The Addition of KNO3 and (NH4)3PO4 Maybe Improves the Ability to Accumulate Cd by Regulating the Redistribution of Nutrients in Bermudagrass

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Abstract

The application of fertilizer can improve the Cd enrichment efficiency of plants, but the changes of nitrogen (N), phosphorus (P), potassium (K) in the growth medium have different impacts on Cd enrichment efficiency of plants. In this study, hydroponic experiment by adding different KNO_3 and $(NH_4)_3PO_4$ was carried out to study whether and how the addition of available N, P and K can improve Cd accumulation. The four treatments were CK, 0.4 mg kg^{-1} Cd (Cd), 0.4 mg kg^{-1} Cd and KNO_3 (COK), 0.4 mg kg^{-1} Cd and $(NH_4)_3PO_4$ (CNP). The results showed that the chlorophyll contents, PSII maximum photochemical quantum efficiency (Fv/Fm) and PSII potential activity (Fv/Fo) of COK and CNP were higher than that of CD. There was no significant difference between CK and CNP ($P > 0.05$). The N, P and K contents and Cd concentrations of roots and stems of COK and CNP were significantly higher than those of CD(*P*□0.05).The BCF of COK and CNP were also significantly higher than that of CD, increasing by 39.8% and 37.6%, respectively. The root, stem and leaf dry weight of COK and the root and stem dry weight of CNP were higher than that of CD, and the biomass of the whole basin was significantly higher than that of CD (*P*□0.05), but with no significant difference from that of CK (*P*□0.05). In addition to stem K accumulation of CNP, N, P and K allocated to root and stem in COK and CNP were significantly higher than that in CD (*P*□0.05). There was no significant difference in pH and water soluble Cd content among CD, COK and CNP (*P*□0.05). it was concluded that plants can improve their Cd accumulation ability by changing biomass and nutrient allocation. The conclusion provides a theoretical basis for improving soil environmental factors by improving fertilization strategies of related plants so as to improve Cd enrichment ability.

Introduction

The heavy metal cadmium (Cd) is a non-essential element for animals, plants and humans. Cadmium will enter the organism through the food chain and endanger human health (Zhou et al. 2014). Phytoremediation is one of the most important technologies for Cd remediation exhibits features of no damaging soil ecological environment, low cost and no secondary pollution (Mahar et al. 2016). The application of fertilizer is an effective way to increase Cd accumulation capacity in plants. Application of eleven kinds of nitrogen (N) can improve the Cd accumulation efficiency by raising vegetative organs Cd concentration or biomass of *Rorippa globosa*, and NH_4HCO_3 and NH_4Cl application have the best effect (Wei et al. 2015). The Cd accumulation ability of *Solanum nigrum* could be improved by the combination of different N, phosphorus (P) and potassium (K) (Wang et al. 2019). The Cd accumulation ability of plants will be different due to the different N forms in growth media. Some studies have pointed out that the increase of ammonia nitrogen (NH_4^+-N) can improve the Cd absorption by flower bud (*Carpobrotus rossii*), nightshade and wheat (Cheng et al. 2016; Cheng et al. 2017; Cheng et al. 2018). However, other studies have indicated that the increase of nitrate nitrogen (NO_3^--N) is more conducive to the Cd absorption by plants than the increase of NH_4^+-N (Xie et al. 2008; Luo et al. 2012). Phosphorus inhibits the Cd absorption by rice (*Oryza sativa* L.) (Zhao et al. 2019). Previous studies have found that soil NH_4^+-

N, available P and K were positively correlated with the Cd bioconcentration factor of bermudagrass (Chen et al. 2019). Moreover, the use of N, P and K fertilizer can change the Cd accumulation efficiency of plants by adjusting the soil pH and the available Cd (Xue et al. 2019; Ata-Ul-Karim et al. 2020). The biomass and N and P accumulation of roots, stems and leaves of osmanthus showed a tendency to decrease with the increase of Cd concentration, Cd changed the distribution pattern of plant biomass and N, P accumulation (Wu et al. 2010). When chamomile (*Matricaria chamomilla*), alfalfa (*Medicago truncatula*) and rice (*Oryza sativa*) were stressed by Cd, the distribution pattern of nutrients also changed (Liu et al. 2003; Xu et al. 2009; Kovacik et al. 2014). Therefore, different conclusions have been drawn on the Cd accumulation efficiency of plants because the N, P and K change in the growth medium and the allocation patten difference in plants.

