

# Metabolism biochemical of young plants of Ucuúba (*Virola surinamensis*) in the presence of cadmium

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## Research article

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# Abstract

The increased cadmium (Cd) concentration in the environment, caused especially by mining residues and excessive use of phosphate fertilizers, can cause serious imbalances in terrestrial and aquatic ecosystems. High levels of Cd in the soil commonly causes many stress symptoms in plants. and nutritional imbalance between carbon and nitrogen metabolism. The aim of this study was to assess the Nitrogen and Carbon metabolism of young plants of Ucuúba (*Viola surinamensis*) in the presence of cadmium with the perspective of the phytoremediation of contaminated environments. The used experimental design was a completely randomized design with five Cd concentrations (0, 15, 30, 45, and 60 mg L<sup>-1</sup>), for 60 days. In general, Cd did not affect nitrate concentration in the root but had a positive effect on leaves. The reduction of nitrate reductase (NR) in plants exposed to Cd was followed by a decrease in ammonia, total soluble amino acids (TSA), and total soluble proteins (TSP). Cd promoted an increase in the concentration of total soluble carbohydrates (TSC), proline, sucrose, and reducing sugars in the plants. *V. surinamensis* presented a higher self-protection capacity in the form of bioaccumulation of total soluble carbohydrates, sucrose, and proline, important for its tolerance to the presence of Cd.

## 1. Background

Increased cadmium (Cd) concentration in the environment, caused especially by mining residues and excessive use of phosphate fertilizers, promotes serious imbalances in terrestrial and aquatic ecosystems because it is highly toxic and persistent in the environment, as well as present a high mobility in the soil for plants, being incorporated and bioaccumulated to other components of the food chain, rapidly affecting the growing number of organisms (Zayneb et al. 2015).

High levels of Cd in the soil commonly causes many stress symptoms in plants such as the reduction of the photosynthetic rate, which may result in alterations in the concentration of starch and soluble carbohydrates in plants tissues (He et al. 2013; Elloumi et al. 2014), and nutritional imbalance between carbon and nitrogen metabolism (Ci et al. 2009), thus reducing biomass production and total plant growth. The lower nitrate absorption (NO<sub>3</sub><sup>-</sup>) (Nasraoui-Hajaji et al. 2011; Nikolić et al. 2017), changes in nitrate reductase (NR) activity (Song et al. 2016), proline (Wang et al. 2016; Nikolić et al. 2017), total soluble proteins (PST) and total soluble amino acids (TSA) (Rahoui et al. 2015) in plants under the effect of Cd have also been observed.

It has been postulated that higher plants are more sensitive to Cd stress (Xie et al. 2014). However, study conducted by Andrade Júnior et al. (2019) demonstrated medium and high tolerance of *Viola surinamensis* to Cd. Variations in Cd tolerance in plants may be associated with changes in nitrogen and or carbon metabolism. Differential Cd tolerance can be attributed to differential accumulation of amino acids such as proline, and sugars, which serve as compatible osmolytes and antioxidants or are involved in other plant defense pathways against stress (Xie et al. 2014).

*V. surinamensis* (Ucuúba) a forest species with economic and medicinal interest, besides being useful for recomposition of altered areas. Is a species widely distributed and adapted to the lowland and igapó ecosystems in the Amazon (Andrade Júnior et al. 2019). These ecosystems are constantly susceptible to heavy metal contamination (Khan et al. 2017), indicating that the plant has strategies to tolerate environments contaminated by heavy metals.

Recent study showed that, in addition to tolerance to Cd, *V. surinamensis* had a greater ability to extract and accumulate metal in the root, restricting its transport to the aerial part (Andrade Júnior et al. 2019). Species with these characteristics are promising for phytostabilization of metals. Thus, we tested the hypothesis that *V. surinamensis* develops biochemical strategies capable of tolerating and accumulate high Cd concentrations. Thus, this study aimed to assess the Nitrogen and Carbon metabolism of young plants of *V. surinamensis* in the presence of cadmium, in order to contribute to understand the potential of *V. surinamensis* against Cd stress.

## 2. Results

### 2.1. Effect of Cd on the concentrations of nitrate, nitrate reductase, and free ammonium

Nitrate concentrations in the roots were not significantly affected by Cd, except for the dose of 15 mg L<sup>-1</sup> of Cd (Fig. 1a). In the leaves, nitrate concentrations were significantly affected by Cd (Fig. 1b). In the roots, Cd concentrations reached 0.045 and 0.04 μmol NO<sub>3</sub><sup>-</sup> g<sup>-1</sup> DM in the control treatment (0 mg L<sup>-1</sup> of Cd) and at a dose of 15 mg L<sup>-1</sup> of Cd, respectively (Fig. 1a), corresponding to a reduction of 11.11% when compared to the control. In the leaves, values of 0.01 and 0.02 μmol NO<sub>3</sub><sup>-</sup> g<sup>-1</sup> DM were obtained in the control plants (0 mg L<sup>-1</sup> of Cd) and at the highest dose of Cd (60 mg L<sup>-1</sup> of Cd), respectively (Fig. 1b), characterizing an increase of 100% in the treatment of 60 mg L<sup>-1</sup> of Cd when compared to the control treatment.

