadmium contaminants in soils include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, municipal and industrial waste disposal sites, application of phosphorous fertilizers, atmospheric deposition and electronic industries [2, 3, 4, 5].

Cadmium is an irritant to the respiratory tract and exposure to this pollutant can lead to anaemia, renal damage, or osseous disease with effects similar to osteoporosis. Cd is a very toxic element for plants and animals and no essential biological functions were found until now [6]. Extreme cases of chronic Cd toxicity can result in osteomalacia and bone fractures, as characterized by the disease called Itai- Itai in Japan in the 1950s and 1960s, where local populations were exposed to Cd-contaminated food crops, principally rice. Many studies have shown that some garden vegetables are capable of accumulating relatively high levels of Cd from the soil [7, 8, 9.10], and accumulation of Cd in plant tissues is influenced by the availability of Cd in the soil [6, 11, 12, 13]. Moreover, its uptake and accumulation in plants poses a serious health threat to humans via the food chain [14].

The presence of excessive amounts of Cd in soil commonly elicits many stress symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism [15], and may thus strongly reduce biomass production. Although Cd is used in a number of industrial applications, the main source of Cd intake is through smoking and food [16].

The induction of some enzymes is considered to play a significant role in the stress metabolism, induced by the metal toxicity [17]. One possible mechanism via which elevated

concentrations of heavy metals may damage plant tissues is the stimulation of free radical production, by imposing oxidative stress [18]. The direct evidence of heavy metal induced oxidative stress is an enhanced level of lipid peroxidation and hydrogen peroxide production in both roots and leaves [19, 20].

Nitrogen (N) is an important component of many structural, genetic and metabolic compounds in plant cells. Nitrate ($NO_3$) is an abundant source of N in most soils and is readily assimilated by higher plants [21]. Basta et al. (1998[22] found that N fertilization may increase Cd accumulation in plants, and the effect varied markedly with changes in N source and application rate [23]. Reports available on effect of N form on Cd toxicity and uptake are inconsistent [23, 24].

Sierra Leone has undergone eleven years of senseless war and is now witnessing rapid industrialization and urbanization during the last one decade. As a consequence, environmental pollution has become an important issue that affects public health. In recent years, Cd contamination of soils, food crops and vegetables have been reported frequently in other parts of the world [25].

Urban and Peri-urban farmers in Sierra Leone often use sewage sludge and municipal solid waste as a means to supply required nutrient for growing vegetables, hence are particularly vulnerable to metal contamination. A more relevant approach towards solving this menace is to assess the risk of Cd concentration exceeding the national limits in different vegetables.

Scarlet eggplant, African eggplant or garden egg (Solanum aethiopicum L.) is an important solanaceous crop in West Africa, grown as a commercial crop for domestic consumption and also for export [26].

Our hypothesis is that different nitrogen forms on African Egg Plants (*Solanum aethiopicum*) 'Bitter Balls' can induce changes on plant growth, metal accumulation, oxidative and antioxidative enzymes in *Solanum aethiopicum* plant when exposed to soils spiked with cadmium

**Plant Materials and Treatments**

**Pot Preparation**

A pot experiment was conducted using agricultural soil collected from the experimental farm on Huajiachi Campus Zhejiang university （Hangzhou, China）during June and August 2010. The soil was dried under natural condition and crashed, sieved using 2mm wire mesh and then divided into 24 pots with three replicates each of eight sets.

Seeds of African Egg Plants (*Solanum aethiopicum*) 'Bitter Balls' were obtained from certified vegetable gardeners in Moyamba town, Moyamba District, southern Sierra Leone. The seeds were surfaced sterilized in 0.1% mercuric chloride solution and washed four times with distilled water followed by deionised water. The seeds were nursed in specially prepared pots and watered as required to maintain moisture. After five weeks, seedlings that were healthy and of uniform size were carefully selected and five seedlings transplanted into the already prepared pots containing 4.5kg of clayey silt with pH at 6.38, organic matter 1.73%, total nitrogen about 0.072%, available P 84.51mg/kg and K 265.51 mg/kg respectively. The temperature during the experimental period indicated an average minimum and maximum around 25 to 39°C respectively.

Cadmium was added once as $CdCl_3$ in solution to the soil before transplanting, and 500ml water for each of the control pots and 500ml g $L^{-1}$ $CdCl_2$ solution per pot added for Cd treatments. The soil was pre-incubated for two weeks before transplanting, after which it was followed by the addition of 3mM of different nitrogen forms,$(NH_4)_2SO_4$,(1) $CO(NH_2)_2$,(2) $NH_4NO_3$,(3) and $Ca(NO_3)_2$ (4) respectively. Treatment with nutrition solution containing CO $(NH_2)_2$(126.7 g $L^{-1}$), $(NH_4)_2SO_4$, (101.9 g $L^{-1}$), $NH_4NO_3$（61.78 g $L^{-1}$）, Ca $(NO_3)_2$. $4H_2O$(182.4 g $L^{-1}$), $K_2SO_4$(23.85 g $L^{-1}$), $CaSO_4.2H_2O$(59.2 g $L^{-1}$), $KH_2PO_4$(24.8 g $L^{-1}$), $MgCl_2.6H_2O$(110.9 g $L^{-1}$), $CaCl_2$(40.5 g $L^{-1}$), and $MgSO_4$(65.9 g $L^{-1}$) were added twice with the first application at the growth stage and the second at the vegetative stage ensuring that the different nitrogen forms were considered according to the experimental design.

After 14 days at room temperature, seedlings were thinned to leave 3 uniform and healthy seedlings in each pot and continued to be watered as required to maintain moist. Treatments were arranged in a completely randomized block design with 3 replications and on alternate weeks the pots were shifted to minimize any positional effects and seedlings were maintained in the green house at room temperature. All reagents were analytical grade and all stock solutions were made with deionized water.

After 20 days (d) of heavy metal application during the first stage (which was 56 d after sowing) and 53 d of heavy metal applications in the case of the second stage (which was 89 d after sowing) plants were sampled for the determination of the following traits.

**Sampling and Measurement.**

Measurement of metal uptakes in plant parts, and Plant height, leaf number, and DW (Root, stem, leaf)

After 56 and 89 days of sowing, plants were carefully harvested at the vegetative stage ensuring minimal damage to the roots. Immediately, the plants were washed well with tap water and dipped into EDTA for 3 hours and then washed again with tap water and rinsed in deionised water and separated into terrestrial parts (stems and leaves) and underground parts (roots). Plant height and leaf number were simultaneously measured then oven dried at 70˚C for 48 h to a constant weight. Dried weight was measured and the samples were powdered with mortar and pestle, then ashed at 550˚C for 12 h. The ash was digested with 5ml 30% $HNO_3$ for 8hours and then diluted using deionized water [27]. Cd and mineral concentration (K, Ca, Mg, Fe, Cu, Mn, and Zn) were determined using a flame atomic absorption spectrometry (SHIMADZU AA-6300).

Determination of MDA content and antioxidative enzymes activities (SOD, POD, CAT, and APX) Leaves.

After 20 and 53 days of treatment, leaves were collected (weight 0.5000g) with 4 replicates, washed thoroughly with deionised water, and immediately frozen in liquid nitrogen and then stored frozen at -80°C for the determination of MDA contents and antioxidative enzyme activities.

The level of Lipid peroxidation in plant tissues was estimated by the formation of MDA, as described by [28] with slight modification. Plant samples (0. 5000g; leaf) were homogenized in 9 ml of 0.1% trichloro acetic acid (TCA). The homogenate was centrifuged at 10,000 for 10 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. Mixture was heated at 95°C for 30 min and then cooled quickly on ice bath. The resulting mixture was centrifuged at 12,000g for 15 min and the absorbance of the supernatant was taken at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

The activity of POD (EC 1.11.1.7) was assayed according to the method described by [28] using pyrogallol as a substrate. One unit of POD activity was defined as the amount of enzyme necessary to obtain 1 mg of purpurogallin from pyrogallol in 20 s, at 420 nm. A control set was prepared by using distilled water instead of enzyme extract

The total activity of SOD (EC 1.15.1.1) was assayed by using the method described by [28] with slight modification.