Although Cd accumulative plants such as *Solanum nigrum* L., *Sedum alfredii* Hance, *Phytolacca americana* L. and so forth have been found, they cannot be applied and promoted due to their small biomass or low accumulation efficiency (Liu et al. 2010; Cheng et al. 2016; Tian et al. 2017). Bermudagrass is a heavy metal Cd enrichment plant and strong stress resistance (Tan et al. 2013; Tan et al. 2017). Previous studies have found that Cd stress alters the nutrients allocation of bermudagrass organs. The accumulation of N, P and K in the stem was positively correlated with the Cd accumulation in the stem, and the accumulation of N, P and K in the root, stem and leaf was positively correlated with the Cd accumulation in the leaf (Tan et al. 2020). Bermudagrass can better grow, reproduce and adapt to Cd through the allocation of nutrient resources in different organs and structural functions (Tan et al. 2020).

In this paper, a certain amount of KNO_3 and $(NH_4)_3PO_4$ were added at the same Cd concentration to change the available N, P and K contents in the hydroponic simulation experiment. It was to verify that the increase of nutrient availability could improve the effect of Cd accumulation ability by measuring the physiological and ecological indexes and the contents of N, P and K in various organs of plants, pH and the available Cd contents of water. Overall, the target is to provide some theoretical basis for enhancing Cd enrichment ability of plants by improving soil environmental factors and fertilization strategies of relevant plants in Cd contaminated areas.

Materials And Methods

Plant materials

Bermuda grass seeds were purchased from Hunan Tianquan Grass Industry Development Co. LTD., Changsha, Hunan Province, China. The floating seedling trays were 180 holes (540mm x 340mm x 50mm) used in the experiment. The water tanks were thickened plastic logistics box with a capacity of 60 L (600mm x 400mm x 340mm). The 1/2 Hoagland nutrient solution is as follows: $Ca(NO_3)_2$4H_2O 945 mg $L^{-1}$$KNO_3$ 607 mg L^{-1} $(NH4)_3PO_4$3H_2O 115 mg $L^{-1}$$MgSO_4$7 H_2O 493 mg L^{-1}Fe-EDTA, adjusting the nutrient solution pH to 6.8-7.0 with 0.1M NaOH or 0.1M HCl. The culture medium was vermiculite. The total contents of N, P and K in vermiculite were 0.098, 0.243 and 9.807g kg^{-1} respectively.

Experimental design

The plump and consistent seeds of Bermuda grass had been in the sun for three days for improving the germination rate. The seeds were then evenly placed on vermiculite in a hydroponic seedling tray with 10 seeds per hole. The seedling trays were placed in 15 water culture tanks respectively containing 50 L 1/2 Hoagland nutrient solution. When the seedling entered the growth stage, only three plants were left in each hole. After one week of seedling thinning, the experimental treatment began.

There were four treatments with three replicates. The four treatments were CK, 0.4 mg kg^{-1} Cd (Cd), 0.4 mg kg^{-1} Cd and KNO_3 (COK), 0.4 mg kg^{-1} Cd and $(NH_4)_3PO_4$ (CNP). The nutrient availability of each treatment was shown in table 1, in which the available P and available K were all water-soluble nutrients. The content of available K in COK was twice than that in CD, and the content of available P in CNP was three times than that in CD. The nutrient solution used for plant culture was 1/2 Hoagland nutrient solution. The treatment time was 15 d. During the experiment, the floating seedling tray was taken out every day. The amount of 1/2 Hoagland nutrient solution evaporated on that day was added to the water tank, and then the floating seedling tray was put back into the water tank. In order to avoid the effects of other factors, no pesticide and fertilizer were applied during the experiment.

Morphological indices

Nine plants were selected for each treatment and washed carefully with tap water and distilled water respectively. The samples were put into the oven and dried to constant weight at 80°C. The dry weights of roots, stems, leaves and the biomass were determined.

