Carbon And Nitrogen Metabolism of Young Plants of Ucuúba (*Virola surinamensis*) in the Presence Of Cadmium

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Research article

Keywords: Nitrate reductase, total soluble carbohydrates, proline, sucrose, reducing sugars

Posted Date: December 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-34697/v3

Version of Record: A version of this preprint was published at BMC Plant Biology on March 24th, 2021. See the published version at https://doi.org/10.1186/s12870-021-02912-y.

Abstract

Virola surinamensis is a forest species widely distributed in the estuaries of the Amazon. These ecosystems are susceptible to contamination by Cadmium (Cd), indicating that the plant has strategies for tolerating this metal. This study aimed to assess the nitrogen and carbon metabolism of young plants of Ucuúba (*Virola surinamensis*) in the presence of cadmium with the perspective of the phytoremediation of contaminated environments. The experimental design was completely randomized with five Cd concentrations (0, 15, 30, 45, and 60 mg L^{-1}) 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 total soluble carbohydrates (TSC), proline, sucrose, and reducing sugars in the plants. The increase in TSC, sucrose and proline suggests a metabolic regulatory mechanism of *V. surinamensis* against Cd stress.

1. Background

Increased cadmium (Cd) concentration in the environment, caused mainly by mining residues and excessive use of phosphate fertilizers, promotes severe imbalances in terrestrial and aquatic ecosystems because of its high toxicity and persistence in the environment. Besides, this element presents high mobility in the soil for plants, being incorporated and bioaccumulated to other food chain components, rapidly affecting the growing number of organisms (Zayneb et al. 2015).

High Cd levels in the soil commonly cause many stress symptoms in plants sucs as alterations in the concentrations of starch and soluble carbohydrates in plant tissues (He et al. 2013; Elloumi et al. 2014). The lower nitrate absorption (NO_3^-) (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, a study conducted by Andrade Júnior et al. (2019) demonstrated medium and high tolerance of *Virola surinamensis* to Cd. Variations in Cd tolerance in plants are possibly associated with changes in nitrogen and or carbon metabolism. Moreover, they relate 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) is a forest species with economic and medicinal interest, besides being useful for the recomposition of altered areas. It is widely distributed and adapted to the lowland igapó ecosystems in the Amazon (Andrade Júnior et al. 2019). These ecosystems are frequently susceptible to heavy metal contamination, including Cd (Nascimento et al. 2006; Oliveira et al. 2015; Khan et al. 2017), indicating that the plant has strategies to tolerate such contaminated environments.

A recent study showed that, in addition to Cd tolerance, *V. surinamensis* had a remarkable 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 the 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 Cd expanding the knowledge on the resistance of this species against metal 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 Cd dose of 15 mg L^{-1} (Fig. 1a). In the leaves, nitrate concentrations were significantly affected by Cd (Fig. 1b). In the roots, Cd concentrations reached 0.045 and 0.040 µmol NO_3^- g^{-1} DM in the control (0 mg L^{-1} of Cd) and at a Cd dose of 15 mg L^{-1}, respectively (Fig. 1a), corresponding to a reduction of 11.11%. In the leaves, values of 0.01 and 0.02 µmol NO_3^- g^{-1} DM were obtained in the control plants (0 mg L^{-1} of Cd) and at the highest dose of Cd (60 mg L^{-1} of Cd), respectively (Fig. 1b), characterizing an increase of 100% in the treatment of 60 mg L^{-1} 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_2^- g^{-1} FM h^{-1}) was observed at a dose of 60 mg L^{-1} of Cd, representing a 56% reduction when compared to the control treatment (0.76 µmol NO_2^- g^{-1} FM h^{-1}) (Fig. 1c). The reduction was sharper in the leaves, reaching a value of 0.02 µmol NO_2^- g^{-1} FM h^{-1} at a dose of 60 mg L^{-1} of Cd, corresponding to a decrease of 97.47% when compared to the control treatment (0.79 µmol NO_2^- g^{-1} FM h^{-1}) (Fig. 1d).