The oxidation of ascorbate was initiated by $H_2O_2$, and the decrease at 290nm was monitored for 1.5 min. One unit of APX was defined as the amount of enzyme required to oxidize 1 mM of ascorbate

Activity of CAT (EC 1.11.1.6) in leaves was measured by the method as described in [29]. CAT activity was determined by monitoring the disappearance of $H_2O_2$ at 240 nm on UV–vis spectrophotometer and expressed in enzyme units (mg protein)$^{-1}$ by using the extinction coefficient ($\varepsilon$) 0.036mM$^{-1}$ cm$^{-1}$. One unit of enzyme is the amount necessary to decompose 1 _mol of $H_2O_2$ per min at 25 ◦C

**Data analysis**

Statistical analysis was performed with the DPS statistical software [30]. The significance of differences between the means of the treatments was evaluated by two-way analysis of variance followed by the least significant difference (LSD) test using the fisher method. Correlation analysis was performed to determine the relationships between the different metals accumulated in the leaves, roots, and shoots interaction respectively. Data are shown as the mean ± standard deviation (SD)

**Results**

Effect of different nitrogen forms in alleviating cadmium-induced changes on SPAD Value, Plant height, leaf number, and DW (Root, stem, leaf)

Effect of different nitrogen forms in alleviating cadmium-induced changes had a distinct effect on SPAD value, and the effect was also dependent on Cd treatment and growth stage (Table 1). It was observed that CO $(NH_2)_2$ and $NH_4$ $(SO_4)_2$, at first stage were almost at equilibrium, but significantly higher than Ca $(NO_3)_2$ and $NH_4NO_3$. However, the story was completely different at the second growth stage as $(NH_4)_2SO_4$ was significantly greater than the other three N forms. It was further observed that for the plants growing under normal condition (no Cd addition), SPAD value in first and second growth stage was higher for CO

$(NH_2)_2$-treated plants than those of $NH_4$ ( $SO_4)_2$, $NH_4NO_3$and Ca $(NO_3)_2$ treatments, at both growth stages.

Only root, stem and leaves were examined for growth parameters, for the plants were at the vegetative growth stage without fruit development. There were no visual symptoms of metal toxicity such as chlorosis or necrosis on the plants observed even though the soil was spiked with Cd.

The result showed that there was a significant difference in plant height among four N forms, with CO $(NH_2)_2$ −-treated plants having the highest compared with the other N forms under Cd treatments in the first growth stage. It was observed that the addition of Cd significantly increased the plant height in $NH_4$ $(SO_4)_2$ -treated plants in comparison to the other three N forms with Urea and $NH_4NO_3$-treated plants at equilibrium.

From the results, it was observed that plants growing under normal conditions (no Cd addition) had no significant differences among three N forms except for $NH_4NO_3$ that was slightly lower at the first growth stage in terms of the number of leaves acquired. However, the addition of Cd to the soil resulted to significant differences among the N forms with $NH_4NO_3$ and $(NH_4)_2SO_4$.-treated plants markedly increased in comparison to the other N forms. Meanwhile, at the second growth stage $(NH_4)_2SO_4$ was significantly increased in comparison to the other N forms with CO $(NH_2)_2$ and Ca $(NO_3)_2$ having no significant differences and $NH_4NO_3$.-treated plants slightly been reduced in the control plants. However, the addition of Cd to the soils showed increased in $NH_4$ $(SO_4)_2$, followed by CO $(NH_2)_2$ then Ca $(NO_3)_2$ and $NH_4NO_3$.-treated plants accordingly.

The root, stem and leaf dry weight showed that at both growth stages of the normal plants (no Cd addition) indicated that CO $(NH_2)_2$ was significantly higher than the other N forms. The story for the first growth stage for plants treated with Cd indicated that CO $(NH_2)_2$.-treated plants markedly increased in comparison to the other N forms. We observed that the second growth stage showed that the root, stem and leaf dry weight in $(NH_4)_2SO_4$- treated plants was significantly higher than the other N forms.

Effect of different nitrogen forms in alleviating cadmium-induced changes on root, stem and leaf macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the first and second growth stage

The result indicated that the effect of different nitrogen forms in alleviating cadmium-induced changes on root, stem and leaf macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum*

*aethiopicum* at the first（1st） and second (2nd) growth stages varied depending on the N form used respectively (tables,3,4,5,6,7,8).

We observed that when the plants grow in the soil without Cd addition, Cd was not available in the CO $(NH_2)_2$, $NH_4$ $(SO_4)_2$, $NH_4NO_3$and Ca $(NO_3)_2$ -treated plants. Our result showed that Cd addition into the soil significantly increased Cd concentrations in plant tissues with the roots in both stages accounting for the highest. Our result showed that Cd was available in CO $(NH_2)_2$ (2.3%), $NH_4$ $(SO_4)_2$ (53.8%), $NH_4NO_3$(24.9%) and Ca $(NO_3)_2$ (19.0)%) -treated plants on the average at the first growth stage, while at the second growth stage the amount of Cd accumulated was CO $(NH_2)_2$ (6.3%), $NH_4$ ( $SO_4)_2$ (52.1%), $NH_4NO_3$(24.6%) and Ca $(NO_3)_2$ (16.9%) in the roots respectively on the average. The result showed that Ca, Mg, K, Mn and Cu was significantly increased in the $NH_4$ $(SO_4)_2$-treated plants when compared with the other N forms, while Fe was significantly increased in CO $(NH_2)_2$-treated plants with Zn being highest in Ca $(NO_3)_2$-treated plants at the first growth stage in the roots in comparison to the other N forms without Cd addition (Control).

Cd addition(1st growth stage) resulted to significant increase in Mg, Fe, Mn and Cu in $NH_4$ $(SO_4)_2$ -treated plants, while Ca, K, and Zn was significantly increased in $NH_4NO_3$-treated plants at the first growth stage in the roots in comparison to the other N forms. Meanwhile, at the second growth stage in the roots, the result indicated that Ca, Mg and K was highest in Ca $(NO_3)_2$-treated plants, Fe and Mn was significantly increased in $NH_4NO_3$-treated plants and Cu and Zn was markedly increased in the $NH_4$ ( $SO_4)_2$ -treated plants when compared with the other N forms without Cd addition ( Control).

However, Cd addition (2nd growth stage) resulted to significant increase in Fe, Mn, Cu and Zn in $NH_4$ $(SO_4)_2$ -treated plants, Mg and K in $NH_4NO_3$-treated plants and Ca in Ca $(NO_3)_2$-treated plants when compared with the other N forms respectively.

From our result it was shown that Cd was available in CO $(NH_2)_2$ (56.7%), $NH_4$ ( $SO_4)_2$ (32.5%), $NH_4NO_3$(4.3%) and Ca $(NO_3)_2$ (6.6)%) -treated plants on the average at the first growth stage(Table5), while at the second growth stage the amount of Cd accumulated was CO $(NH_2)_2$ (23.9%), $NH_4$ ( $SO_4)_2$ (32.4%), $NH_4NO_3$(19.0%) and Ca $(NO_3)_2$ (24.7%) in the stems(Table6) respectively on the average. The results indicated that at the control level, Ca, K, Cu and Zn and Mg, Fe and Mn were significantly increased in $NH_4NO_3$ and CO $(NH_2)_2$ respectively when compared with the other N forms in the first growth stage. However, at the second growth stage, Ca, Mg and K, and Fe, Cu and Zn were significantly increased in Ca $(NO_3)_2$-treated plants and $NH_4(SO_4)_2$ treated plants respectively..

The result revealed that the addition of Cd showed significant increases in Ca, Mg, Fe and Mg content in $NH_4$ ( $SO_4)_2$ -treated plants when compared with the other N forms, with K highest in Ca $(NO_3)_2$ -treated plants and Cu and Zn recorded significantly in CO $(NH_2)_2$-treated plants respectively at the first growth stage. Meanwhile at the second growth stage, Ca, Mg, Fe, Mn, and Zn was significantly increased CO $(NH_2)_2$-treated plants respectively when compared with other N forms. However K and Cu were recorded highest in Ca $(NO_3)_2$ -treated plants and $NH_4$ $(SO_4)_2$ treated plants respectively.