Determination of chlorophyll content and chlorophyll fluorescence parameters

The relative chlorophyll content (SPDA) of the fully expanded leaves of each treatment was determined by SPDA-502 Plus portable chlorophyll meter (Konica Minolta Japan). The new leaves of PSII maximum photochemical quantum efficiency (Fv/Fm) and PSII potential activity (Fv/Fo) after full dark adaptation were determined using rapid plant stress measurement instrument (OS-30p+ Opti-sciences, America).

Determination of pH and available Cd in water

10 ml water samples were taken, pH was determined by pH meter (Ray-magnetic PHS-3G), and the content of water-soluble Cd was measured by ICP-MS.

Determination of N, P and K in plant organs

The plant samples were carefully cleaned with tap water and distilled water respectively, all samples were put into the oven and heated to 105°C for fixation, to stay for half an hour, then the samples were dried to a constant weight at 80°C. Plant samples from different treatments were divided into root, stem and leaf, and crushed by grinder. Samples of crushed roots, stems and leaves were digested by heating with concentrated H_2SO_4-H_2O_2 (analytical reagent). 10 ml digestion solution was used to determine the content of N and P in different organs by flow analyzer (AA3, German SEAL). The other 10 ml digestion solution was used to determine K content in various organs of plants by atomic absorption spectrophotometer (NOVAA350 German Jena Company).

Cd determination of soil and plants

The leaves and stems are the aboveground parts, and the root system was underground part. Take 2.6 finely ground root, stem and leaf samples were heated and digested with concentrated HNO_3 and $HClO_4$ (3:1 v/v, analytical reagent). ICP-MS was used to determine the total Cd content of roots, stems and leaves.

Statistical analysis

All the results were the mean ± standard deviations (SD) of three replications. Microsoft Excel 2016 was used for data statistics and tabulation, and SPSS 20.0 was used for normal distribution and T test. Data was analyzed using SPSS 20.0 for the ANOVA test and LSD multiple comparative analysis. The figures were drawn using Origin 9.0.

Results

Effects of different treatments on chlorophyll, Fv/Fm, Fv/Fo and Cd accumulation of bermudagrass

Under the same Cd concentration, the chlorophyll content of the CD treatment was significantly lower than that of CK. The chlorophyll content of COK and CNP was higher than that of the CD, and there was no significant difference (*P*0.05) (Fig. 1 A).

CD had the smallest Fv/Fm, which showed the most serious Cd stress. There was no significant difference in Fv/Fm between COK and CD. Fv/Fm of CNP was significantly higher than that of CD, and there was no significant difference between CNP and CK (*P*0.05) (Fig. 1 B). The CK Fv/Fo was the largest and showed the strongest potential activity, while the Fv/Fo of CD was the smallest and showed the weakest potential activity. There was no significant difference in Fv/Fo between COK and CD. Fv/Fo of CNP was significantly higher than that of CD, but there was no significant difference between CNP and CK (*P*0.05) (Fig. 1C).

The BCF of COK and CNP was significantly higher than that of CD, increased by 39.8% and 37.6% respectively. The TCF of COK and CNP was significantly lower than that of CD (*P*0.05)(Fig. 1 D).

Effects of different treatments on biomass of bermudagrass

In agricultural activities, the application of KNO_3 and $(NH_4)_3PO_4$ fertilizers can increase the biomass of crops and thus the yield. In this experiment, under the stress of the same Cd concentration, the root dry weight of COK and CNP was higher than that of CD, but the difference was not significant (*P*0.05) (Fig. 2A).

The stem dry weight of COK was significantly higher than that of CD. There was no significant difference between COK and CNP (*P*0.05) (Fig. 2B). There was no significant difference in leaf dry weight between CD and CK (*P* 0.05) (Fig. 2C). However, the biomass of COK and CNP was significantly higher than that of CD, and there was no significant difference with that of CK (*P*0.05) (Fig. 2D). It can be seen that the addition of KNO_3 and $(NH_4)_3PO_4$ can significantly increase the biomass of bermudagrass under Cd stress.