The nitrate reductase activity (NRA) was significantly affected by Cd, both in roots and leaves (Fig. 1c, d). In the roots, the lowest value (0.33 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> FM h<sup>-1</sup>) was observed at a dose of 60 mg L<sup>-1</sup> of Cd, representing a 56% reduction when compared to the control treatment (0.76 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> FM h<sup>-1</sup>) (Fig. 1c). The reduction was more accentuated in the leaves, reaching a value of 0.02 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> FM h<sup>-1</sup> at a dose of 60 mg L<sup>-1</sup> of Cd, corresponding a decrease of 97.47% when compared to the control treatment (0.79 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> FM h<sup>-1</sup>) (Fig. 1d).

Cd significantly affected free ammonia, both in roots and leaves (Fig. 1e, f). In the roots, values of 9.52 mmol NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> DM (0 mg L<sup>-1</sup> of Cd) and 1.39 mmol of NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> DM (60 mg L<sup>-1</sup> of Cd) were obtained, representing a 85.4% reduction at the highest dose of Cd when compared to the control treatment (Fig. 1e). In the leaves, Cd effect was more significant, promoting a reduction of 87.77% in

ammonia concentration at a dose of 60 mg L<sup>-1</sup> of Cd (2.38 mmol of NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> DM) when compared to the control treatment (19.47 mmol of NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> DM) (Fig. 1f).

## 2.2. Effect of Cd on the concentrations of total soluble amino acids, total soluble proteins and proline concentration

The concentration of total soluble amino acids in roots and leaves was significantly affected by Cd (Fig. 2). In the roots, the concentration was 330 and 243 μmol AA g<sup>-1</sup> DM in the control treatment (0 mg L<sup>-1</sup> of Cd) and at a dose of 60 mg L<sup>-1</sup> of Cd, respectively (Fig. 2a), corresponding to a reduction of 26.36% at the highest Cd dose when compared to the control treatment. In the leaves, values of 337 and 215 μmol AA g<sup>-1</sup> DM were obtained in the control plants (0 mg L<sup>-1</sup> of Cd) and at the highest Cd dose (60 mg L<sup>-1</sup> of Cd), respectively (Fig. 2b), characterizing a 36.2% reduction in the treatment of 60 mg L<sup>-1</sup> of Cd when compared to the control treatment.

The concentrations of total soluble proteins in plants submitted to the presence of Cd were significantly reduced in both roots and leaves (Fig. 2c, d). The highest and lowest concentrations of proteins in roots occurred in the control treatment (0.54 mg protein g<sup>-1</sup> DM) and at a dose of 60 mg L<sup>-1</sup> of Cd (0.35 mg protein g<sup>-1</sup> DM), with a 35.18% reduction at the highest Cd dose when compared to the control treatment (Fig. 2c). In the leaves, the values obtained were 0.37 mg protein g<sup>-1</sup> DM (control treatment) and 0.15 mg protein g<sup>-1</sup> DM (60 mg L<sup>-1</sup> of Cd), corresponding to a decrease of 59.46% in the lowest Cd dose when compared to the control treatment (Fig. 2d).

Proline concentrations in roots and leaves of plants submitted to Cd doses increased significantly (Fig. 2). Values of 0.60 μmol Pro g<sup>-1</sup> DM (0 mg L<sup>-1</sup> of Cd) and 0.76 μmol Pro g<sup>-1</sup> DM (60 mg L<sup>-1</sup> of Cd) were obtained in the roots, representing a 26.7% increase at the highest Cd dose when compared to the control treatment (Fig. 2e). In the leaves of control plants and at a dose of 60 mg L<sup>-1</sup> of Cd, proline concentrations were 0.81 and 1.06 μmol Pro g<sup>-1</sup> DM, respectively, demonstrating a 30.86% increase of proline in plants with the highest Cd dose when compared to the control treatment (Fig. 2f).

## 2.3. Concentration of total soluble carbohydrates, sucrose, and reducing sugars in the presence of Cd

The concentrations of total soluble carbohydrates in Cd-treated plants increased significantly in both roots and leaves (Fig. 3a, b). The lowest and highest concentrations of carbohydrates in the roots were observed in the control treatment (0.06 mmol Glu g<sup>-1</sup>) and at a dose of 60 mg L<sup>-1</sup> of Cd (0.1 mmol Glu g<sup>-1</sup>), with an increase of 83.3% at the highest Cd dose when compared to the control treatment (Fig. 3a). In the leaves, the obtained values were 0.09 mmol Glu g<sup>-1</sup> (control treatment) and 0.1 mmol Glu g<sup>-1</sup>

(15 mg L<sup>-1</sup> of Cd), corresponding to an 11.11% increase at the lowest Cd dose when compared to the control treatment (Fig. 3b).

