

Influence of light-irradiated *Noccaea caerulea* on the characteristics of dissolved organic matter in its rhizospheric soil during phytoremediation

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Research Article

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Abstract

It has been observed that suitable light irradiation can improve the phytoremediation efficiency of various plants by enhancing their growth rate and metal uptake capacity. However, the mechanisms underlying the effects of light irradiation on metal mobilization and translocation in soils have rarely been reported. This experiment was conducted to evaluate the variation in dissolved organic matter (DOM) in the rhizosphere of *Noccaea caerulescens* when irradiated with different combinations of red (0, 25, 50, 90, and 100 % red) and blue light. *N. caerulescens* was induced to secrete significantly more DOM, relative to the control, into its rhizosphere after being irradiated with pure red light and other red light combinations; this increased the bioavailability of soil Cd. Moreover, the concentrations and proportions of the hydrophilic DOM fractions, particularly hydrophilic acid, which exhibited a high affinity for Cd, increased with increasing ratios of the red light. Furthermore, DOM secreted because of the light irradiation treatments exhibited a significantly higher Cd extraction ability compared with that of the untreated control; this consequently increased the Cd uptake capacity of *N. caerulescens*. The results demonstrated that the secretion of more DOM, particularly hydrophilic acid, plays a pivotal role in improving the phytoremediation efficiency of *N. caerulescens*.

1. Introduction

Soil plays an essential role in supporting ecosystems and the human society by providing a habitat for the majority of the Earth's species and serving as a medium for grain production (Hou et al., 2020). A national evaluation of soil environment reported that measurements for approximately 19 % of the area of farmland exceeded the contamination thresholds in China. Moreover, Cd in particular, ranked at the top in comparison with other heavy metals such as Cu, Cr, Ni, and Hg (Cui et al., 2020).

Heavy metals are one of the most dangerous contaminants because of their great persistence, high biotoxicity, and high bioavailability in various environmental media. They pose great threats to the health of higher animals like humans through the food web (Fei et al., 2019). Cd is ranked seventh on the priority list of hazardous materials established by the US Environmental Protection Agency. It is a highly toxic metal and can enter the human organs via food consumption (Shi et al., 2019). Soil pollution has recently gained increasing interest because it causes a threat to food security. In China, the area of derelict land increases at the rate of 46,700 ha every year, and food security has deteriorated acutely with the pollution of agricultural lands (Yang et al., 2020). Therefore, soil remediation measures are urgently required. The Chinese government has decontaminated approximately 700,000 ha of heavily polluted fields in 2020 and plans to restore approximately 90 % of the contaminated fields within a decade (Hou et al., 2020). Some methods such as isolation, chemical leaching, inerting, and electrokinetic remediation have been developed to clean the metal-impacted fields. However, these technologies are expensive and might cause soil degradation, thus resulting in secondary pollution by leaching (Robinson et al., 2000).

Alternatively, phytoremediation is more acceptable owing to its environmental friendliness and economic feasibility. It utilizes plants to decrease the activity and toxicity of metals in soils (phytostabilization) or to

help metals migrate from soils to other harvestable parts (phytoextraction). However, the low growth rate of plants, especially hyperaccumulators, restricts the wide use of phytoremediation in real fields. Some supplementary measures such as organic acid application, microorganism inoculation, electric field addition, and genetic modification have been taken to improve the metal accumulation capacity and growth rate of plants (Mahmood-ul-Hassan et al., 2017; Xiao et al., 2017). The application of these methods not only increases the cost of phytoremediation but also induces potential environmental risks such as metal leaching, acidification, and soil erosion (Luo et al., 2016).

It has been reported that suitable light irradiation can improve the growth rate and enhance the nutrient extraction ability of various plants. Moreover, light irradiation is a physical trigger in plants rather than a chemical trigger (Luo et al., 2020). Previous studies focused only on the effects of light irradiation on plants themselves. The influence of light irradiation on soil characteristics, especially on the concentration and fractionation of dissolved organic matter (DOM) in soil, has rarely been reported. DOM can activate metals in the soil by generating soluble complexes with them or by competing with metals for adsorption sites on exchangeable surfaces of soil particles (Chen et al., 2018).