Effects of different treatments on N, P, K distribution and Cd accumulation of bermudagrass

The N, P and K distributions of roots and stems of COK and CNP were significantly higher than those of CD(*P*0.05). The N, P and K distributions of leaves were not significantly different from those of CD (*P* 0.05). Cd accumulation of roots and stems of COK and CNP was significantly higher than that of CD, but Cd accumulation of leaves was not significantly different from that of Cd (*P*0.05).

Effects of different treatments on effective Cd and pH of water

Existing studies have shown that the effective Cd and pH are important factors affecting the Cd enrichment ability of plants. In this study, the pH of the four treatments before the start of the test (V1) was neutral with no significant difference. pH of CD, COK and CNP was acidic at the end of the experiment (V2) except CK was neutral, but there was no significant difference among the three treatments. The pH difference at the early stage and the later period of CD, COK and CNP was significantly higher than that of CK, but there was no significant difference among the three treatments (*P* 0.05). There was no significant difference in effective Cd content in the early or late periods among CD, COK and CNP (*P*0.05). Thus, the addition of KNO_3 and $(NH_4)_3PO_4$ did not significantly change the pH value and the content of effective Cd when bermudagrass was stressed by Cd (Table 2).

Discussion

In the Cd contaminated soil, when applying NH_4^+-N fertilizer, plant roots will release H^+ into the medium. When applied with NO_3^--N fertilizer, the roots would release OH^- or HCO_3^- into the medium. NH_4^+-N and NO_3^--N would decrease pH in the medium. Cheng et al. (2017) showed that under two different acidic soil, the application of NH_4^+-N can reduce pH and improve the content of available Cd in soil, thus increasing the Cd enrichment by plants. The addition of different N, P and K fertilizer combinations would lead to a significant decrease in soil pH and promote the enrichment of Cd by *Solanum nigrum* (Wang et al. 2019). Wei et al. (2015) found that by adding 11 different types of N fertilizer experiments including $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$ with P elements, different N types increased plant Cd enrichment but the enrichment efficiency was different, NH_4HCO_3 and NH_4Cl can increase the biomass of *Rorippa globosa*, and improve Cd enrichment ability at the same time, but there was no significant difference in the soil pH.

In this study, the addition of $(NH_4)_3PO_4$ broke the distribution pattern of nutrients, significantly increased the accumulation of N, P, K and Cd in the root and the accumulation of N, P and Cd in the stem (Fig. 3). it was found that $(NH_4)_3PO_4$, as the N and P source of NH_4^+-N and effective P, could also significantly improve the Cd enrichment ability and biomass of plants, although it did not significantly change the content of effective Cd and pH of water. However, increasing the available N and P content in soil could indeed improve the Cd enrichment ability of bermudagrass. The addition of $(NH_4)_3PO_4$ improved the Cd accumulation ability of plants by increasing the biomass and allocating high N, P, and K to roots and stems in the experiment, while reduce the Cd effect on photosynthetic system because the chlorophyll, Fv/Fm, and Fv/Fo all increased (Fig. 1). Since the content of NH_4^+-N and available P in CNP was three times higher than that of CD. It was the effect of N and P altogether, which can only explain the overall effect of the available N and P on the plant Cd enrichment ability. The addition of $(NH_4)_3PO_4$ did not change TCF, which may be attributed to the lower value of NH_4^+-N/NO_3^--N (0.030-0.098) in the addition. Studies have shown that Cd content in amaranth decreases significantly with the decrease of NH_4^+-N/NO_3^--N, and increases with the increase of NH_4^+-N/NO_3^--N (Wu et al. 2007). NH_4^+-N/NO_3^--N value will affect the Cd enrichment by rice and maize (Mihailovi 2010; Wu et al. 2018). In previous studies, the larger the ratio of NH_4^+-N/NO_3^--N (0.06-0.68), the higher the TCF (Chen et al. 2019).