Sucrose concentrations in Cd-treated plants increased significantly in both roots and leaves (Fig. 3c, d). In the roots, the values were 1.16 mg sucrose g<sup>-1</sup> DM (0 mg L<sup>-1</sup> of Cd) and 2.11 mg sucrose g<sup>-1</sup> DM (60 mg L<sup>-1</sup> of Cd), representing an increase of 81.9% at the highest Cd dose when compared to the control treatment (Fig. 3c). The lowest and highest concentrations of sucrose in the leaves were observed in the control treatment (0.57 mg sucrose g<sup>-1</sup> DM) and at a dose of 60 mg L<sup>-1</sup> of Cd (2.38 mg sucrose g<sup>-1</sup> DM), with a 317.54% increase at the highest Cd dose when compared to the control treatment (Fig. 3d).

The concentrations of reducing sugars increased in the roots and reduced significantly in the leaves of plants submitted to the presence of Cd (Fig. 3e, f). Values of 0.83 μmol carb g<sup>-1</sup> DM (0 mg L<sup>-1</sup> of Cd) and 1.42 μmol carb g<sup>-1</sup> DM (45 mg L<sup>-1</sup> of Cd) were obtained in the roots, representing a 71.08% increase at a dose of 45 mg L<sup>-1</sup> of Cd when compared to the control treatment (Fig. 3e). The concentrations in the leaves of control plants and at a dose of 15 mg L<sup>-1</sup> of Cd were 1.57 and 1.27 μmol carb g<sup>-1</sup> DM, respectively, demonstrating a 19.11% reduction of reducing sugars in plants with the lowest Cd dose when compared to the control treatment (Fig. 3f).

### 3. Discussion

NO<sub>3</sub><sup>-</sup>, an important N source, is actively absorbed by the plasma membrane of epidermal and cortical cells of roots through nitrate carrier proteins, but in plants exposed to Cd, there is an inhibition of the activities of these proteins (Dai et al. 2013) because Cd damages the normal function of the proton pump (H<sup>+</sup> ATPase) in the plasmalemma (Mehes-Smith et al. 2013, Hasanuzzaman et al. 2017). However, in general, no reduction of NO<sub>3</sub><sup>-</sup> was observed in the roots of *V. surinamensis* (Fig. 1a), indicating that the presence of Cd probably did not affect the activity of NO<sub>3</sub><sup>-</sup> carrier proteins, which is in accordance with the study performed by (Hernández et al. 2015), who showed an increase of the total ATPase in the root and stem of *Cucumis sativus* in the presence of Cd.

In healthy plants, once absorbed by roots, NO<sub>3</sub><sup>-</sup> is transported to the leaves, stored in the vacuoles or reduced into nitrite (NO<sub>2</sub><sup>-</sup>) by NAD(P)H-dependent cytosolic NR activity (Mao et al. 2014). In this study, the increase of NO<sub>3</sub><sup>-</sup> in the leaves of *V. surinamensis* (Fig. 1b) suggests that Cd did not interfere with the translocation of the nitrogen compound to the shoot. The assimilation of NO<sub>3</sub><sup>-</sup> into the cytosol of mesophyll cells may have been affected by the NRA inactivation caused by Cd. The reduction of NRA with the increasing Cd doses in the nutrient solution may be an efficient energy-saving mechanism to reduce the effect of stress and not to decrease NO<sub>3</sub><sup>-</sup> in the plant.

NR is the key enzyme in the process of NO<sub>3</sub><sup>-</sup> assimilation (Nikolić et al. 2017) and is regulated by the presence of NO<sub>3</sub><sup>-</sup> (Van der Ent et al. 2013), its degradation, activation or inactivation. Plants exposed to

Cd have a reduced NRA, leading to a decreased  $\text{NO}_3^-$  assimilation because the metal causes a lower  $\text{NO}_3^-$  absorption by plant roots (Nasraoui-Hajaji et al. 2011, Nikolić et al. 2017). In this study, the marked reduction of NRA with the increasing Cd concentration (Fig. 1c) did not appear to have been caused by substrate availability ( $\text{NO}_3^-$ ) since there was no reduction of the nitrogen compound in the plant root and shoot, suggesting a direct effect of Cd on NR activity, i.e. the interaction of the metal with the thiol group ( $-\text{SH}$ ) in the active site of the enzyme would result in the inactivation. It is suggested that reduction of NRA affected  $\text{NO}_3^-$  assimilation and influenced N metabolism, resulting in the reduction of ammonia (Fig. 1e, f), amino acids (Fig. 2a, b), and TSP (Fig. 2c, d) both in the roots and in the shoot of *V. surinamensis*. Reduction of nitrate reductase activity was also observed in other tree species (Nikolić et al. 2017) exposed to Cd.