It was observed that Cd was accumulated in the leaves (Table 7,8) by CO $(NH_2)_2$ (11.0%), $NH_4$ ( $SO_4)_2$ (35.7%), $NH_4NO_3$(38.7%) and Ca $(NO_3)_2$ (14.7)%) -treated plants on the average at the first growth stage, while at the second growth stage the amount of Cd accumulated was CO $(NH_2)_2$ (29.9%), $NH_4$ ( $SO_4)_2$ (27.5%), $NH_4NO_3$(28.8%) and Ca $(NO_3)_2$ (13.8%) in the leaves respectively on the average. For Ca accumulation, no significant differences was recorded for $NH_4$ $(NO_3)_2$ -treated plants and $NH_4$ $(SO_4)_2$ treated plants respectively, whereas for Mg, no significant differences was recorded for Ca $(NO_3)_2$ -treated plants and CO $(NH_2)_2$-treated plants respectively. Meanwhile, K, Mn and Cu was highest in $NH_4$ $(SO_4)_2$- treated plants and Fe and Zn highest in $NH_4$ $(NO_3)_2$ -treated plants respectively in the first growth stage without Cd addition (Control).

At the second growth stage, Ca, Zn and Cu was observed to be significantly increased in Ca $(NO_3)_2$ -treated plants with K showing no significant differences for plants treated with CO $(NH_2)_2$ , $NH_4$ ( $SO_4)_2$ , and Ca $(NO_3)_2$ respectively. However, Mg, Fe and Mn were significantly increased in $NH_4$ $NO_3$ -treated plants respectively in the second growth stage without Cd addition (Control).

The addition of Cd to the soil significantly resulted to the increase in Ca, Mg, Fe and Mn $NH_4$ $(SO_4)_2$- treated plants at both growth stages. It was observed that K was significantly increased in CO $(NH_2)_2$-treated plants and Ca $(NO_3)_2$ -treated plants in the first and second growth stages according. However, we observed that Cu and Zn was significantly increased in CO $(NH_2)_2$-treated plants at the first growth stage and in $NH_4$ $(SO_4)_2$- treated plants in the second growth stage respectively.

The effect of different nitrogen forms in alleviating cadmium-induced changes on SOD and APX at the first (k and m) and second (l and n) growth stage, POD and CAT at first (q and s) and second (r and t) growth stage and MDA at first (o) and second (p) growth stages in *Solanum aethiopicum*.

From the results, it was observed that at both growth stages, Ca $(NO_3)_2$ -treated plants showed significant increases in leaf MDA activity for the plants grown under normal condition (Fig. (o) and (p)). However, for the plants exposed to Cd stress, the significant difference among four N fertilizers in leaf MDA activity was found, with $NH_4$ $NO_3$ -treated plants having the highest at the first growth stage. While $NH_4$ $(SO_4)_2$- treated plants at the second growth stage showed significant increases in MDA activity when compared with the other N forms accordingly.

In addition, POD activity was highest in CO $(NH_2)_2$-treated plants in the first growth stage (Fig. q). Whereas in the second growth stage, Ca $(NO_3)_2$ -treated plants showed significant increases in leaf POD activity for the plants grown under Cd condition (Fig. r). For CAT activity at the first growth stage Ca $(NO_3)_2$ -treated plants showed significant increases , while at the second growth stage CAT activity was highest in $NH_4$ $NO_3$ -treated plants under Cd stress condition(Fig. s, t).. Also Cd addition resulted to significant increases of SOD activity in Ca $(NO_3)_2$ -treated plants when compared with the other N forms (Fig. k).. However, in the second growth stage, $NH_4$ $NO_3$ -treated plants and $NH_4$ $(SO_4)_2$- treated plants showed no significant differences when compared with the other N forms accordingly (Fig. l ). For APx activity under Cd stress conditions, Ca $(NO_3)_2$ -treated plants and $NH_4$ $(SO_4)_2$- treated plants were increased in the first and second growth stages respectively when compared with the other N forms(Fig. m, n)..

**Correlations among the Eight Elements**

The relationship among the eight elements was analyzed for their root, stem and leaf concentrations (Table 9, 10, 11, 12, 13, 14). In the roots in the first growth stage a very strongly positive correlation with Mg was only detected in Cd. We observed that there was significant correlation between K and Ca, Mg. Furthermore, Fe was very strongly positively correlated with Cd, Mg. The significantly positive correlation was also observed between Mn and Cd, Mg, Fe and Cu very strongly positively correlated with Cd, Mg, Fe and Mn and significantly correlated with K. Also Zn was significantly positively correlated with Ca, Mg and Cu, and very strongly positively correlated with K respectively. Meanwhile, at the second growth stage we observed that Cd was significantly positively correlated with Mg, Fe, and Zn and that Fe was strongly positively correlated with Mn However, there was strong negative correlation between Zn and K. Meanwhile, in the stem at the first growth stage a significant positive correlation occurred between Mg and Ca, between Zn and K, between Fe and Ca, Mg, between Mn and Ca, Mg, Fe, Between Cu and Cd, and between Zn and Cd, Cu. The

results also showed that K was strongly negatively correlated with Cd respectively. Furthermore, at the second growth stage Mg was significantly correlated with Ca. We also observed a very strong correlation between Fe and Mg, between Mn and Ca, Mg, Fe and between Zn and Mg, Fe, Mn accordingly. The results for the leaf at the first growth stage showed that Mg was strongly positively correlated with Ca and Cd, respectively. Very strong significantly positive correlation was observed between Fe and Cd, Mg, between Mn and Cd, Ca, Mg, between Cu and Ca, Mg, Fe and that Cu showed significant negative correlation with K respectively. However, at the second growth stage a positively strongly correlation was observed between Mg and Cd, Ca, between Fe and Ca, Mg, Mn and Ca, Mg, Fe, between Cu and Cd, Ca, Fe and Mg, and between Zn and Cd, Ca, Fe and Mg. We also observed that there was strong negative correlation between Zn and K, between K and Ca, Mg and between Mn and K respectively.

**Discussion**

Depending on the content of reduced or oxygenated forms of nitrogen, or accompanying nutrients, fertilizers can invoke varied physiological effects in plants, e.g. on the level of nitrate accumulation ( Smoleń et al. 2006) or heavy metal accumulation [23, 31].

Cd toxicity may cause essential nutrient deficiency and changes in the concentration of basic nutrients such as N and P in plant tissues and it has been revealed that Cd is strongly phytotoxic, and causes growth inhibition and even plant death due to its interaction with photosynthesis, respiration and nitrogen assimilation in plants [32]. No visual symptoms of metal toxicity of chlorosis and necrosis were observed on the leaves of *Solanum aethiopicum* at the first ($1^{st}$) and second ($2^{nd}$) growth stages grown in 500ml 1g $L^{-1}$ $CdCl_2$ solution per pot added for Cd treatments to the soil before transplanting respectively.

From the results, it was observed that there was a significant difference in plant height among four N forms, with CO $(NH_2)_2$ −-treated plants having the highest compared with the other N forms under Cd treatments in the first growth stage, while it was observed at the second growth stage significant increase in the plant height in $(NH_4)_2SO_4$-treated plants in comparison to the other three N forms..

From the results, it was also observed that Cd addition had an antagonistic effect on plant height, leaf number, root, stem and leaf dry weight and SPAD value, at the first growth stage respectively. Meanwhile at the second growth stage for plant height, leaf number, SPAD value, root, stem and leaf dry weight, were drastically reduced in the different N forms when compared with the control. Our result finding was in tandem with other researchers. [33] in

bean, [34] in barley, and [31] in rice who also reported drastic reduction in plant growth as indicated by the leaf number and plant biomass.

Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil [35]. The result finding was in agreement with them as it was observed that when the plants were grown in the soil without Cd addition, Cd was not available in the CO $(NH_2)_2$, $NH_4$ $(SO_4)_2$, $NH_4NO_3$and Ca $(NO_3)_2$ -treated plants, while Cd addition into the soil significantly increased Cd concentrations in plant tissues with the roots in both stages accounting for the highest i.e. , $NH_4$ $(SO_4)_2$ >$NH_4NO_3$> Ca $(NO_3)_2$ >CO $(NH_2)_2$ in the order of accumulation respectively. The result findings was in total agreement with (31) who also reported similar Cd concentration in the roots of rice

From the results, addition of Cd showed that N was significantly increased in Ca $(NO_3)_2$ -treated plants at the first growth stage respectively which was contrary to the second growth stage, as the addition of Cd resulted to significant increases of N in CO $(NH_2)_2$-treated plants respectively. The result findings was completely in agreement with other research results [36] who also reported that CO $(NH_2)_2$ -treated plants have more N accumulation when compared with $(NH_4)_2SO_4$-treated plants and $NH_4NO_3$.-treated plants .In agreement with [31] and [36] it was observed in the results that the form of N significantly affected both N and Cd accumulation in the leaves of plant. The result findings further indicated that the age of the plants played significant roles in the ability of the plant to accumulate Cd and N in the leaves as it was shown in the case of CO $(NH_2)_2$ -treated plants that significantly improved in their accumulation in the second growth stage, while Ca $(NO_3)_2$ -treated plants also significantly reduced in their accumulations reasons that need further investigation.

Cd in plants can cause oxidative stress by favoring the production of reactive oxygen species and lipid peroxidation [37]. Cd is known to disrupt the plant defense system against naturally occurring reactive oxygen species. This antioxidant defense system mainly includes the antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) and many more. Our result showed that MDA content was drastically reduced in the plants implying that Cd caused oxidative stress in the plants at the first growth stage. Our result findings was in agreement with [38], who reported that Cd toxicity in plants has been shown to be mediated by the inhibition of antioxidases resulting in lipid peroxidation and superoxide anion generation thereby causing serious stress to the plants. Meanwhile at the second growth stage, MDA was enhanced accordingly when compared with those plants that where grown without the addition of Cd (Control) Increased

MDA in Cd treatments, accordingly, in agreement with our previous findings [39] suggests that Cd stimulate lipid peroxidation, resulting in oxidative stress

Exposure to Cd has the propensity to stress induction in which the role of oxidative stress and reactive oxygen species (ROS) production may be involved. Furthermore, relating to enzymatic antioxidants, different or even controversial patterns of heavy metal toxicity on the activity of antioxidant enzymes scavenging ROS, including superoxide dismutase (SOD; EC1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11), have been reported [28] in different plant species, tissues analyzed, concentration, and duration of metal exposure. For example, it has been demonstrated that Cd activated CAT [38], SOD and POD [28], and APX [28, 39]. Conversely, a decrease in SOD [40], CAT [41], APX and GR [42] activities were also reported under Cd exposure.

Antioxidative enzymes are the most important components in the scavenging system of ROS. Superoxide dismutase (SOD; EC 1.15.1.1) is a major scavenger of superoxide (O2_+), and its enzymatic action results in the formation of $H_2O_2$ and $O_2$. The hydrogen peroxide produced is then scavenged by catalase (EC 1.11.1.6) and a variety of peroxidases (POD: EC 1.11.1.7). Catalase, which is apparently absent in the chloroplast, dismutates $H_2O_2$ into water and molecular oxygen [43], whereas POD decomposes $H_2O_2$ by oxidation of co-substrates such as phenolic compounds and/or antioxidants.

. From our results finding, it was shown that Cd addition activated both POD and CAT at the second growth stage in all of the different N forms. Meanwhile, $NH_4 (SO_4)_2$, and Ca $(NO_3)_2$ -treated plants, Cd treated plants activated SOD activities, whereas in CO $(NH_2)_2$ -treated plants SOD was repressed. Also in $NH_4 (SO_4)_2$, and Ca $(NO_3)_2$ -treated plants, APX activities was activated accordingly. In tandem with our previous findings [39], Cd stress induced increase in SOD, POD, and APX activities accordingly.

ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids, creating oxidative injury that results in a reduction of plant growth and development [44]. The result findings was in agreement with their own findings as the plant biomass at both growth stages under Cd stress irrespective of the N forms were reduced in comparison to the controls ( without Cd addition).

The result indicated that the effect of different nitrogen forms in alleviating cadmium-induced changes on root, stem and leaf macro- and micronutrients concentration varied depending on the N form used respectively. From our results, we observed that though $(NH_4)_2SO_4$-treated

plants showed significant increases in terms of Cd accumulation in the roots, yet poor translocation of Cd into the leaves was recorded with just 15.7 and 12.8% of the total accumulated in the roots accrued in the leaves. Inversely, in terms of plant height, soluble sugar and plant biomass, the effect of Cd addition was more adverse on $(NH_4)_2SO_4$-treated plants

In conclusion, this study showed that Cd addition into the soil significantly increased Cd concentrations in plant tissues with the roots in both stages accounting for the highest i.e. , $NH_4 (SO_4)_2$ >$NH_4NO_3$> Ca $(NO_3)_2$ >CO $(NH_2)_2$ in the order of accumulation respectively. Also, there was a significant difference of SOD, POD, CAT and APX activities and MDA content in the two growth stages under different N forms and it could be ascribed to the difference in mechanisms underlying oxidative stress injury and subsequent tolerance to Cd. The observed poor translocation of Cd in the leaves of $(NH_4)_2SO_4$-treated plants may be attributed to the function of sulfur in ameliorating Cd stress.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (30671256).

**References**

[1]. O .V. Singh, S. Labana,G. Pandey, R. Budhiraja, , R.K. Jain, Phytoremediation: an overview of metallicion decontamination from soil. Applied Microbiology and Biotechnology, 61,(2003). 405–412.

[2]. B. J. Alloway, Soil processes and the behavior of metals. In: Alloway B. J. (Ed), Heavy metals in soils (pp. 38–57). (1995). London: Blackie.

[3]. R.M. Harrison M.B. Chirgawi, The assessment of air and soil as contributors of some trace-metals to vegetable plants. 3. Experiments with field-grown plants. Science of the Total Environment 83(1989), 47–62.

[4]. A.P. Jackson, B.J. Alloway The bioavailability of cadmium to lettuce and cabbage in soils previously treated with sewage sludge's. Plant and Soil 132(1991)., 179–186

[5]. S.J Kim, A.C. Chang, A.L. Page, J.E. Warneke, Relative concentrations of cadmium and zinc in tissue of selected food plants grown on sludge-treated soils. Journal of Environmental Quality 17(1988), 568–573

[6]. B.J. Alloway, A.P. Jackson, H. Morgan, The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources. The Science of the Total Environment 91(1990), 223–236.

[7]. B.J Alloway, I, Thornton, G.A. Smart, J.C. Sherlock, M.J Quinn, Metal availability. The Science of the Total Environment 75(1988), 41–69.

[8]. T.E. Bahemuka, E.B. Mubofu, Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi rivers in Dar es Salaam, Tanzania. Food Chemistry 66(1999), 63–66.

[9]. G.P. Cobb, K. Sands, M .Waters, B.G. Wixson, E. Dorward-King, Accumulation of heavy metals by vegetables grown in mine wastes. Environmental Toxicology and Chemistry 19(2000), 600–607.

[10]. D.H. Khan, B. Frankland, Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil-profile and plant. Plant and Soil 70(1983), 335–345.

[11]. I. Ahumada, J. Mendoza, E. Navarrete, L. Ascar, Sequential extraction of heavy metals in soils irrigated with wastewater. Communications in Soil Science and Plant Analysis 30(1999), 1507–1519.

[12]. S.L. Brown, R.L. Chaney, J.S. Angle, J.A. Ryan, The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. Journal of Environmental Quality 27(1998), 1071–1078.

[13]. J. Hart, R. Welch, W. Norvell, L. Kochian, Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. Physiologia Plantarum 116(2002), 73–78.

[14]. G.J .Wagner, Accumulation of cadmium in crop plants and its consequences to human health. -Adv. Agron. 51. (1993). 173-212.

[15]. J.R. Moya, I. Ros, and Picazo, Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photosynthesis Research* 36(1993), 75–80.

[16]. L. Järup, M. Berglund, C.G. Elinder, G. Nordberg, M. Vahter, Health effects of cadmium exposure--a review of the literature and a risk estimate Scandinavian journal of work, environment & health 24(1998), Suppl 1:1-51

[17]. K. Smeets, A. Cuypers, A. Lambrechts, B. Semane,P. Hoet, A.V., Laere, J. Vangronsveld, Induction of oxidative stress and antioxidative mechanisms in Phaseolus vulgaris after Cd application. Plant Physiol. Biochem. 43(2005), 437–444.