Cd significantly affected free ammonia, both in roots and leaves (Fig. 1e, f). The roots exhibited 9.52 mmol NH_4^+ kg^{-1} DM (0 mg L^{-1} of Cd) and 1.39 mmol of NH_4^+ kg^{-1} DM (60 mg L^{-1} of Cd), representing an 85.4% reduction compared to the control plants (Fig. 1e). In the leaves, the Cd effect was more significant, promoting a reduction of 87.77% in ammonia concentration at a dose of 60 mg L^{-1} of Cd (2.38 mmol of NH_4^+ kg^{-1} DM) when compared to the control treatment (19.47 mmol of NH_4^+ kg^{-1} 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^{-1} DM in the control treatment (0 mg L^{-1} of Cd) and at a dose of 60 mg L^{-1} 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^{-1} DM were obtained in the control plants (0 mg L^{-1} of Cd) and at the highest Cd dose (60 mg L^{-1} of Cd), respectively (Fig. 2b), characterizing a 36.2% reduction in the 60 mg L^{-1} of Cd dose compared to the control treatment.

The concentrations of total soluble proteins in plants under the presence of Cd significantly decreased 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^{-1} DM) and at a dose of 60 mg L^{-1} of Cd (0.35 mg protein g^{-1} 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^{-1} DM (control treatment) and 0.15 mg protein g^{-1} DM (60 mg L^{-1} 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^{-1} DM (0 mg L^{-1} of Cd) and 0.76 µmol Pro g^{-1} DM (60 mg L^{-1} 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^{-1} of Cd, proline concentrations were 0.81 and 1.06 µmol Pro g^{-1} 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 levels 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^{-1}) and at a dose of 60 mg L^{-1} of Cd (0.1 mmol Glu g^{-1}), 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^{-1} (control treatment) and 0.1 mmol Glu g^{-1} (15 mg L^{-1} 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^{-1} DM (0 mg L^{-1} of Cd) and 2.11 mg sucrose g^{-1} DM (60 mg L^{-1} 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^{-1} DM) and at a dose of 60 mg L^{-1} of Cd (2.38 mg sucrose g^{-1} 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^{-1} DM (0 mg L^{-1} of Cd) and 1.42 µmol carb g^{-1} DM (45 mg L^{-1} of Cd) were obtained in the roots, representing a 71.08% increase at a dose of 45 mg L^{-1} 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^{-1} of Cd were 1.57 and 1.27 µmol carb g^{-1} DM, respectively, demonstrating a 19.11% reduction of reducing sugars in plants containing the lowest Cd dose when compared to the control treatment (Fig. 3f).

3. Discussion

NO_3^-, a crucial N source, is actively absorbed by the plasma membrane of epidermal and cortical cells of roots through nitrate carrier proteins. Still, in plants exposed to Cd, the activities of these proteins are inhibited (Dai et al. 2013) because Cd damages the normal function of the proton pump (H^+ ATPase) in the plasmalemma (Mehes-Smith et al. 2013, Hasanuzzaman et al. 2017). However, no reduction of NO_3^- occurred in the roots of most *V. surinamensis* (Fig. 1a); hence, the Cd probably did not affect the activity of NO_3^- carrier proteins. This result corroborates the study of Hernández et al. (2015), who showed an increase in total ATPase in the root and stem of *Cucumis sativus* in the presence of Cd.

In healthy plants, once absorbed by roots, NO_3^- is transported to the leaves, stored in the vacuoles, or reduced into nitrite (NO_2^-) by NAD(P)H-dependent cytosolic NR activity (Mao et al. 2014). In this study, the increase of NO_3^- 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_3^- 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 stress and prevent the decrease of NO_3^- in the plant.

NR is the key enzyme in the process of NO_3^- assimilation (Nikolić et al. 2017) and is regulated by its presence (Van der Ent et al. 2013), degradation, activation, or inactivation. Plants exposed to Cd have a reduced NRA, leading to a decreased NO_3^- assimilation because the metal causes a lower 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) apparently was independent of the substrate availability (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 (–SH) in the active site of the enzyme would result in the inactivation. Reduction of nitrate reductase activity also occurred in other tree species (Nikolić et al. 2017) exposed to Cd.

The ammonium ion is a central intermediate in the nitrogen metabolism in plants. It is produced during nitrate assimilation, deamination of amino acids, and photorespiration (Huang; Xiong, 2009). Since there are several biosynthesis routes for all aminoacids from ammonia (Zemanová et al. 2013), this study suggests that the decrease in ammonia levels (Fig. 1e, f) in plants submitted to Cd possibly relates to the reduction of TSA (Fig. 2a, b). Another explanation lies in the increase in the synthesis of specific amino acids, those for protection and stress regulation, such as proline (Fig. 2e, f).