The stress due to Cd activates the biochemical defense mechanisms in plants, such as sugar accumulation, which activate the NADPH-generating reactions through the pentose-phosphate cycle to eliminate or keep ROS levels under control and then repair the phytotoxic effects of oxidants (El-Beltagi et al. 2013). Thus, the increase of TSC in *V. surinamensis* exposed to Cd (Fig. 3a, b) may have been a protection mechanism to reduce the toxicity of this metal by ROS detoxification through the chelation of Cd. Moreover, a high concentration of sugars leads to a reduced mobilization of fatty acids and the accumulation of ROS is less intense (Yan et al. 2015). Therefore, an alteration in TSC can be considered as an important indicator of oxidative stress in plants. The increase in TSC may have worked as a compatible solute, which would help the plant in the osmotic adjustment against Cd stress (Singh et al. 2016), i.e. the accumulation of TSC may have contributed to the maintenance of the water status of the plant, favoring tissue protection and physiological processes, which is an important mechanism in the tolerance of *V. surinamensis* to the presence of Cd, at least during the experimental period.

The highest proline content in plants exposed to Cd occurred by de novo synthesis or decreased degradation and/or both processes (Singh et al. 2016). In this study, the increased free proline concentration with the increasing Cd doses (Fig. 2a, b) may be related to its osmoprotective role for osmotic adjustment, which would help to diminish the water potential of tissues, avoiding dehydration. Suggests that proline has acted on stomatal closure in order to restrict Cd absorption and translocation through transpiration, minimizing the metal damage. Proline accumulation may also have contributed to stabilize and protect subcellular structures against oxidative damage caused by free radicals (Wang et al. 2016, Singh et al. 2016, Solti et al. 2016). The increase of proline in plants in the presence of Cd would be due to degradation of proteins by proteolytic enzymes (Raldugina et al. 2016) and the accumulation of this amino acid and formation of a non-toxic Cd-proline complex in tissues would be a plant response to reduce the phytotoxicity of the metal (Chen et al. 2001, Aslam et al. 2014). The increase of proline induced by Cd was evidenced in other forest species (He et al. 2013, Wang et al. 2016, Yadav and Srivastava 2017).

Sucrose is a disaccharide consisting of glucose and fructose and, by means of the invertase activity, plays an important metabolic role as a donor of glycosyl and fructosyl for the synthesis of

polysaccharides (Sharma et al. 2006) and amino acids in plants (Todd et al. 2016). Therefore, the increase in sucrose concentration (Fig. 3c, d) in *V. surinamensis* exposed to Cd may be due to the inhibition of invertase activity, interfering with carbon and nitrogen metabolism, especially in proline accumulation (Fig. 2a, b). Another explanation for sucrose accumulation would be because the metal positively affects the activity of sucrose phosphate synthase (SPS) and negatively affects the sucrose synthase (SuSy) (Fryzova et al. 2017). In addition, the increase in sucrose concentration in *V. surinamensis* exposed to Cd may be related to the degradation of starch by the activity of the enzymes  $\alpha$ - and  $\beta$ -amylase hydrolases although heavy metals have an inhibitory effect on these enzymes (Reyes et al. 2018). The higher concentration of sucrose in the plant exposed to Cd could be related to a reduction in the cell metabolism of this carbohydrate (Badr et al. 2015) as a form of energy saving since sucrose accumulation in plants submitted to Cd would be a form of tolerance to the metal (Rahoui et al. 2015), which is attributed to chelation of Cd by sucrose and activation of specific ROS elimination systems, with a subsequent reduction of oxidative damage caused by the metal and/or the sucrose would act in the osmotic adjustment for the conservation of the internal water flow of the plant, thus maintaining the water potential under sufficient conditions. Thus, high concentrations of sucrose in *V. surinamensis* suggest a good metabolic regulatory state of the plant in the presence of Cd. The high concentration of sucrose was also observed in other species of plants exposed to Cd.

The highest concentration of reducing sugars in plants under stress caused by Cd (Fig. 3e, f) indicates energy savings by plants or even the presence of Cd negatively affecting cell respiration of root and shoot. The results are consistent with those obtained by Xie et al. (2014), who suggested the increase of reducing sugars due to the lower utilization of these carbohydrates in plants exposed to Cd. The highest accumulation of reducing sugar in the root (Fig. 3e) suggests an increase in the transport of these carbohydrates from the shoot to the growing cells of the root system, indicating that Cd may not have affected the transport system of assimilates of *V. surinamensis*. In addition, the sugar transported to the roots because of starch degradation would be an essential energy substrate for the resumption of respiration, conferring a mechanism of tolerance of the plant against the phytotoxic effect of Cd (Rahoui et al. 2015). Studies conducted by Andrade Júnior et al. (2019) indicated that *V. surinamensis* presented medium and high tolerance to Cd. Similar results in the reducing sugar concentration have been found in other species (Shah et al. 2017).

## 4. Conclusion

In general, treatment with Cd affects nitrogen assimilation and metabolism to a greater extent in *V. surinamensis* leaves.