It is safe to hypothesize that under the influence of light irradiation, plants can alter the content and fractionation of DOM in the rhizosphere. Therefore, the major goals of this study were to (i) estimate the influence of light irradiation on the dry weight and Cd uptake efficiency of *Noccaea caerulea*, a well-known Cd hyperaccumulator; (ii) evaluate the effects of light treatments on DOM concentrations and fractions in the rhizosphere of the species, and (iii) reveal the mechanisms by which light irradiation improves the Cd decontamination effect of *N. caerulea*.

2. Materials And Methods

2.1 Source of soil sample

The soil samples remediated in this experiment were collected from Guiyu, an electronic waste (e-waste) recycling town located in southern China. Soils in the town are heavily polluted by metals and persistent organic pollutants due to the prevalence of dismantling and recycling activities without essential protection means (Jiang et al., 2019). The e-waste recycling business is detrimental to the local inhabitants, particularly vulnerable populations like children and pregnant women (Kim et al., 2019). Therefore, it is necessary to remediate the soils in the town. Considering the topography and background of the study region, 200 soils (0–20 cm) were sampled. After drying on a filter paper, unwanted materials including stone and organic debris were removed using 2-mm meshes. The sieved soils were homogenized to obtain a composite substrate that represented the overall characteristics of the region. The mixed substrate was underwent three cycles of wetting and drying to ensure an even distribution of Cd in it. When chemical equilibrium was achieved, the composite soil was divided into aliquots of 6 kg each for plant growth. The Cd content did not vary significantly due to complete homogenization. It ranged from 1.02 to 1.18 mg kg⁻¹ with an average value of 1.13 mg kg⁻¹ and a variable coefficient of 5.6 %. The

content severely exceeded the statutory value of 0.3 mg kg^{-1} stipulated by the Ministry of Ecology and Environment of China.

2.2 Experimental design

Seeds of *N. caerulea* were surface-sterilized using 5 % HClO. After cleaning with deionized water to remove the residual disinfectant, the seeds were grown in Petri dishes under an 8/16-h dark/daytime rhythm. During the daytime, the intensity of light was set at $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

After the emergence of four true leaves, ten plants with similar morphophysiological characteristics were transplanted into soil-padded containers for 15 d for acclimation. At the beginning of the light irradiation, five seedlings were maintained in each container. Red and blue light treatments with different proportions of the red light (0, 25, 50, 90, and 100 % red, simplified as R_0 , R_{25} , R_{50} , R_{90} , and R_{100} light treatment groups) generated by LED arrays were used to irradiate the transplanted plants for 16 h each day for a span of 120 days. During the experiment, the strength of each light combination was set at $220 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Plants irradiated with incandescent lamps were used as the control. Water was added to the tray placed under each container to maintain an 80 % soil water-holding capacity. Five replicates were performed for each light treatment including the control. The soil pH was analyzed using a pH meter in a 1:2.5 ratio of CaCl_2 and soil suspension.

2.3 Cd analysis

The harvested *N. caerulea* was separated into roots and aerial parts. Roots were cleaned using tap water to eliminate foreign materials and then immersed in a 10 mM ethylenediaminetetraacetic acid (EDTA) solution to remove the adsorbed ions. The shoots were rinsed using tap water and then soaked in CaCl_2 to remove the adsorbate. The cleaned samples were dried at 70°C in an oven until no weight change was observed. Rhizosphere soil in each container was sampled by shaking the plant roots according to the procedure described by Liu et al. (2004).

The dried plant tissues and rhizosphere soils were pulverized and passed through 74- μm nylon meshes. The powders were solubilized using the aqua regia digestion method (Ok et al., 2011). Briefly, the prepared samples were dissolved in a mixture of hydrochloric acid and nitric acid at a volumetric ratio of 3:1 at 130°C for 100 min. The cooled solution was filtered using a 0.45- μm membrane, and the concentration of Cd in the supernatant was measured using ICP-MS (Agilent 7700, USA).

The pots designed by Martínez-Alcalá et al. (2016) allow the separation of the bulk soil and rhizosphere to reveal the variation in the pseudototal Cd in the soil after the growth of *N. caerulea*. They found that the species failed to change the content of the soil Cd during the 132-day treatment. Therefore, the duration of the present experiment was not long enough for *N. caerulea* to change the concentration of Cd in the composite substrate. Thus, the Cd content was determined at the beginning of the treatment to ensure the homogeneity of the soil.