Cd stress in plants causes protein degradation and affects amino acid metabolism (Dinakar et al. 2007). The reduction in TSP (Fig. 2c, d) in *V. surinamensis* under Cd may be due to the activation of proteases that degrade proteins for specific amino acid biosynthesis, such as proline (Fig. 2e, f). Thus, the degradation of TSP could function as a self-protection mechanism combined or not with cell signaling against Cd stress. Another explanation for reduced TSP in plants exposed to Cd would be the direct effect of the metal on the NRA that affected the concentration of TSP. This assumption is supported by the significant positive correlation coefficient ($r = 0.784$; $p = 0.0367$) between these variables in plants under Cd, i.e., the decrease in TSP in *V. surinamensis* under Cd would be associated with a reduction in NRA. The results obtained herein concerning the total soluble proteins agree with Anand et al. (2017).

The highest proline content in plants exposed to Cd occurred by the synthesis or decreased degradation or both processes (Singh et al. 2016). In this study, the increase of proline (Fig. 2a, b) in plants in the presence of Cd possibly depends on the NO_3^- concentration (Fig. 1a. b) since there was a significant positive correlation coefficient ($r = 0.801$; $P = 0.0304$) between these variables. On the other hand, the 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 could be a plant response to reduce the phytotoxicity of the metal (Chen et al. 2001, Aslam et al. 2014). Other forest species also showed an increase of proline induced by Cd (He et al. 2013, Wang et al. 2016, Yadav and Srivastava 2017).

The TSC increase in *V. surinamensis* exposed to Cd (Fig. 3a, b) may have worked as a compatible solute, helping the plant in the osmotic adjustment against Cd stress (Singh et al. 2016). When the TSC accumulates, it may contribute to maintaining the plant's water status, favoring tissue protection and physiological processes, an essential mechanism in the tolerance of *V. surinamensis* to Cd, at least during the experimental period. The total soluble carbohydrate results obtained in this study corroborate with Anand et al. (2017).

Sucrose is a disaccharide consisting of glucose and fructose. Through the invertase activity, it 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 a positive effect on the activity of sucrose phosphate synthase (SPS) and a negative effect on the sucrose synthase (SuSy) caused by the metal (Fryzova et al. 2017). Besides, 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 α- and β-amylase hydrolases; however, heavy metals have an inhibitory effect on these enzymes (Reyes et al. 2018). The cell metabolism involving sucrose possibly reduces in plants exposed to Cd (Badr et al. 2015), accumulating this carbohydrate as an energy-saving mechanism, and improving Cd tolerance (Rahoui et al. 2015) because it chelates with sucrose. Thus, high concentrations of sucrose in *V. surinamensis* suggest a good

metabolic regulatory state of the plant in the presence of Cd. More species exposed to Cd also contained high sucrose concentrations (Devi et al. 2007, Kapoor et al. 2016).

The higher concentration of reducing sugars in plants under Cd stress (Fig. 3e, f) indicates energy savings 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). They 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*. Also, 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). Similar reducing sugar concentrations have been found in other species (Shah et al. 2017).

4. Conclusion

The reduction of NR, ammonia, TSA, and TSP activity evidenced the Cd effect and suggested metal toxicity, at least in part, in the nitrogen assimilation and metabolism of *V. surinamensis*.

The increase in TSC, sucrose, and proline suggested a metabolic regulatory mechanism in *V. surinamensis* exposed to 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, an average temperature of 25.9 °C, an average relative humidity of 86.8%, and a wind speed of 1.35 m s^{-1}.

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).

Authorization is not necessary to collect seeds from this area, 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 widespread species, easily identified by the local workers of Embrapa Amazônia Oriental on many previous occasions.