*V. surinamensis* presented a higher self-protection capacity in the form of bioaccumulation of total soluble carbohydrates, sucrose, and proline, important for its tolerance to the presence of Cd.

## 5. Methods



## 5.1. Experiment location

The experiment was conducted in a greenhouse at the Federal Rural University of Amazonia (UFRA) in Belém, State of Pará, Brazil (01°27'21" S, 48°30'16" W) from 15 September 2017 to 14 November 2017. According to the climate classification of Köppen, the climate is type Af (Tropical rainforest), with an annual average precipitation of 2921.7 mm, average temperature of 25.9 °C, average relative humidity of 86.8% and wind speed of 1.35 m s<sup>-1</sup>.

## 5.2. Plant material and growth condition

Seeds of *V. surinamensis* were collected in the area of the Brazilian Agricultural Research Corporation (Embrapa Eastern Amazon), located in Belém, State of Pará, Brazil (01°26'44.2" S, 48°25'03.8" W).

To collect seeds from this area, authorization is not necessary as it is not a Forest Reserve. At the time of collection, no botanical sample was taken for the IAN Herbarium of Embrapa Amazônia Oriental because it is a very common species and is easily identified.

The identifications were carried out by the employees of Embrapa Amazônia Oriental, in relation to the verification of the samples, the herbarium of this institution is in quarantine due to COVID-19, with no expected return. Each country has its own rules of access to its genetic resources and in Brazil this access is more flexible for Universities and Research Institutions.

These seeds were sown in 5-L polyethylene trays containing sand and sterilized sawdust (1:1, v/v), and maintained under mean air temperature (T<sub>air</sub>) and relative air humidity (RH) of 28 °C and 90%. After emergence, the seedlings containing the first pair of eophylls were transplanted to 10-L polyethylene pots containing yellow latosol and poultry litter (3:1, v/v). The seedlings grown were in a greenhouse for 180 days, being irrigated daily to replace the water lost by evapotranspiration. Subsequently, the young plants were removed and their roots washed with deionized water and transferred to 5-L Leonard pots containing sterilized and washed sand and 800 mL of nutrient solution, replaced weekly and constituted of (μM): KH<sub>2</sub>PO<sub>4</sub>, 400; KNO<sub>3</sub>, 2000; Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 2000; MgSO<sub>4</sub>·7H<sub>2</sub>O, 800; FeEDTA, 400; H<sub>3</sub>BO<sub>3</sub>, 400; MnCl<sub>2</sub>·4H<sub>2</sub>O, 400; ZnCl<sub>2</sub>, 400; CuCl<sub>2</sub>·2H<sub>2</sub>O, 400; and H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O, 400. The pH was maintained at 5.9 ± 0.2 using HCl and NaOH. The ionic strength was initiated in 25% (10 days) and then increased to 50% (35 days), remaining for a period of acclimatization of 45 days.

## 5.3. Experimental design and treatment evaluation

After 45 days of cultivation, we selected the most uniform seedling considering height, stem diameter, number of leaves and submitted to five Cd concentrations (treatments) as following: 0 mg L<sup>-1</sup> of CdCl<sub>2</sub> (control), 15, 30, 45, and 60 mg L<sup>-1</sup> of CdCl<sub>2</sub>. The doses of Cd were determined based on the Resolution 420 of the National Council of the Environment, which establishes criteria and guiding values of soil quality regarding the presence of chemical substances. The experimental design was a completely randomized design with seven replications, per each treatment, totaling 35 experimental units. Asingle

plant per pot was considered a replicate. All variables for treatment comparisons were assessed 60 days after Cd treatment differentiation.

## 5.4. Biochemical assessments

The biochemical analyses were performed at the Laboratory of EBPS of UFRA. The following variables were determined: contents of nitrate ( $\text{NO}_3^-$ ) and free ammonium ( $\text{NH}_4^+$ ) (Weatherburn 1967), activity of the enzyme nitrate reductase ( $\text{RNO}_3^-$ ) (Hageman et al. 1971); total soluble amino acids (TSA) (Peoples, 1989), total soluble proteins (TSP) (Bradford 1976); proline (Bates 1973), total soluble carbohydrates (TSC) (Dubois 1956); sucrose (Van Handel 1968); and reducing sugars (Rinner et al. 2012).

## 5.5. Data analyses

The experimental data were assessed for the normality and homogeneity of variances by the Shapiro–Wilk and Bartlett tests, respectively. For parametric variables, the means of treatments were submitted to PROC GLM, post hoc Tukey’s HSD test and correlation between variables by the PROC CORR linear of Pearson using the software SAS 9.1.3 (SAS 2007). For non-parametric variables, the data were assessed by the Kruskal–Wallis test with Bonferroni correction by the software RStudio version 1.1.383. The experimental data of all analyses were assessed at 5% significance.