2.4 Content and fraction of DOM

The DOM in the soil was extracted according to the method described by Jones and Willett (2006). Briefly, 2.5 g of the composite soil was shaken with 25 mL of deionized water for 2 h and then centrifuged at $10,000 \times g$ for 20 min. The solution was passed through a 0.45- μm filter, and the concentration of DOM in the filtrate was measured using a total organic carbon (TOC) analyzer. Furthermore, six DOM fractions including hydrophobic acid (HoA), hydrophobic base (HoB), hydrophobic neutral (HoN), hydrophilic acid (HiA), hydrophilic base (HiB), and hydrophilic neutral (HiN) were extracted using the cation exchange resins Amberlite XAD-8, Amberlite XAD-4, and Duolite A-7, according to the methods described by Leenheer and Croue (2003). The concentrations of these fractions in the extraction solution were determined using the same TOC analyzer.

2.5 Cd extraction ability

An Amberlite cation exchange H resin (Sigma-Aldrich, USA) was used to eliminate the cations in the DOM extracted from different light irradiation treatments. After dilution with deionized water to 100 mg L^{-1} , the DOM dilution was centrifuged with 2 g of the composite soil at $8000 \times g$ for 30 min. The suspension was sieved using a 0.45- μm membrane, and the Cd content in the filtrate was analyzed using ICP-MS. Deionized water was used as a control to extract Cd in the composite soil. Two reference materials, GBW07410 for the soil and GBW10012 for the plant, were used in the analysis processes to ensure the accuracy of the measurement.

2.6 Statistical analysis

All data in this study are exhibited as the average of the replicates. The influences of light irradiation on the dry weight of *N. caerulea*, DOM content and fraction, and Cd extraction ability of DOM from different treatments were judged using one-way analysis of variance. SPSS 15.0 was used for the statistical analyses.

3. Results And Discussion

3.1 Variation in soil pH

At the termination of the experiment, the initial soil pH decreased from 6.2 to 5.5, 5.6, 5.2, 5.5, 5.1, and 5.6 in the control, R_0 , R_{25} , R_{50} , R_{90} , and R_{100} light treatment groups, respectively, without significant differences. Inconsistent findings have been observed in the literature for the changes in the pH of plant-growing rhizospheres. For instance, *Nicotiana tabacum* reduced the soil pH (Loosemore et al., 2004), as expected, while *Brassica juncea* increased the pH in both alkaline and acidic soils (Kim et al., 2010). Variation of the soil pH is an intricate procedure determined by soil characteristics, plant species, and microbial activities. In general, Cd exists as an oxyanion in the soil, and plants can release OH^- into the soil. This results in an increase in the soil pH that helps maintain the charge equilibrium of the soil when the soil Cd oxyanion is extracted. In contrast, plants can secrete organic acid/s to decrease the soil pH. The results suggested that soils may buffer the hyperaccumulator-induced pH variations via different pathways, and the reduction of soil pH might not be a unique mechanism for Cd mobilization in the

rhizosphere of *N. caerulea*. Thus, the concentration and fractionation of DOM can influence the bioavailability of Cd in soil, and these parameters have been discussed in this study.

3.2 Characteristics of DOM

As shown in Fig. 1, the initial DOM concentration in the composite substrate was significantly lower than that in all the growing treatments of *N. caerulea* including the control. This result indicated that the cultivation of this species increased the content of DOM in its rhizospheric soil via the secretion of organic materials into the soil; this observation was consistent with the results of previous studies (Jing et al., 2017; Smith et al., 2018). Relative to the control, the R₀ and R₂₅ light treatments slightly decreased the DOM levels, while the R₅₀, R₉₀, and R₁₀₀ light treatment significantly increased the concentrations of soil DOM by 19.0, 26.5, and 46.9 %, respectively, compared with the control. No significant differences were observed in the soil DOM levels of the R₅₀ and R₉₀ light treatment groups; R₁₀₀ had significantly higher soil DOM levels than the R₅₀ and R₉₀ light treatment groups (Fig. 1). Martin et al. (2017) found that the exudation of dissolved organic carbon (DOC) and protein-like DOM by *Halophila ovalis* grown under continuous light irradiation is more than that by the control; this supports the current findings.