Concerning botanical verification, this institution's herbarium 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, it 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), maintained under mean air temperature (Tair) 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_2PO_4, 400; KNO_3, 2000; $Ca(NO_3)_2 \cdot 4H_2O$, 2000; $MgSO_4 \cdot 7H_2O$, 800; FeEDTA, 400; H_3BO_3, 400; $MnCl_2 \cdot 4H_2O$, 400; $ZnCl_2$, 400; $CuCl_2 \cdot 2H_2O$, 400; and $H_2MoO_4 \cdot H_2O$, 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^{-1} of $CdCl_2$ (control), 15, 30, 45, and 60 mg L^{-1} of $CdCl_2$. 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 completely randomized with seven replications per treatment, totaling 35 experimental units. A single 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 (NO_3^-) and free ammonium (NH_4^+) (Weatherburn 1967); activity of the enzyme nitrate reductase (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 analysis

The experimental data were evaluated for normality and homogeneity of variances by the Shapiro-Wilk and Bartlett tests. The analyzes were performed using the SAS 9.1.3 software (SAS 2007) and the Rstudio 1.1.383 software. For the parametric variables, the comparison of means was performed in PROC GLM, from SAS, with the method of least squares of adjustment of general linear models, with the

MEANS instruction of multiple comparisons for means of the main effect with the command *lines Tukey* (Tukey's studentized range test (HSD). For the correlation analysis, the PROC CORR procedure of the SAS, was used (Pearson linear and Spearman moment product, for the parametric and nonparametric variables, respectively). The significance of these correlations was performed by the t-student test. The Kruskal-Wallis test with Bonferroni correction was used for nonparametric variables in the control of Type I error in RStudio. All statistical analyzes were performed at a 5% significance level.

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; NO3− - Nitrate; RNO3− - Nitrate Reductase; DM – Dry Mass; FM – Fresh Mass; Glu – Glutamine; NH4+ - 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 not been 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.

Funding

not applicable

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

Acknowledgments

The authors are grateful to the Group of Studies on Biodiversity in Upper Plants of the Federal Rural University of Amazonia for the collaborations of researchers.

Coordination of the Improvement of Higher Education Personnel in Brazil (CAPES).