Potassium is one of the main nutrients needed in plant growth and is considered to be a quality factor in crop production. Potassium can promote the photosynthesis and has important impacts on the normal growth and development, stress resistance, yield, etc. (Pettigrew 2008; Jin et al. 2011; Wang et al. 2013). Adding different kinds of K to the medium has different effects on Cd enrichment. Chen et al. (2007) showed that the application of K_2SO_4 reduced the Cd absorption in different parts and reduced the plant availability of Cd and Pb in wheat. Liu et al. (2013) indicated that K deficiency protected rice seedlings from Cd stress. The available K content of COK was 2 times higher than that in CD, which proved that changing the K availability in the medium could improve the enrichment Cd of plants. The available Cd content and pH of water were not significantly changed, which reduced the Cd effect on photosynthetic system because the chlorophyll, Fv/Fm, and Fv/Fo all increased. Meanwhile, the biomass was significantly increased and more N, P and K were allocated to plant roots and stems. It was preliminarily verified that changes in K availability increased Cd enrichment by increasing biomass and giving more N, P, and K contents to plant roots and stems.

Conclusion

The addition of KNO_3 and $(NH_4)_3PO_4$ can significantly increase the biomass, break the nutrient distribution pattern of plants and allocate more N, P and K to the roots and stems. Under low Cd concentration, the addition of KNO_3 and $(NH_4)_3PO_4$ can remarkably improve the Cd enrichment ability of plants, reduce the inhibition of Cd stress on chlorophyll synthesis to a certain extent. When bermudagrass was stressed by Cd, the addition of KNO_3 could not significantly alleviate the Cd stress on plants, could significantly increase the Cd enrichment by plants conversely. The addition of $(NH_4)_3PO_4$ can significantly alleviate the Cd stress on plants and increase the Cd enrichment in plants. However, the addition of KNO_3

and $(NH_4)_3PO_4$ did not improve the Cd transfer capacity and change the contents of effective Cd and pH of water significantly.

In conclusion, the addition of KNO_3 and $(NH_4)_3PO_4$ can significantly increase the biomass, adjust the distribution pattern of plant nutrients, allocate more N, P and K to roots and stems to improve the Cd accumulation ability, alleviate the Cd inhibition on chlorophyll synthesis and plants to some extent.

Declarations

Authors' contributions Shuduan Tan has contributed to conceptualization, methodology and writing. Bin Chen has contributed to methodology, investigation, statistics analysis and writing. Zhongshu Liu has contributed to investigation and indices determination, Meng Dong has contributed to methodology and writing, Hongbing Yu has contributed to statistics analysis and writing, Xiaomei Zhou has contributed to supervision and validation, Yili Ge has contributed to investigation and indices determination, Tongtong Hua has contributed to indices determination. All authors read and approved the final manuscript. The authors declare that they have no competing interests.

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Data availability The data and materials will be available on request.

Ethics approval and consent to participate Not applicable**Consent for publication** Not applicable

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Tables

Table1 Nutrient and cadmium contents in different treatments. Values are means±SE, n=4.

Treatment	Cd (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	available phosphorus (mg kg^{-1})	available potassium (mg kg^{-1})
CK	0	4.85±1.22b	170.96±18.07a	7.80±2.02b	95.83±12.21b
CD	0.4	5.27±0.58b	174.42±13.31a	9.23±1.05b	96.50±13.95b
COK	0.4	5.63±0.99b	179.55±5.14a	9.25±2.45b	184.00±14.56a
CNP	0.4	15.83±0.66a	161.76±2.64a	27.24±4.17a	85.59±5.82b

Different lowercase letters of each column are significantly different at *p*<0.05 according to LSD test among different treatments (CK, CD, COK and CNP).

Table 2 Effects of different treatments on available Cd and pH. Values are means±SE, n=4.

Treatments	pH (V_1)	pH (V_2)	pH (V_1-V_2)	Effective Cd (V_1)	Effective Cd (V_2)	Effective Cd (V_1-V_2)
CK	7.04±0.09a	6.54±0.01a	0.49±0.11b	–	–	–
CD	7.23±0.14a	6.43±0.02b	0.81±0.15a	0.19±0.01a	0.14±0.02a	0.05±0.02a
COK	7.30±0.14a	6.39±0.08b	0.91±0.13a	0.18±0.02a	0.13±0.03a	0.05±0.01a
CNP	7.25±0.11a	6.33±0.05b	0.91±0.11a	0.16±0.01a	0.14±0.01a	0.03±0.02a

Different lowercase letters of each column are significantly different at *p*<0.05 according to LSD test among different treatments (CK, CD, COK and CNP).