## Abbreviations

NR – Nitrate Reductase; TSA – Total Soluble Aminoacids; TSP – Total Soluble Proteins; TSC – Total Soluble Carbohydrates; Cd – Cadmium; AA – Aminoacids; ROS – Reactive Oxygen Species; SPS – Sucrose Phosphate Synthase; T air – Air; Temperature; RH – Relative Air Humidity;  $\text{NO}_3^-$  - Nitrate;  $\text{RNO}_3^-$  - Nitrate Reductase; DM – Dry Mass; FM – Fresh Mass; Glu – Glutamine;  $\text{NH}_4^+$  - Ammonia; NRA – Nitrate Reductase Activity.

## Declarations

### Ethics approval and consent to participate

not applicable

### Consent for publication

we consent to the publication of the manuscript for the journal BMC Plant Biology

### Availability of data and materials

not applicable

### Competing Interest

The authors declare that there is no conflict of interest publishing of the paper, that the paper has been not published elsewhere, and not include any form of plagiarism. All the authors mentioned above have approved the manuscript and have agreed with the submission of the manuscript.

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## Authors' Contributions

A.J. : Installation and application of experiment in a greenhouse;  
O. N. : and G.N: Biochemical analysis of the samples in a Plant Physiology laboratory;  
S. F. : production of data tables;  
E. C. : Collection of plant material (seeds) and storage;  
C. A. : Biochemical Analysis;  
S. V. : Statistical analysis of the data;  
V. B. : Translation of the scientific text;  
D. S and J. T. : Withdrawal of the greenhouse experiment