The fractionations of DOM in the rhizosphere of *N. caerulea* exhibited different concentration characteristics under different light irradiation treatments. Generally, the concentrations of all six fractions, especially the hydrophilic fraction consisting of HoA, HoB, and HoN, increased with increasing proportions of the red light (Fig. 2). However, monochromatic (R₀) and 75 % (R₂₅) blue light irradiation treatments showed slightly lower concentrations of DOM fractions than the control. Additionally, the concentration of acid fractions consisting of HiA and HoA was significantly higher in the R₁₀₀ light treatment group than that in other treatment groups (Fig. 2). The proportion of hydrophilic fractions consisting of HiA, HiB, and HiN in the rhizosphere of *N. caerulea* increased with an increase in the ratio of the red light until it reached a peak of 64.7 % at R₂₅. At this point, the proportions reduced significantly. Because the metal affinity of the hydrophilic fractions was higher than that of the hydrophobic fractions (Huang et al., 2019), we hypothesized that the chemical properties of DOM extracted from soils under the R₉₀ and R₁₀₀ light treatment groups were more propitious for the activation of soil Cd. A Cd extraction experiment was performed to verify the correctness of this assumption.

3.3 Cd extraction ability of DOM

The DOM from soils planted with *N. caerulea* had a significantly higher Cd extraction capacity relative to that of deionized water regardless of light irradiation; however, the exception was R₀ light treatment group (Fig. 3). The amount of Cd extracted using DOM from the control, R₀, R₂₅, R₅₀, R₉₀, and R₁₀₀ light treatment groups was 27.6 %, 6.9 %, 24.1 %, 37.9 %, 103.4 %, and 110.3 % greater, respectively, than that obtained using deionized water. The results indicated that monochromatic blue light could reduce the Cd extraction ability of DOM, while an increasing ratio of the red light enhanced the Cd extraction capacity. Chen et al. (2018) evaluated the response of Zn extractability and transfer by diffusion to DOM after maize straw amendment. They reported that the DOM derived from straw

increased the content of DTPA-extractable Zn by forming a Zn-DOM complex. Krishnamurti et al. (2004) found that the Cd-DOM complexes were absorbed in an intact form by alga and plants resulting in higher Cd toxicity. Combined with the results of previous studies, the current Cd extraction experiment might explain the reason for the greater mobilization of Cd in the rhizosphere of *N. caerulea*, relative to the control, by the DOM from light irradiation treatments (except monochromatic blue light). This phenomenon might influence the phytoremediation efficiency of *N. caerulea*.

The Cd decontamination effect was determined by the dry weight and Cd uptake capacity of the species. The dry weight of the plant roots and aerial parts in the present experiment ranged from 19 to 49 mg and 43 to 125 mg (Fig. 4a), respectively. The lowest root dry weight was observed under monochromatic blue light irradiation (R_0), while the lowest dry weight of the aerial part was observed under monochromatic red light irradiation (R_{100}). The R_{50} light treatment group had the highest dry weight among all species; the dry weights were successively lower in R_{90} , R_{25} , R_0 , control, and R_{100} light treatment groups. In general, R_{100} inhibited the growth of the aerial parts and stimulated the development of the belowground parts. The reduced dry weight of the aerial parts could not be compensated by the enhanced root biomass. Lower growth rate and lower biomass generation ability in plants grown under monochromatic red light have been reported for different plant species. Hogewoning et al. (2010) found that *Cucumis sativus* had a lower dry weight when grown under monochromatic red light compared to its dry weight when grown under red-blue light. They attributed the relatively low growth rate of the species to disorders in the development of the photosynthetic apparatus and suggested that a small dose of blue light could alleviate the symptoms.

The concentration of Cd in the aerial parts of *N. caerulea* increased with a decrease in the ratio of the red light. This trend continued until it reached a peak at R_{50} and then reduced slightly (Fig. 4b). The mechanisms underlying the effects of light irradiation on metal accumulation in plant tissues have rarely been reported. Dong et al. (2014) have reported that a suitable combination of red and blue light improves the activities of the antioxidant enzymes in the plants. This removes the oxygen free radicals resulting from metal stress and helps the plants accumulate a greater amount of metals without obvious oxidative damage. This result can at least partly explain why the R_{50} light treatment group had a higher Cd content in the plant tissues compared with the contents observed in other treatments.