[18]. C.H. Foyer, H. Lopez-Delgado, J.F. Dat, I.M. Scott, Hydron peroxide and glutathione associated mechanism of acclamatory stress tolerance and signaling. Physiol. Plant. 100(1997), 241–254.

[19]. U.H. Cho, N.H. Seo, Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci. 168(2005), 113–120.

[20]. V. Dixit, V. Pandey, R. Shyam, Differential responses to cadmium in roots leaves of pea (*Pisum sativum* L. cv. Azad), J. Exp. Bot. 52 (2001), 1101–1109.

[21]. C.O. Ajakaiye, Influence of soil applications of nitrogen on nitrate reductase activity, leaf and protein content in sorghum. Plant Soil 60(1981), 423–434.

[22]. N.T. Basta, W.R. Raun , F. Gavi, Wheat grain cadmium under long-term fertilization and continuous winter wheat production. Better Crops 82(1998), 14–25.

[23]. M.A. Maier, M.J. McLaughlin, M. Heap, M. Butt, M.K. Smart, Effect of nitrogen source and calcium lime on soil pH and potato yield, leaf chemical composition and tuber cadmium concentration, J. Pl. Nutr. 25 (2002) 523–544.

[24]. P.J. Florijn, J.A. Nelemans, M.L. van Beusichem, The influence of the form of nitrogen nutrition on uptake and distribution of cadmium in lettuce varieties. J. Plant Nutr. 15(1992), 2405–2416.

[25] Y. Li, Y.B. Wang, X. Gou, Y.B. Su, G. Wang, Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, China. Journal of Environmental Sciences-China 18(2006), 1124–1134

[26]. M.C. Daunay, R.N. Lester, G. Ano, Egg plant. In: Charrier, A., Jacquot, M., Hamon, S., Nicholas, D. (Eds.), Tropical Plant Breeding. Science Publishers, Plymouth(2001)..

[27]. W.D. Cheng, G.P. Zhang, H.G. Yao, P. Dominy, W. Wu, R.Y. Wang, Possibility of predicting heavy metal contents in rice grains based on DTPA-extracted levels in soil. Commun Soil Sci Plant Anal 35(2004), 2731–2745.

[28]. F.B. Wu, G.P. Zhang, P. Dominy, Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. Environ Exp Bot 50(2003),67–78

[29]. H .Aebi, Catalase in vitro. Method Enzymol 105(1984), 121-126.

[30]. Q. Tang, M.G..Feng, Practical statistics and its DPS statistical software package. China Agriculture Press, Bejing(1997)

[31]. M.J. Hassan, G. Zhang, Z. Zhu, Influence of cadmium toxicity on plant growth and nitrogen uptake in rice as affected by nitrogen form. J. Plant Nutr. 31(2008), 251-262.

[32]. L. Sanita, diX.X. Toppi, R. Gabbrielli, Response to cadmium in higher plants, Environ. Exp. Bot. 41 (1999) 105–130.

[33]. K. Padmaja, D.D.K. Prasad , A.R.K. Prasad, Inhibition of chlorophyll synthesis in Phaseolus vulgaris seedlings by cadmium acetate. Photosynthetica 24(1990), 399–405

[34]. F.B. Wu , G.P. Zhang, Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedlings. Bull. Environ. Contam. Toxicol. 69(2002), 219–227

[35]. F.M. Marshall, J.Holden, C. Ghose, B.Chisala, E. Kapungwe, J. Volk, M. Agrawal, R. Agrawal, R.K. Sharma, R.P. Singh, *Contaminated Irrigation Water and Food Safety for the Urban and Peri-urban Poor: Appropriate Measures for Monitoring and Control from Field Research in India and Zambia.* Inception Report DFID Enkar R8160, SPRU, University of Sussex. www.pollutionandfood. net (2007).

[36]. M.A. Jalloh J .Chen, F. Zhen, G. Zhang, Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress Journal of Hazardous Materials 162(2009), 1081–1085

[37]. A. Schützendübel, P. Schwanz, T. Teichmann, K. Gross, R. Langenfeld-Heyser, D.L. Godbold, A .Polle, Cadmium induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in scots pine roots. Plant Physiol 127(2001),887–898

[38]. K. Shah, R.G. Kumar, S. Verma, R.S. Dubey, Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings, Plant Sci. 161 (2001) 1135–1144.

[39]. A.M. Bah, H. Dai, J. Zhao, H. Sun, F. Cao, G. Zhang, F.B. Wu. Effects of Cadmium, Chromium and Lead on Growth, Metal Uptake and Antioxidative Capacity in Typha angustifolia. Biol, ele. Res (2010)

[40]. L. Sandalio, H. Dalurzo, M. Gomez, M. Romero-Puertas, L.A. del Rio, Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot 52(2001), 2115–2126

[41]. B.P. Shaw, Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of Phaseolus aureus. Biol Plant 37(4) (1995), 587–596

[42]. S.M. Gallego, M.P. Benavides, M.L. Tomaro, Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Sci 121(1996), 151–159

[ 43] Y. Rusina, N. Kaloyan, L. Christov, P. Petrova, Antioxidative enzymes in barley plants subjected to soil flooding. Environment and Experimental Botany 51(2004), 93–101.

[44]. M.J. Hernandez-jimenz, M.M. Lucas, M.F. Rosario, Antioxidant defense and damage in senescing lupin nodules. Plant Physiology and Biochemistry 40(2002), 645 – 657.