Figures

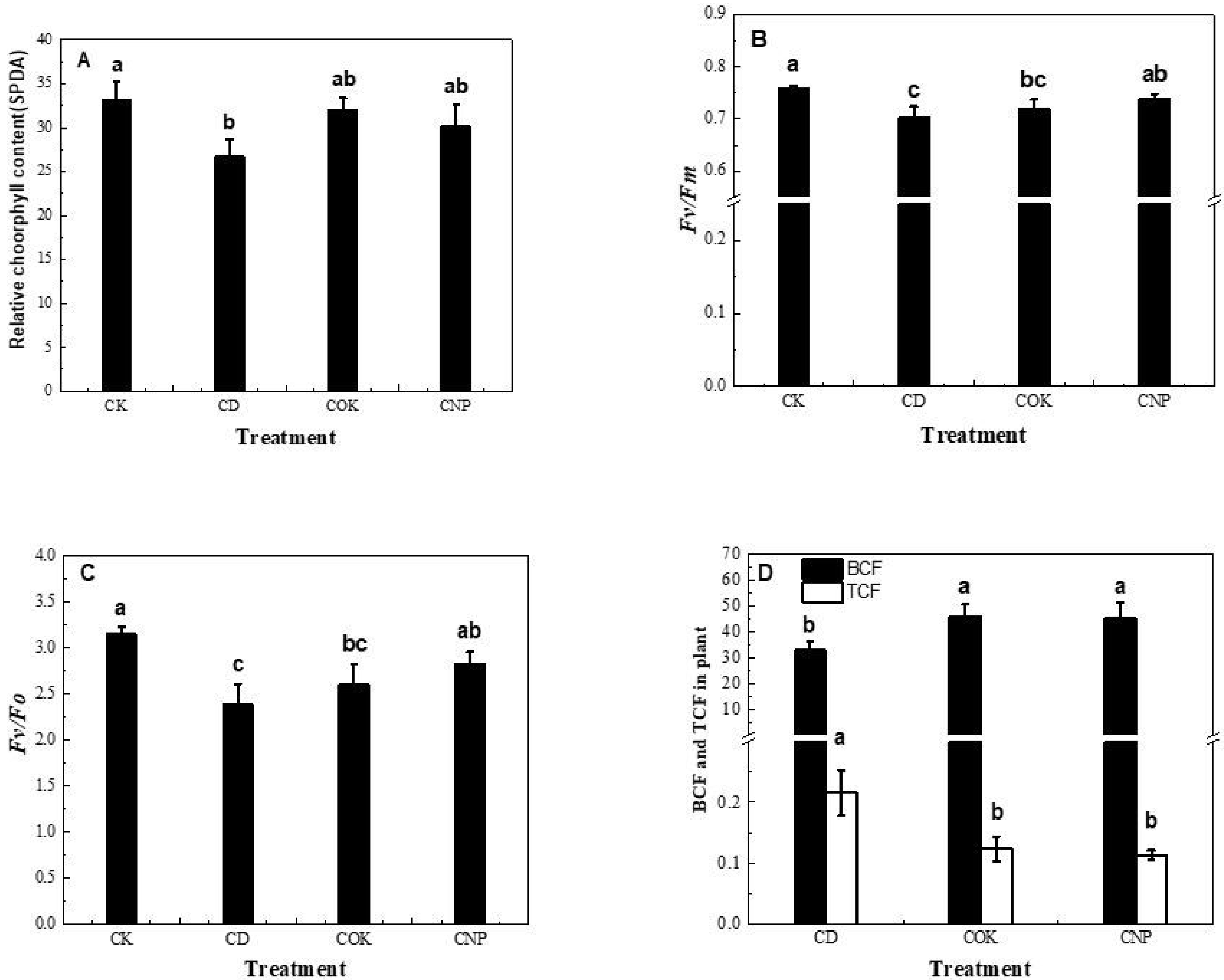

Figure 1

Effects of different treatments on chlorophyll, Fv/Fm, Fv/Fo and Cd enrichment ability of Bermudagrass (n = 4; bars indicate s.e.).