3.4 Phytoremediation efficiency

The Cd uptake efficiency of *N. caerulea* was calculated as the product of the dry weight and Cd content of the species. Based on the calculations, 5.5, 9.4, 7.6, 16.4, 9.3, and 3.7 μg of Cd was extracted from the control, R_0 , R_{25} , R_{50} , R_{90} , and R_{100} light treatment groups, respectively. Considering the initial soil Cd content (1.13 mg kg^{-1}), 183, 107, 132, 61, 108, and 268 planting cycles of *N. caerulea* were required to remove the excess Cd in the composite soil in the control, R_0 , R_{25} , R_{50} , R_{90} , and R_{100} light treatment groups, respectively (Table 1). Under suitable growing conditions, three planting and harvesting cycles can be carried out in one year, and 61, 36, 44, 21, 36, and 90 years would be required to clean the soil in the control, R_0 , R_{25} , R_{50} , R_{90} , and R_{100} light treatment groups, respectively. Suitable light irradiation

decreased the required phytoremediation time by half when compared to that of the control. This study verified that light treatment is an efficient method to enhance the phytoremediation efficiency of *N. caerulescens* from the perspective of soil DOM concentration and fractionation. It is worth noting that, although the chemical properties of DOM extracted from the soils in the R₁₀₀ light treatment group were more favorable for the activation of soil Cd, optimal phytoremediation was not observed with this irradiation strategy. This may be due to the physiological responses of *N. caerulescens* to light irradiation, and this mechanism should be discussed in detail in future work.

Conclusion

This work showed that light irradiation increased the phytoremediation efficiency of *N. caerulescens* via increasing its dry weight and Cd uptake capacity. Relative to the control, the content and acid fraction of DOM were enhanced by light irradiation. The Cd extraction capacity of DOM in R₉₀ and R₁₀₀ were significantly higher than other treatments, increasing Cd content in *N. caerulescens* roots. The present experiment exhibits that the main mechanism to increase Cd phytoremediation efficiency of *N. caerulescens* is the alteration in concentration and fraction of DOM in treated plant, which increases Cd activity in soils.

Declarations

Ethical Approval: Not applicable. This work did not describe experiments with animals, human subjects, or human tissue samples.

Consent to Participate: Not applicable. This work did not describe experiments with animals, human subjects, or human tissue samples.

Consent to Publish: The manuscript entitled, "Influence of light-irradiated *Noccaea caerulescens* on the characteristics of dissolved organic matter in its rhizospheric soil during phytoremediation" is prepared in accordance with the Guide for Authors available on the journal's website and it has not been published elsewhere in part or in its entirety. All authors attest to the validity of its contents, and agree to its submission in Environmental Science and Pollution Research.

Authors Contributions: Conceptualization, Yao Niu, Jie Luo; Data curation, Zhansheng Wang, Jie Luo; Methodology, Hanfei Wang; Funding acquisition, Jie Luo; Writing – original draft, Yao Niu, Xiaoying Yang, Jie Luo; Writing – review & editing, Min Cao. All authors read and approved the final manuscript.

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Competing Interests: None of the authors have any competing interests.

Availability of data and materials: The datasets used or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