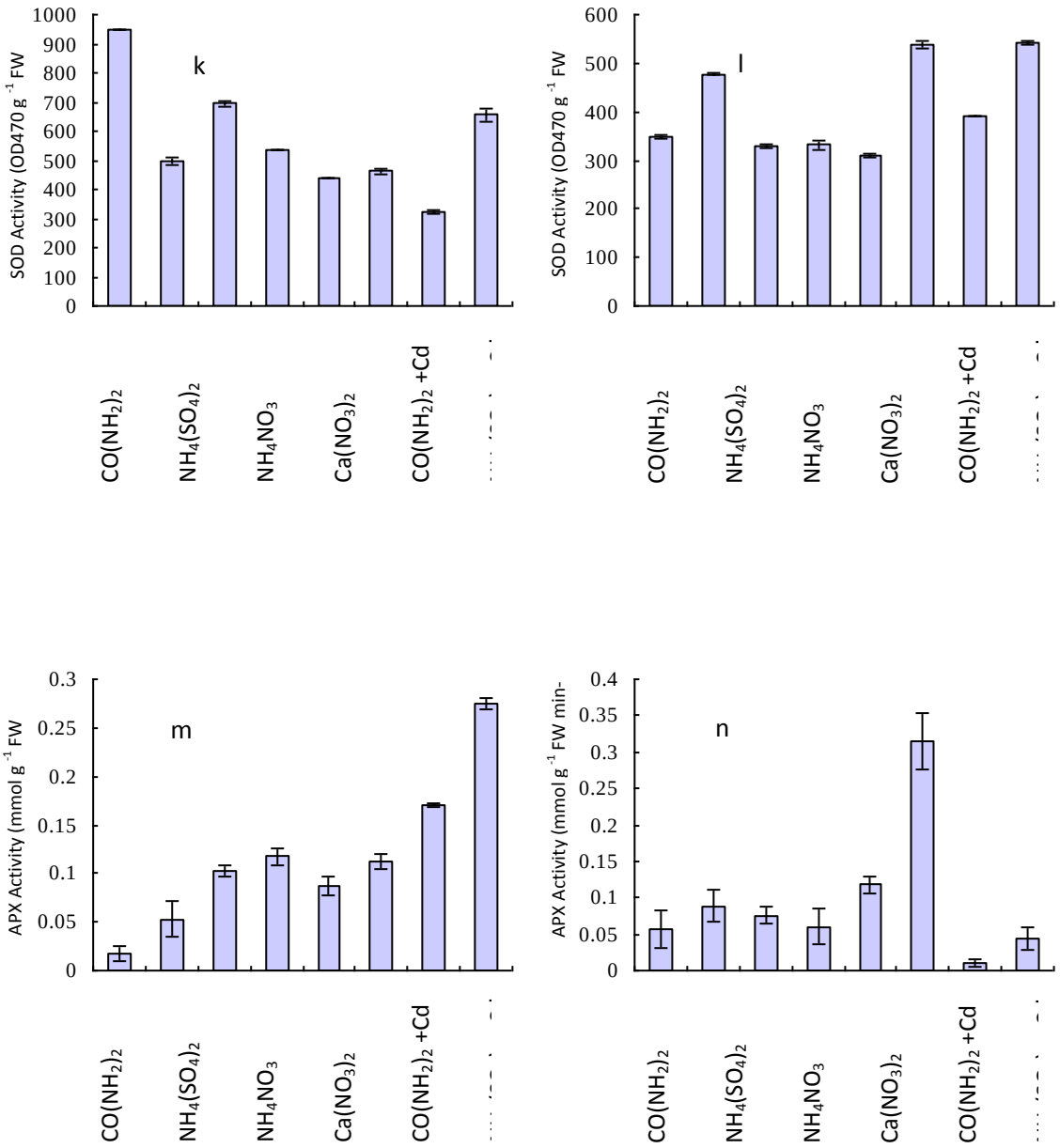


Fig 1. The effect of different nitrogen forms in alleviating cadmium-induced changes of SOD and APX in *Solanum aethiopicum* at the first (k and m) and second (l, and n) growth stage. Data are mean ± SE.

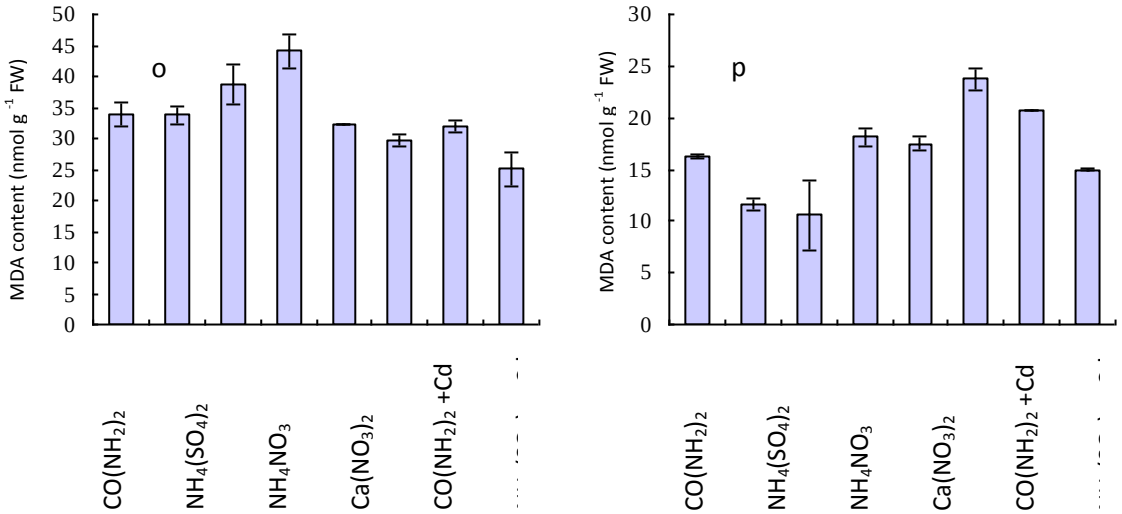


Fig 2. The effect of different nitrogen forms in alleviating cadmium-induced changes of MDA contents in *Solanum aethiopicum* at the first (o) and second (p) growth stage. Data are mean ± SE.

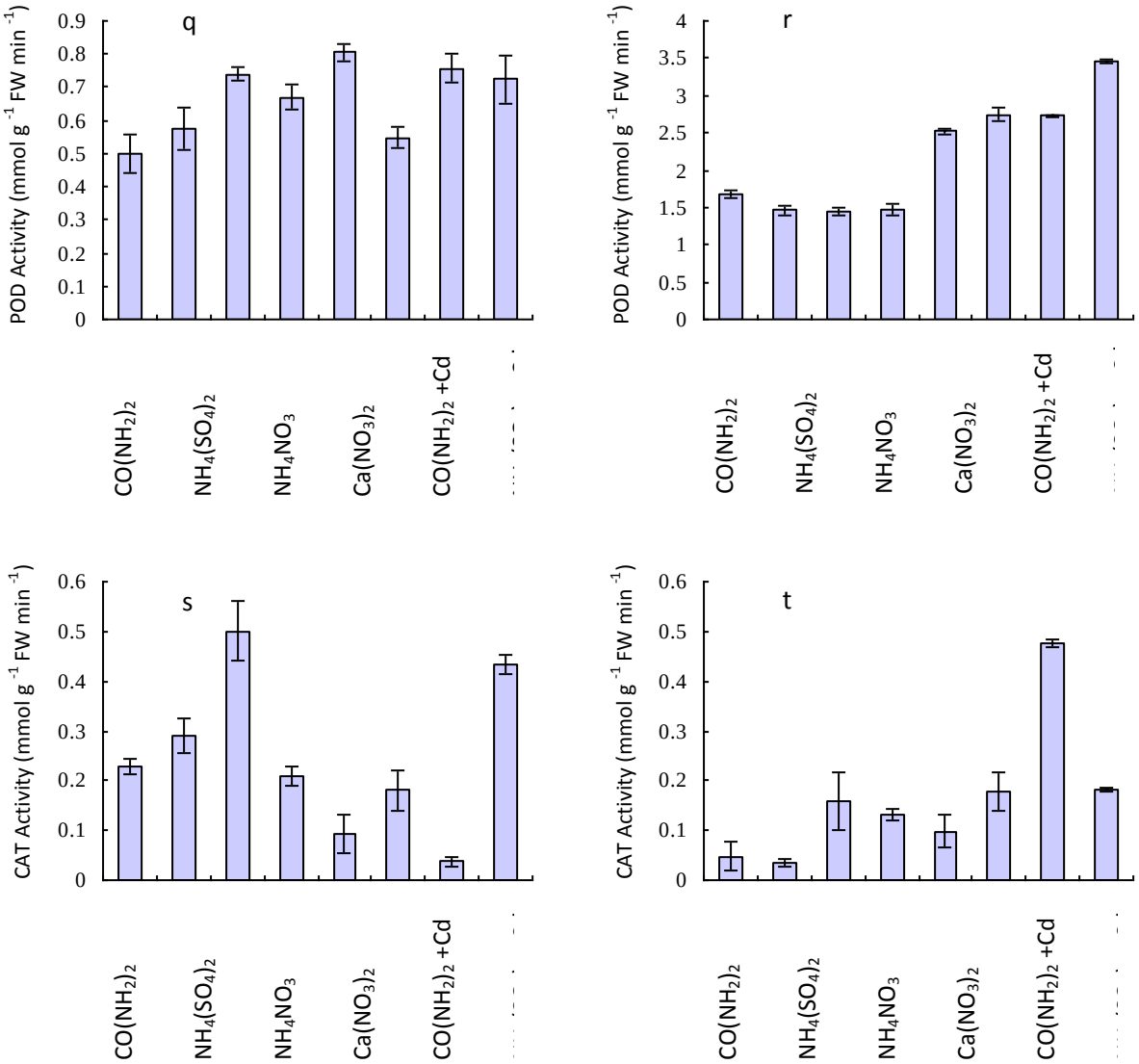


Fig 3. The effect of different nitrogen forms in alleviating cadmium-induced changes of POD and CAT in *Solanum aethiopicum* at the first (q and s) and second (r and t) growth stage. Data are mean ± SE.