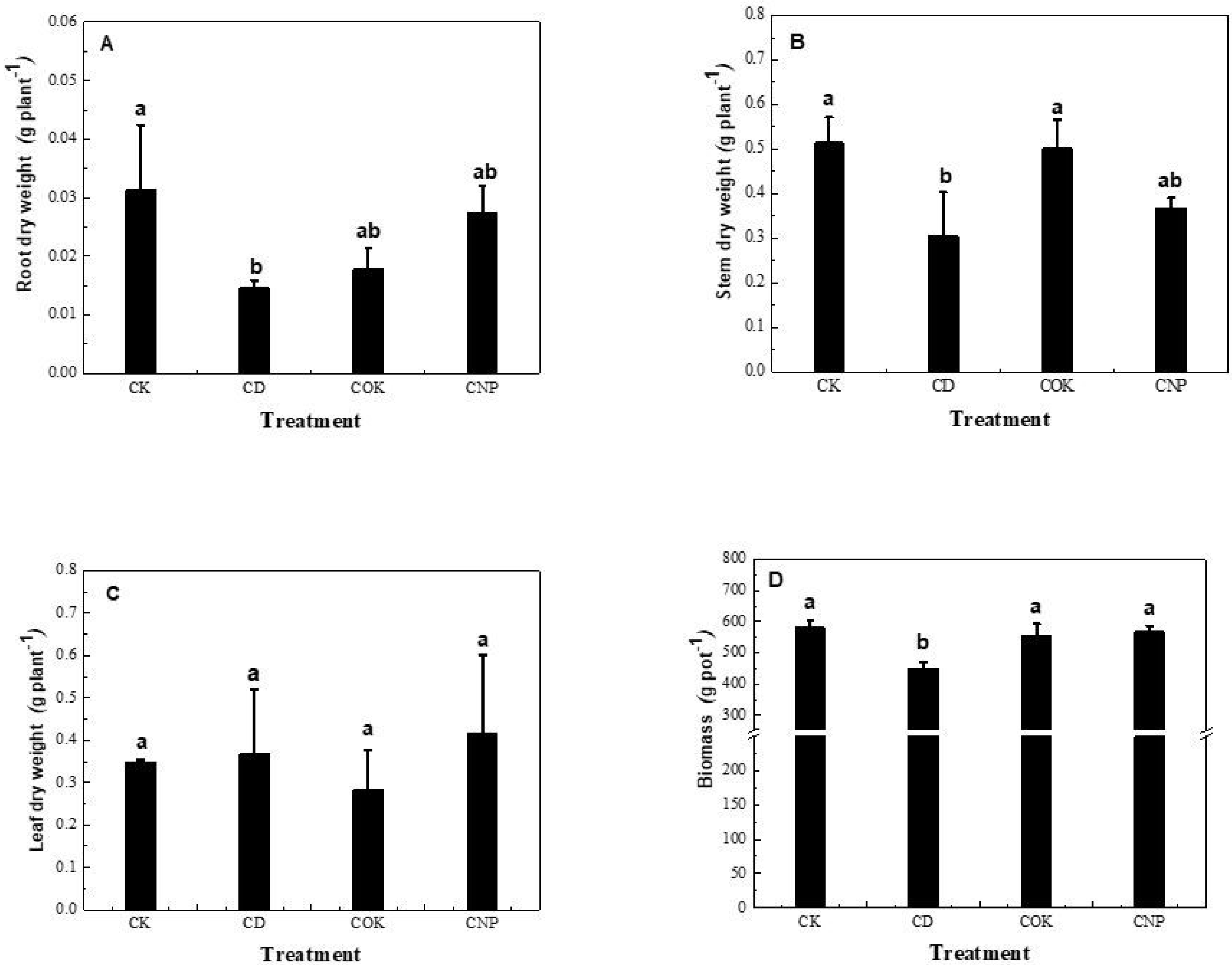

Figure 2

Effects of different treatments on biomass of Bermudagrass (n = 4; bars indicate s.e.).