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Figures

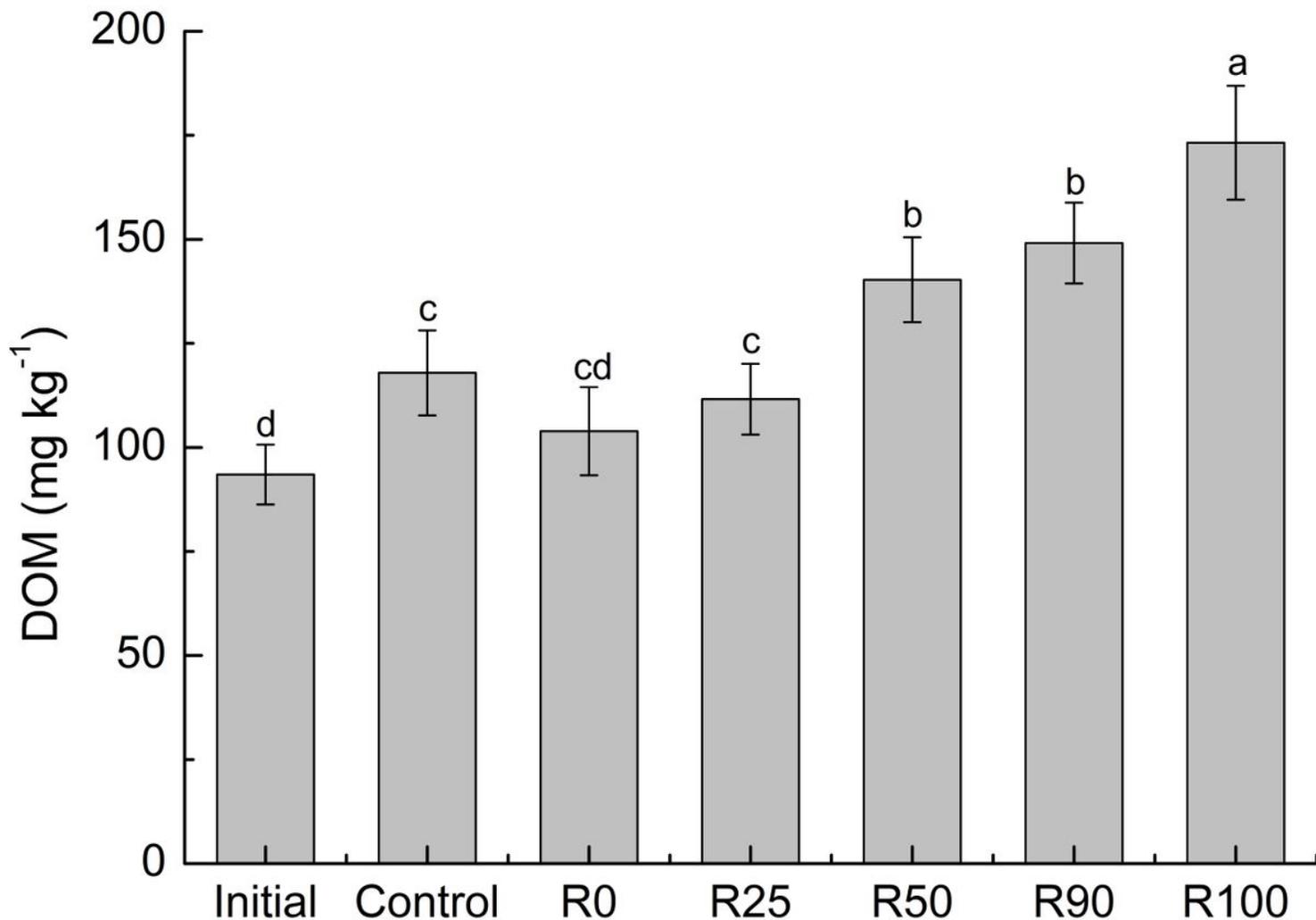


Figure 1

Concentrations of DOM in different light treatment groups Different letters mean significant differences in DOM concentrations ($p < 0.05$) judged using Fisher's LSD post-hoc tests.