Table 1： The effect of different nitrogen forms in alleviating cadmium-induced changes on SPAD Value, Plant Height (cm), Leaf Number , Plant biomass (g) and Nitrogen (%) of *Solanum aethiopicum* at the 1st growth stage

| Treatment | SPAD value | Plant Height (cm) | Leaf Number | Rt DW (g) | Stem DW (g) | Leaf DW (g) | N % |
|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | 37.6 | 9.1 | 5.3 | 0.76 | 0.54 | 1.45 | 2.93 |
| $NH_4(SO_4)_2$ | 35.0 | 7.7 | 5.3 | 0.33 | 0.50 | 0.94 | 2.25 |
| $NH_4NO_3$ | 31.9 | 6.7 | 4.8 | 0.21 | 0.35 | 0.72 | 2.03 |
| $Ca(NO_3)_2$ | 31.0 | 9.6 | 5.5 | 0.23 | 0.51 | 0.92 | 2.20 |
| $CO(NH_2)_2$+ Cd | 33.6 | 6.2 | 4.8 | 0.30 | 0.19 | 0.50 | 2.74 |
| $NH_4(SO_4)_2$+ Cd | 33.5 | 5.2 | 5.0 | 0.09 | 0.14 | 0.31 | 2.22 |
| $NH_4NO_3$ + Cd | 26.3 | 4.0 | 3.8 | 0.08 | 0.12 | 0.17 | 1.68 |
| $Ca(NO_3)_2$+Cd | 27.7 | 4.9 | 4.2 | 0.09 | 0.13 | 0.47 | 2.87 |

Table 2：The effect of different nitrogen forms in alleviating cadmium-induced changes on SPAD Value, Plant Height (cm), Leaf Number ,Plant biomass (g) and, Plant biomass (g) and Nitrogen (%) of *Solanum aethiopicum* at the 2nd growth stage

| Treatment | SPAD value | Plant Height(cm) | Leaf Number | Root DW(g) | Stem DW(g) | Leaf DW(g) | N% |
|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | 43.0 | 16.1 | 7.5 | 1.7 | 2.4 | 6.1 | 2.73 |
| $NH_4(SO_4)_2$ | 41.6 | 15.1 | 8.5 | 0.8 | 1.5 | 3.2 | 2.17 |
| $NH_4NO_3$ | 37.0 | 11.4 | 5.5 | 0.6 | 1.0 | 2.6 | 2.41 |
| $Ca(NO_3)_2$ | 39.2 | 15.9 | 7.3 | 0.6 | 1.1 | 2.4 | 2.75 |
| $CO(NH_2)_2$ + Cd | 37.6 | 9.3 | 4.5 | 0.4 | 0.7 | 2.5 | 3.14 |
| $NH_4(SO_4)_2$ + Cd | 40.8 | 11.7 | 7.0 | 0.7 | 1.3 | 3.0 | 2.31 |
| $NH_4NO_3$ + Cd | 35.5 | 6.5 | 7.0 | 0.2 | 0.3 | 0,5 | 2.30 |
| $Ca(NO_3)_2$+Cd | 37.7 | 9.3 | 5.5 | 0.6 | 0.5 | 2.4 | 2.39 |

Table 3: The effect of different nitrogen forms in alleviating cadmium-induced changes on root macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the first（$1^{st}$） growth stage.

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 978.4a | 3414.6d | 14552.4b | 2092.2 b | 517.9d | 21.1 c | 225.5 b |
| $NH_4(SO_4)_2$ | N/A | 1261.7a | 4662.1cd | 24161.8a | 1854.1b | 529.8d | 23.1bc | 291.4ab |
| $NH_4NO_3$ | N/A | 790.6a | 2918.3d | 20755.9ab | 1793.2b | 434.0e | 14.3d | 238.5ab |
| $Ca(NO_3)_2$ | N/A | 1052.7a | 3381.5d | 21956.4a | 1703. 3b | 348.8f | 20.4 c | 308.5ab |
| $CO(NH_2)_2$ + Cd | 6.7d | 116.4b | 555.6e | 1376.1c | 473.3c | 155.2g | 2.9e | 51.4c |
| $NH_4(SO_4)_2$ + Cd | 160.3a | 808.8a | 9415.5a | 20815.8ab | 4468.6a | 1927.2a | 42.4a | 291.3ab |
| $NH_4NO_3$ + Cd | 74.2b | 816.1a | 6969.8b | 25918.0a | 3734.7a | 1146.2b | 41.3a | 356.7a |
| $Ca(NO_3)_2$+Cd | 56.6c | 762.9a | 5959.2bc | 23451.6a | 3867.3a | 677.9c | 27.0b | 238.6ab |

Table 4: The effect of different nitrogen forms in alleviating cadmium-induced changes on root tissue macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the second ($2^{nd}$) growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 1632.7a | 2556.3f | 10653.9cd | 1438.8b | 230.7g | 16.2f | 233.5ab |
| $NH_4(SO_4)_2$ | N/A | 1471.7a | 2973.5e | 16230.3abc | 1353.0b | 276.4f | 81.3a | 325.3ab |
| $NH_4NO_3$ | N/A | 1107.2a | 3439.2d | 13460.7bc | 2169.0ab | 471.2c | 27.8c | 301.8ab |
| $Ca(NO_3)_2$ | N/A | 1852.8a | 3451.1d | 16345.1abc | 1456.4b | 210.7h | 28.5 c | 245.1ab |
| $CO(NH_2)_2$ + Cd | 24.9d | 1498.5a | 3920.1c | 16707.8abc | 1727.8b | 492.1b | 21.0e | 351.7ab |
| $NH_4(SO_4)_2$ + Cd | 204.7a | 2147.0a | 4854.3b | 6197.8d | 3221.1ab | 1082.4a | 55.4b | 545.3a |
| $NH_4NO_3$ + Cd | 96.7b | 1836.6a | 5221.2 a | 21594.5a | 1916.3ab | 409.5d | 24.0 d | 215.3b |
| $Ca(NO_3)_2$+Cd | 66.5c | 2264.1a | 3548.4d | 16447.9ab | 1931.1ab | 365.5e | 18.3f | 333.5ab |

Table 5: The effect of different nitrogen forms in alleviating cadmium-induced changes on stem macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the 1st growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 2945.3c | 3710.7b | 15281.8e | 167.9 bcd | 217.4c | 28.3c | 278.6b |
| $NH_4(SO_4)_2$ | N/A | 2798.3c | 3062.3b | 15120.2f | 124.1cd | 98.7f | 27.2c | 291.1b |
| NH4NO3 | N/A | 3177.8c | 3701.8b | 29841.4a | 122.2d | 71.6g | 32.2b | 301.3b |
| $Ca(NO_3)_2$ | N/A | 2601.7c | 3078.9b | 29040.0b | 106.1d | 66.0h | 17.2e | 299.1b |
| $CO(NH_2)_2$+ Cd | 30.2a | 2333.0c | 4659.1b | 6943.1h | 256.2bc | 152.3d | 70.5a | 482.5a |
| $NH_4(SO_4)_2$+ Cd | 17.3b | 5912.7a | 7858.0a | 12600.0g | 593.4a | 488.6a | 19.5d | 347.9ab |
| $NH_4NO_3$ + Cd | 2.3d | 3907.0bc | 4480.7b | 19365.5d | 262.8b | 323.8b | 10.8f | 333.9ab |
| $Ca(NO_3)_2$+Cd | 3.5c | 5507.4ab | 4932.6b | 27619.5c | 276.6b | 147.8e | 8.3g | 250.3b |

Table 6: The effect of different nitrogen forms in alleviating cadmium-induced changes on stem macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the 2nd growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 3835.5bc | 4852.2b | 16783.3 e | 120.8 b | 78.1d | 13.9c | 404.8b |
| b$NH_4(SO_4)_2$ | N/A | 4090.8bc | 4929.8b | 15864.2g | 277.2ab | 109.0c | 21.8a | 467.1b |
| $NH_4NO_3$ | N/A | 4355.6bc | 5352.8b | 21322.1d | 110.0b | 113.3bc | 16.8bc | 452.9b |
| $Ca(NO_3)_2$ | N/A | 5793.2ab | 5894.1b | 31107.4a | 106.2b | 48.0e | 18.7ab | 407.3b |
| $CO(NH_2)_2$ + Cd | 34.5b | 3394.9c | 5135.0b | 23330.5b | 131.0b | 113.4bc | 22.1a | 612.9a |
| $NH_4(SO_4)_2$ + Cd | 46.9a | 7197.4ab | 11437.1a | 12062.4h | 409.0a | 808.8a | 19.9 ab | 731.0a |
| $NH_4NO_3$ + Cd | 27.5c | 3753.1bc | 4913.1b | 16492.2f | 131.0b | 116.4b | 17.0bc | 340.2b |
| $Ca(NO_3)_2$+Cd | 35.7 b | 4255.7bc | 5727.7b | 22778.2c | 183.5b | 84.7d | 19.8 ab | 405.5b |