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Figures

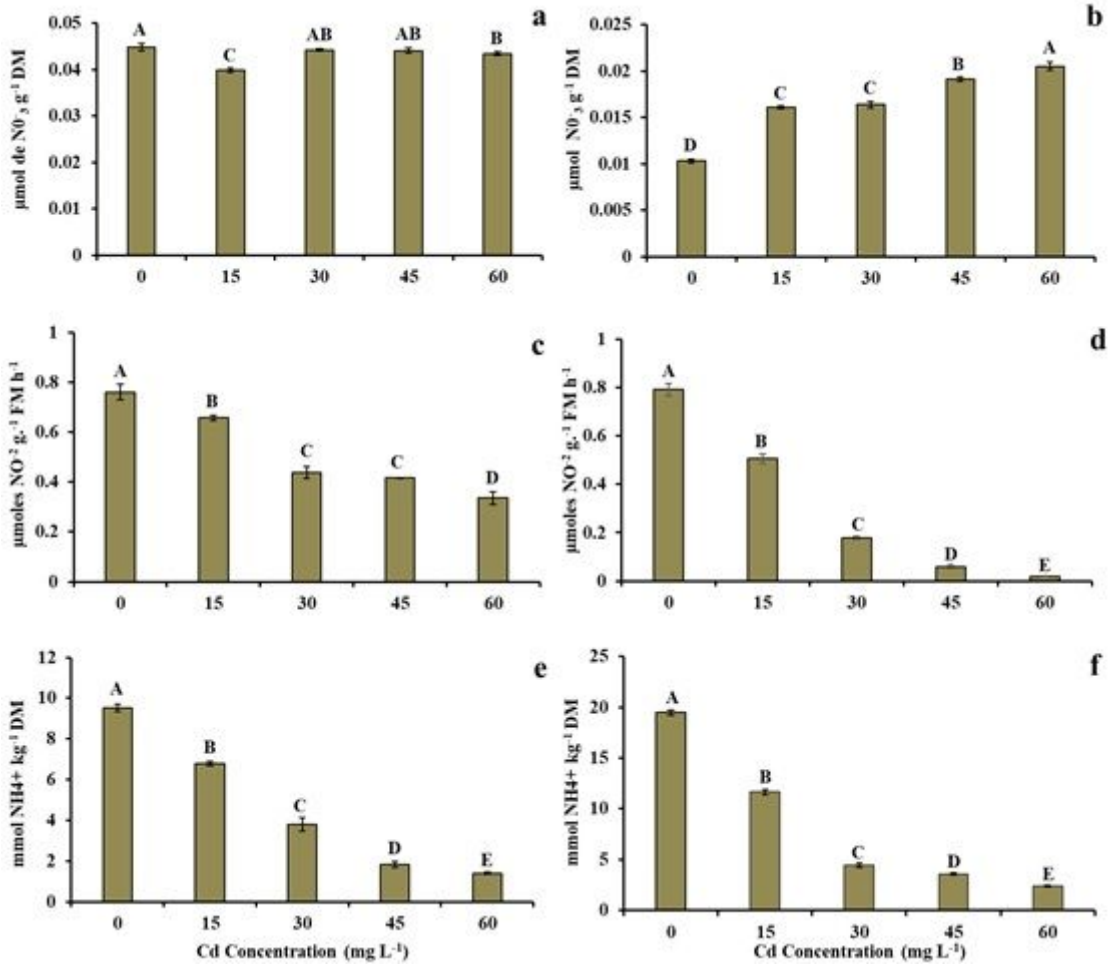

Figure 1

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 ± SD, n = 7.

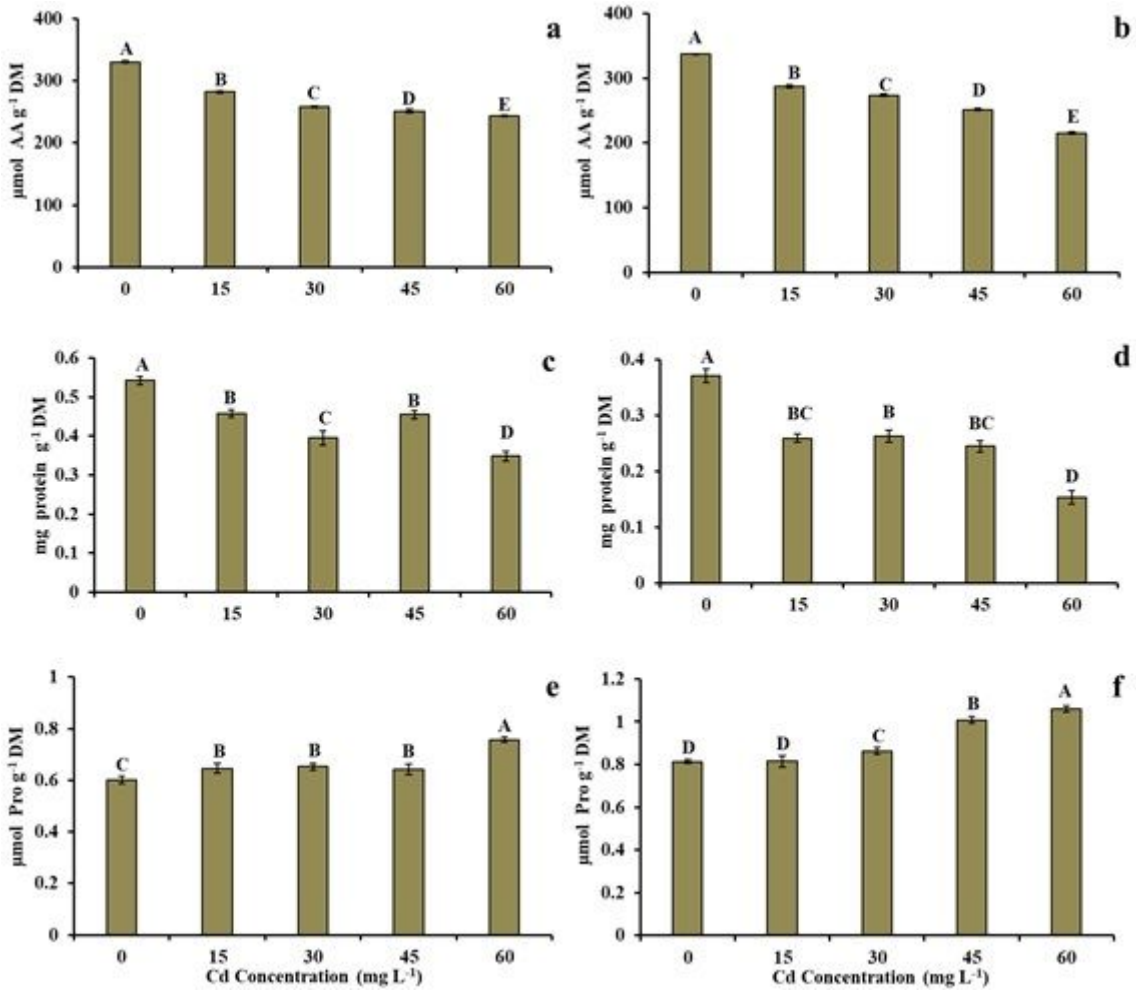

Figure 2

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 ± SD, n = 7.