## Statement

all authors have read and approved the manuscript

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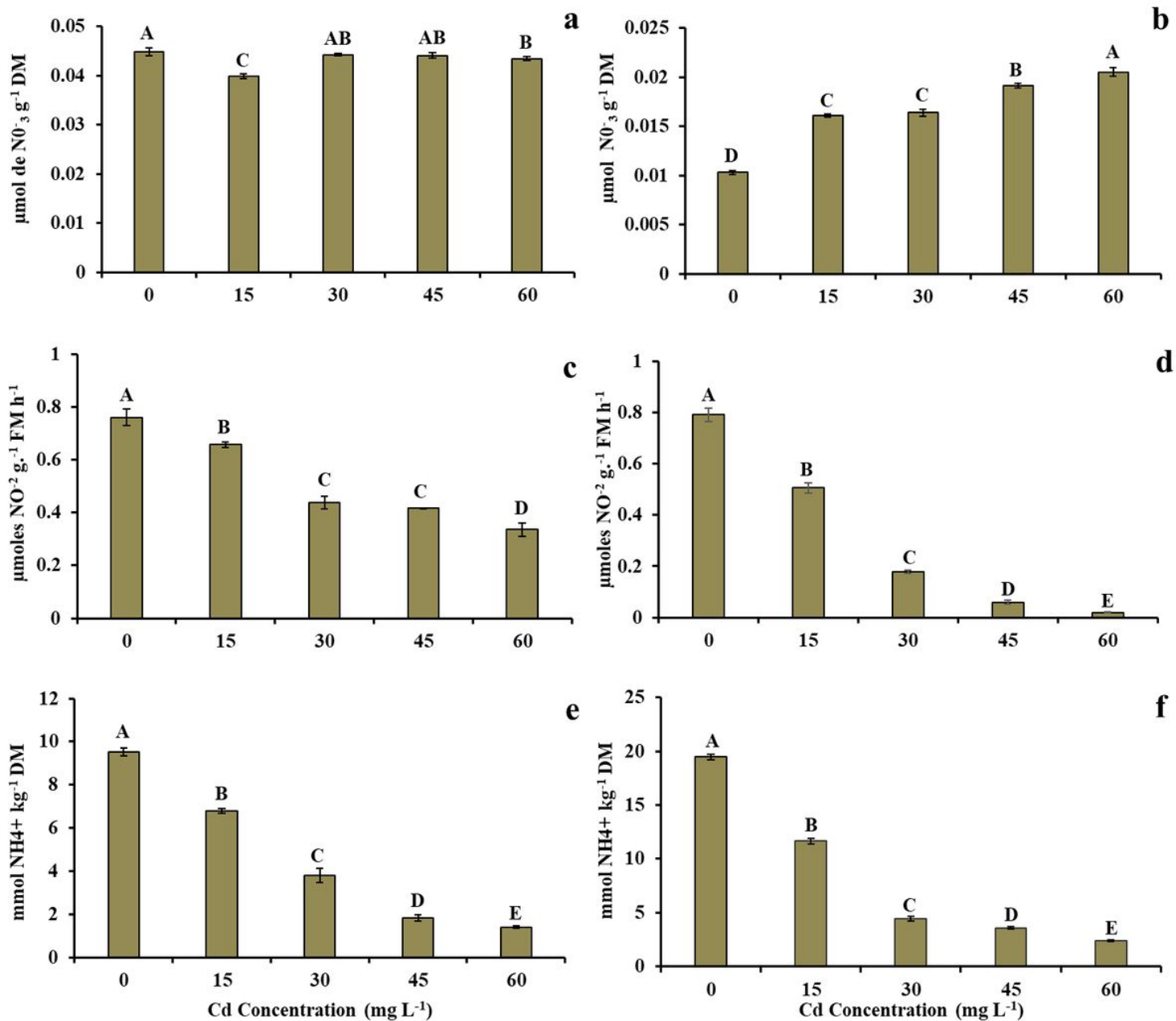
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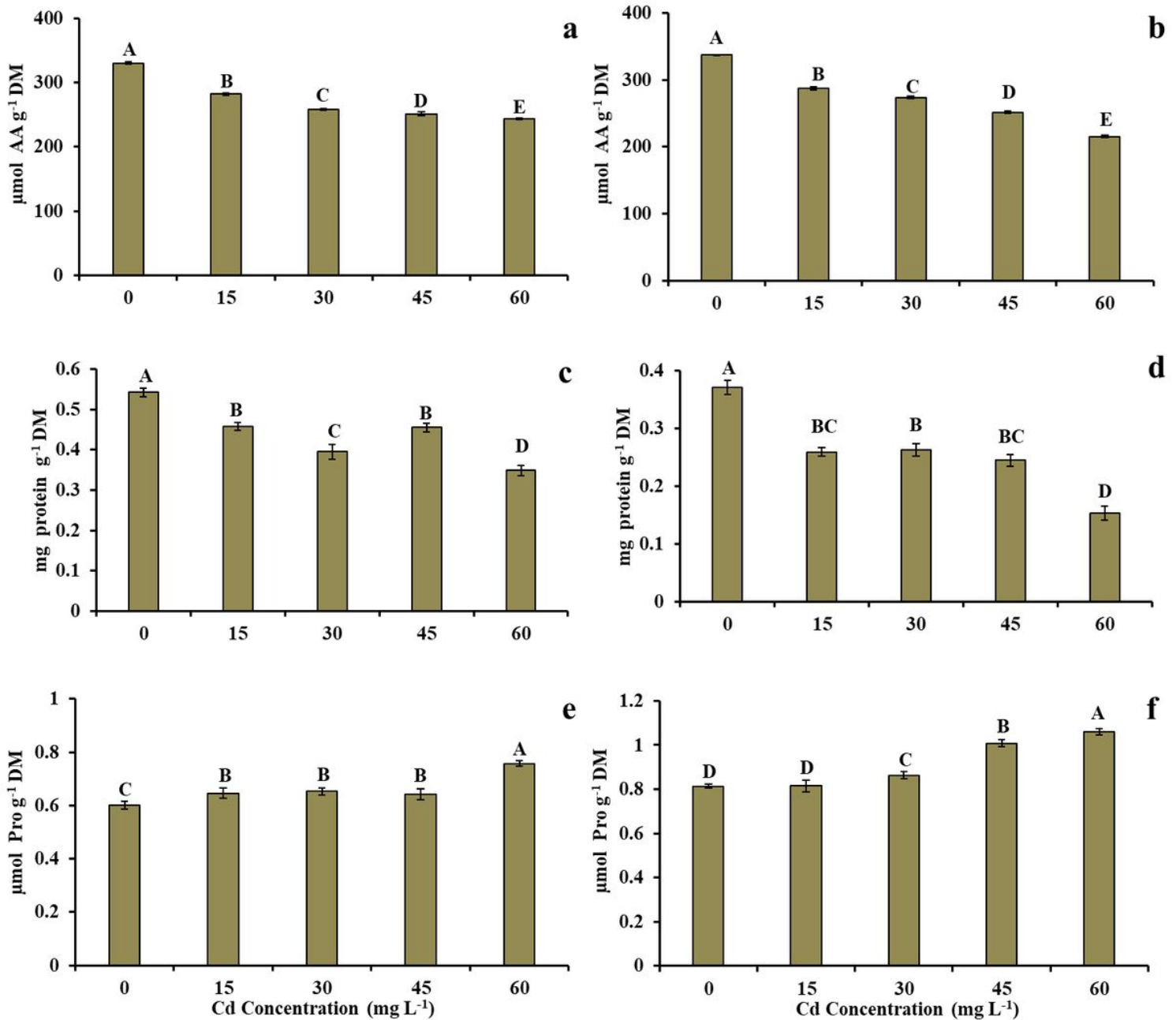
## Figures