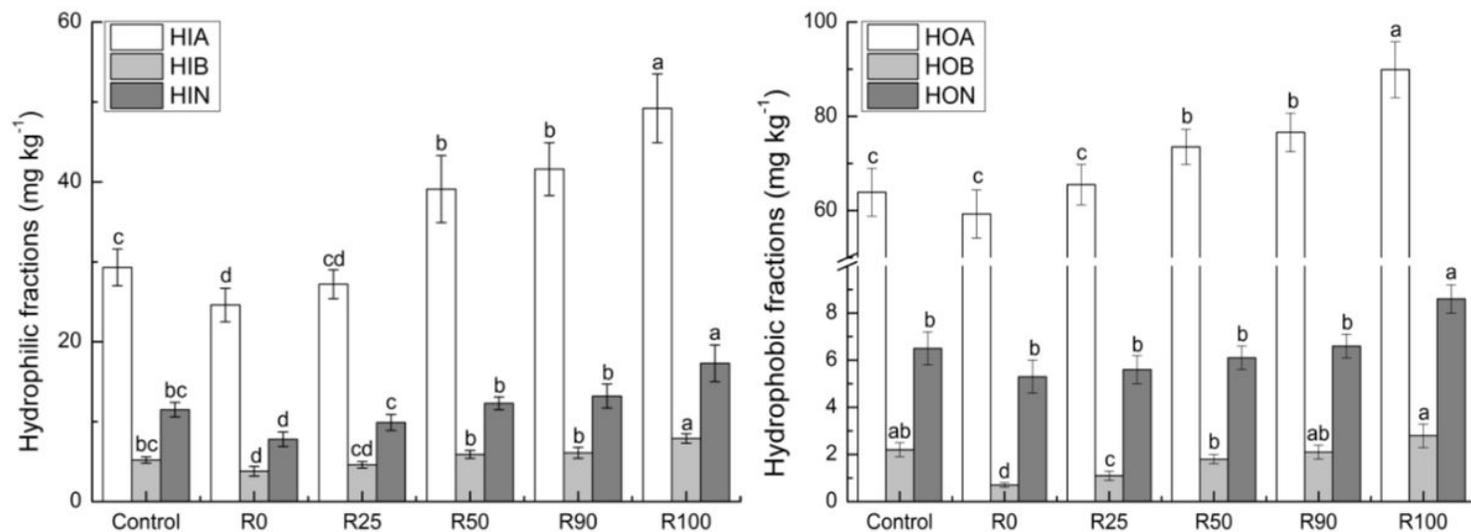


Figure 2

DOM fractions in different light treatment groups Different letters mean significant differences in DOM fractions ($p < 0.05$) judged using Fisher's LSD post-hoc tests.