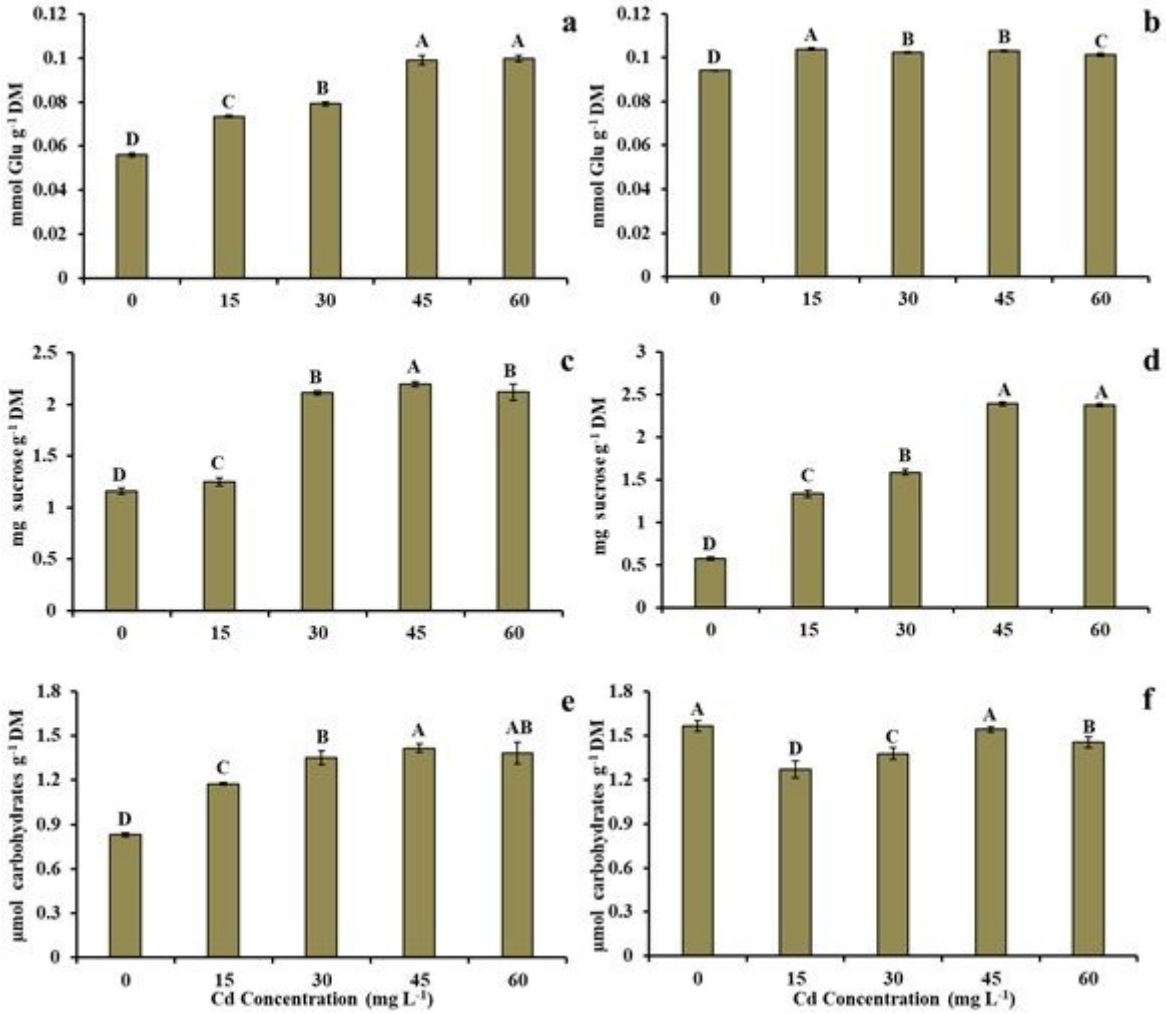

Figure 3

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 ± SD, n = 7.