**Figure 1**

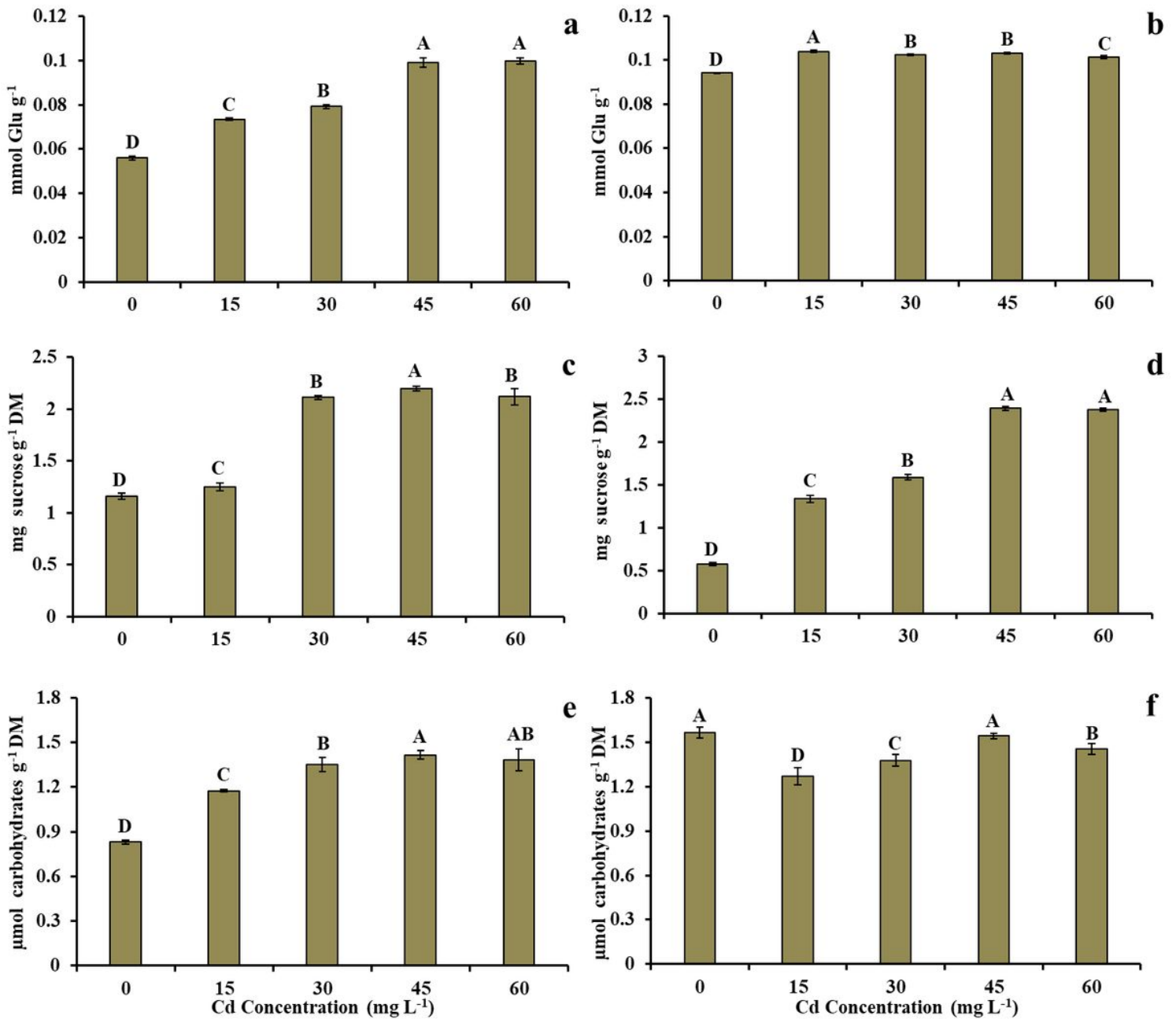
a: Nitrate concentration in the root, b: nitrate concentration in the leaves, c: nitrate reductase concentration in the root, d: nitrate reductase concentration in the leaves, e: ammonium concentration in the root, f: ammonium concentration in the leaves in young plants of *V. surinamensis* exposed to five cadmium concentrations (0, 15, 30, 45, and 60 mg). Different letters for cadmium concentrations in solution indicate significant differences in the Tukey's test ( $P < 0.05$ ). Mean  $\pm$  SD,  $n = 7$ .





**Figure 2**

a: Concentration of total soluble amino acids in the root, b: concentration of total soluble amino acids in the leaves, c: concentration of total soluble proteins in the root, d: concentration of total soluble proteins in the leaves, e: Proline concentration in the root, f: proline concentration in the leaves in young plants of *V. surinamensis* exposed to five cadmium concentrations (0, 15, 30, 45, and 60 mg). Different letters for cadmium concentrations in solution indicate significant differences in the Tukey's test ( $P < 0.05$ ). Mean  $\pm$  SD,  $n = 7$ .



**Figure 3**

a: Concentration of total soluble carbohydrates in the root, b: concentration of total soluble carbohydrates in the leaves, c: sucrose concentration in the root, d: sucrose concentration in the leaves, e: concentration of reducing sugars in the root, f: concentration of reducing sugars in the leaves in young plants of *V. surinamensis* exposed to five cadmium concentrations (0, 15, 30, 45, and 60 mg). Different letters for cadmium concentrations in solution indicate significant differences in the Tukey's test ( $P < 0.05$ ). Mean  $\pm$  SD,  $n = 7$ .