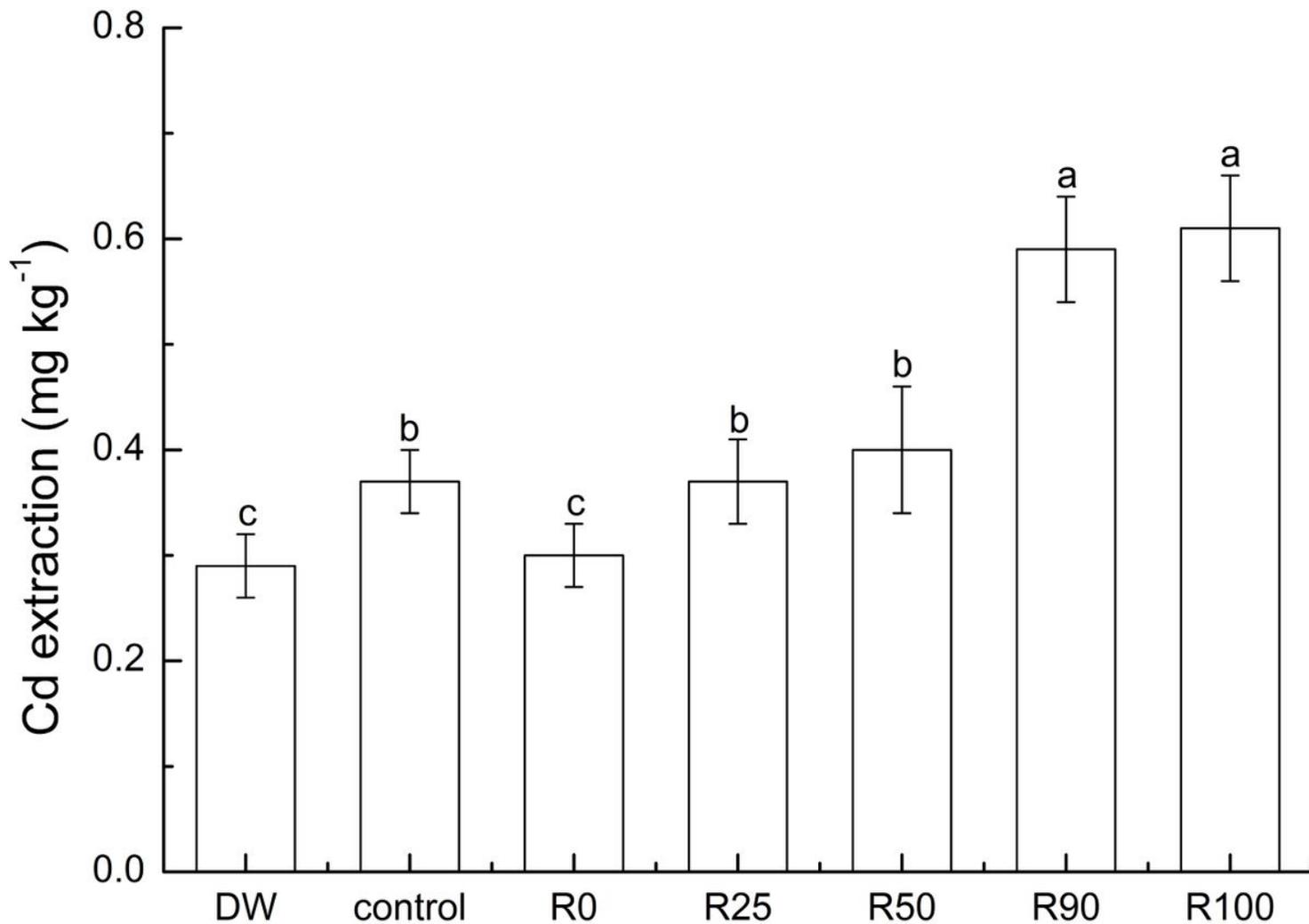


Figure 3

Cd extraction capacity of DOM in different light treatment groups Different letters mean significant differences in Cd extraction ability of DOM ($p < 0.05$) judged using Fisher's LSD post-hoc tests. DW is deionized water.

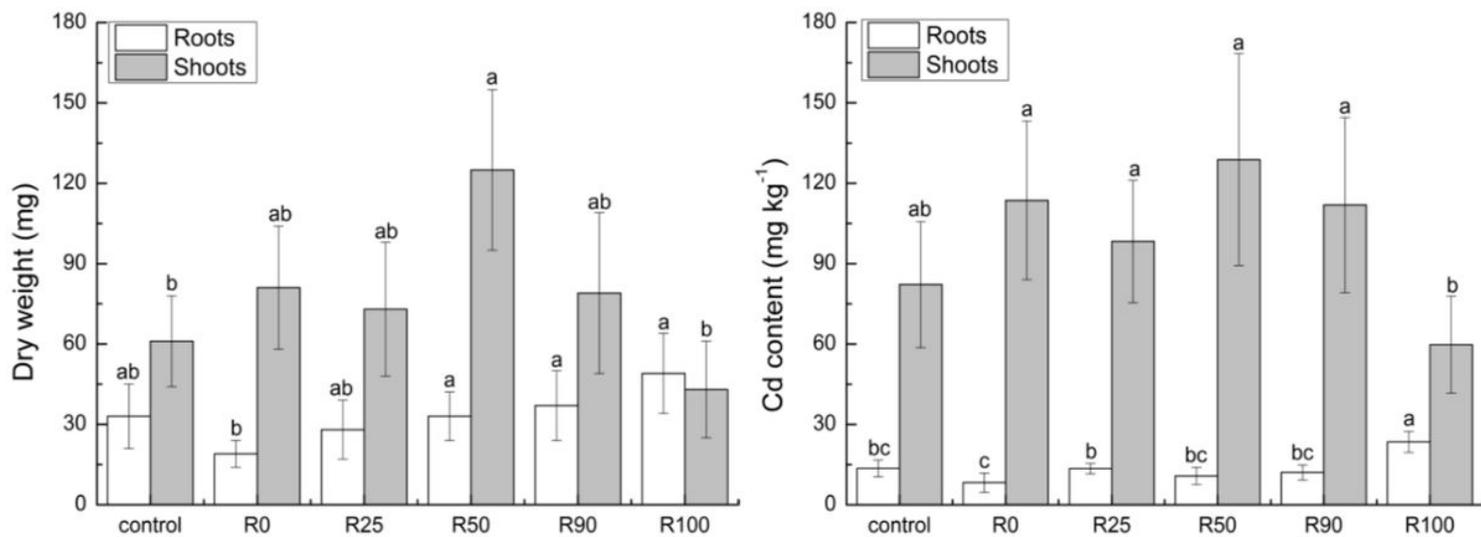


Figure 4

Dry weight (a) and Cd concentration (b) of *N. caerulescens* in different light treatment groups. Different letters mean significant differences in dry weight and Cd content of the species ($p < 0.05$) judged using Fisher's LSD post-hoc tests.