Table 7: The effect of different nitrogen forms in alleviating cadmium-induced changes on leaf macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the 1st growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 3298.4c | 5027.1bc | 30958.9b | 317.6d | 146.6g | 16.7g | 202.4b |
| $NH_4(SO_4)_2$ | N/A | 3899.5bc | 4872.6 bc | 42850.2a | 331.7cd | 387.8e | 24.4d | 479.5a |
| $NH_4NO_3$ | N/A | 3886.4bc | 4521.6c | 33571.4ab | 401.9bcd | 236.9c | 18.9 f | 545.2a |
| $Ca(NO_3)_2$ | N/A | 3744.9bc | 4997.9bc | 36200.1ab | 337.1cd | 239.8e | 21.2e | 214.1b |
| $CO(NH_2)_2$+ Cd | 7.7 c | 5032.0b | 7396.3b | 42593.3a | 653.2ab | 389.3c | 42.4a | 347.0ab |
| $NH_4(SO_4)_2$+ Cd | 25.1a | 6938.3a | 10615.1a | 14620.2c | 664.9a | 1416.8a | 35.9b | 249.2b |
| $NH_4NO_3$ + Cd | 27.2a | 3938.1bc | 6595.5bc | 31106.2b | 582.9abc | 587.9b | 32.4c | 203.3b |
| $Ca(NO_3)_2$+Cd | 10.3b | 3537.4c | 6461.8bc | 40733.9ab | 601.3ab | 338.5d | 23.7d | 211.6b |

Table 8: The effect of different nitrogen forms in alleviating cadmium-induced changes on leaf macro- and micronutrients concentration (mg $kg^{-1}$) of *Solanum aethiopicum* at the 2nd growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| $CO(NH_2)_2$ | N/A | 2660.0c | 4852. 2 b | 30084.3a | 411.3b | 177.6g | 19.6 bc | 205.5b |
| $NH_4(SO_4)_2$ | N/A | 4067.4bc | 4929. 8b | 30481.1a | 317.3b | 199.9e | 20.6bc | 208.7b |
| $NH_4NO_3$ | N/A | 4181.3bc | 5352. 8b | 29626.9a | 422.5b | 259.3c | 22.2bc | 196.8b |
| $Ca(NO_3)_2$ | N/A | 4555.1abc | 5894. 1b | 30382.0a | 302.2b | 142.8h | 22.8bc | 219.2b |
| $CO(NH_2)_2$ + Cd | 28.5a | 4054.7bc | 5135. 0b | 25195.7a | 283.6b | 250.9d | 20.2bc | 383.9ab |
| $NH_4(SO_4)_2$ + Cd | 26.2a | 6482.8a | 11437. 1a | 13177.5b | 1305.8a | 946.4a | 33.5a | 500.6a |
| $NH_4NO_3$ + Cd | 27.5 a | 3948.9bc | 4913. 1b | 31228.2a | 435.4b | 285.8b | 19.5bc | 238.2b |
| $Ca(NO_3)_2$+Cd | 13.2b | 4997.5ab | 5727. 7 b | 27443.9a | 669.5ab | 191.3f | 18.1c | 277.8b |

Table 9: Correlations among eight elements in the Root tissue of *Solanum aethiopicum* at the first（1st）growth stage.

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | -0.08 | 1 | | | | | | |
| Mg | 0.89** | 0.38 | 1 | | | | | |
| K | 0.33 | 0.78* | 0.68* | 1 | | | | |
| Fe | 0.88** | 0.25 | 0.95** | 0.64 | 1 | | | |
| Mn | 0.95** | 0.14 | 0.93** | 0.42 | 0.86** | 1 | | |
| Cu | 0.83** | 0.42 | 0.97** | 0.71* | 0.92** | 0.89** | 1 | |
| Zn | 0.38 | 0.79* | 0.71* | 0.93** | 0.62 | 0.51 | 0.80* | 1 |

Correlation is significant at the p<0.05(*) p<0.01(**) levels, respectively.

Table 10: Correlations among eight elements in the Root tissue of *Solanum aethiopicum* at the second (2nd) growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | 0.64 | 1 | | | | | | |
| Mg | 0.74* | 0.4 | 1 | | | | | |
| K | -0.47 | -0.11 | 0.15 | 1 | | | | |
| Fe | 0.90** | 0.36 | 0.65 | -0.58 | 1 | | | |
| Mn | 0.91** | 0.31 | 0.64 | -0.61 | 0.96** | 1 | | |
| Cu | 0.21 | -0.08 | -0.01 | -0.22 | 0.14 | 0.26 | 1 | |
| Zn | 0.78* | 0.33 | 0.34 | -0.68* | 0.81** | 0.89** | 0.46 | 1 |

Correlation is significant at the p<0.05(*) p<0.01(**) levels, respectively.

Table 11: Correlations among eight elements in the stem tissue of *Solanum aethiopicum* at the 1st growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | 0.01 | 1 | | | | | | |
| Mg | 0.51 | 0.80** | 1 | | | | | |
| K | -0.70* | 0.1 | -0.37 | 1 | | | | |
| Fe | 0.5 | 0.79* | 0.99** | -0.43 | 1 | | | |
| Mn | 0.29 | 0.67* | 0.86** | -0.47 | 0.90** | 1 | | |
| Cu | 0.75* | -0.57 | -0.08 | -0.59 | -0.11 | -0.23 | 1 | |
| Zn | 0.92** | -0.28 | 0.27 | -0.68* | 0.27 | 0.18 | 0.82** | 1 |

Correlation is significant at the $p<0.05$(*) $p<0.01$(**) levels, respectively.

Table 12: Correlations among eight elements in the stem tissue of *Solanum aethiopicum* at the 2nd growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | 0.3 | 1 | | | | | | |
| Mg | 0.61 | 0.89** | 1 | | | | | |
| K | -0.33 | -0.1 | -0.4 | 1 | | | | |
| Fe | 0.51 | 0.65 | 0.81** | -0.66 | 1 | | | |
| Mn | 0.63 | 0.78* | 0.97** | -0.59 | 0.86** | 1 | | |
| Cu | 0.38 | 0.11 | 0.2 | 0.1 | 0.44 | 0.19 | 1 | |
| Zn | 0.54 | 0.54 | 0.77* | -0.34 | 0.70* | 0.80** | 0.51 | 1 |

Correlation is significant at the $p<0.05$(*) $p<0.01$(**) levels, respectively.

**Table 13:** Correlations among eight elements in the leaf tissue of *Solanum aethiopicum* at the 1st growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | 0.55 | 1 | | | | | | |
| Mg | 0.77* | 0.91** | 1 | | | | | |
| K | -0.56 | -0.66 | -0.65 | 1 | | | | |
| Fe | 0.80** | 0.64 | 0.82** | -0.27 | 1 | | | |
| Mn | 0.75* | 0.91** | 0.92** | -0.78* | 0.63 | 1 | | |
| Cu | 0.61 | 0.71* | 0.75* | -0.14 | 0.83** | 0.59 | 1 | |
| Zn | -0.47 | -0.02 | -0.35 | 0.3 | -0.27 | -0.19 | -0.1 | 1 |

Correlation is significant at the $p<0.05$(*) $p<0.01$(**) levels, respectively.

**Table 14:** Correlations among eight elements in the leaf tissue of *Solanum aethiopicum* at the 2nd growth stage

| Treatment | Cd | Ca | Mg | K | Fe | Mn | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Cd | 1 | | | | | | | |
| Ca | 0.4 | 1 | | | | | | |
| Mg | 0.74* | 0.80* | 1 | | | | | |
| K | -0.55 | -0.80** | -0.86** | 1 | | | | |
| Fe | 0.41 | 0.81** | 0.83** | -0.89** | 1 | | | |
| Mn | 0.54 | 0.77* | 0.92** | -0.94** | 0.92** | 1 | | |
| Cu | 0.3 | 0.76* | 0.79* | -0.87** | 0.81** | 0.93** | 1 | |
| Zn | 0.75* | 0.73* | 0.85** | -0.95** | 0.76* | 0.84** | 0.73* | 1 |

Correlation is significant at the $p<0.05$(*) $p<0.01$(**) levels, respectively.