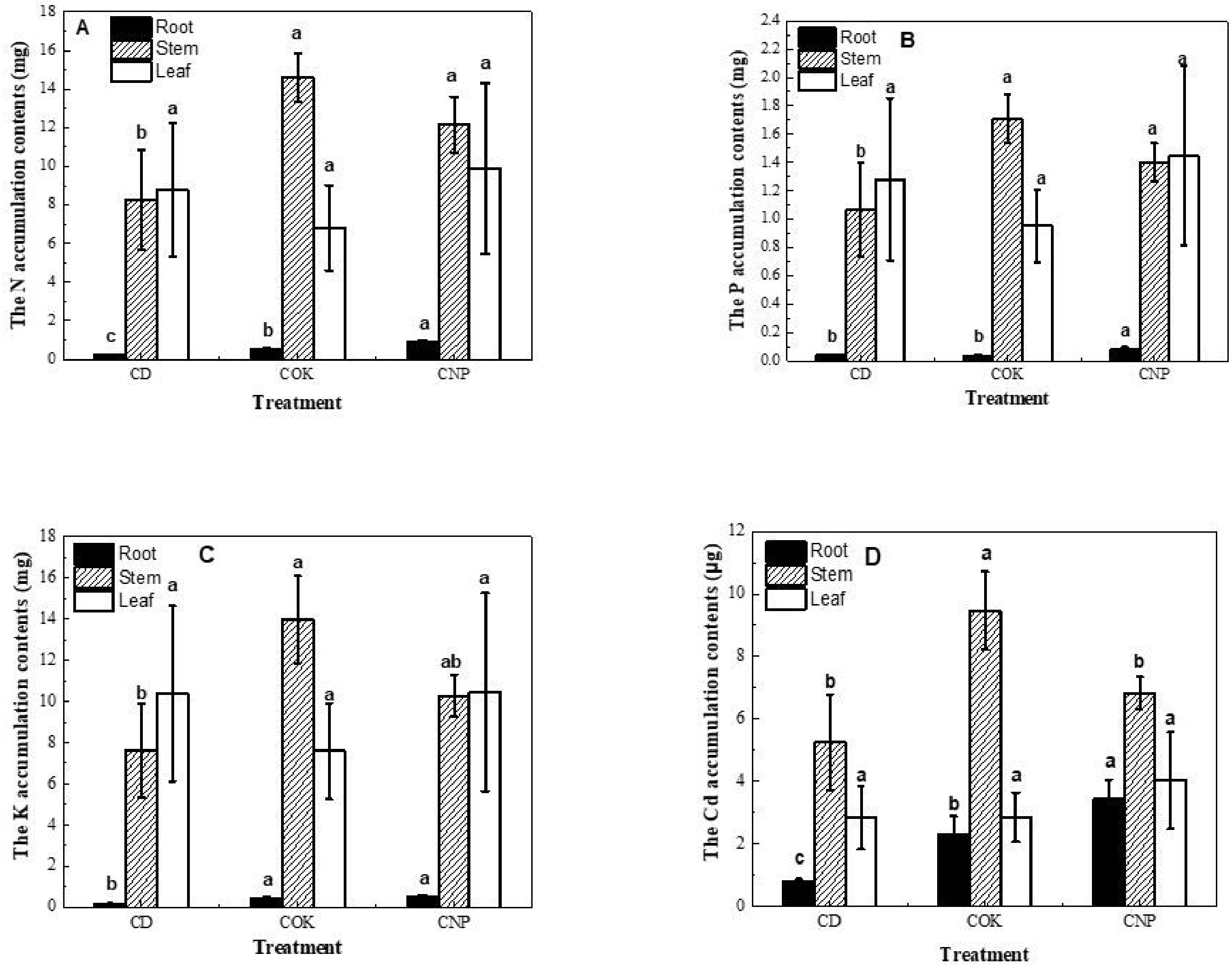

Figure 3

The N, P, K and Cd accumulation content in Plants (n = 4; bars indicate s.e.).