

Original Research

Effects of *Solanum* spp. Straw on Cadmium Accumulation of *Solanum diphyllum*

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Abstract

To screen the straw of *Solanum* spp. for improving the phytoremediation ability of cadmium (Cd) hyperaccumulator plants, a pot experiment was conducted in this study by adding straws from four *Solanum* species (*Solanum alatum*, *Solanum photeinocarpum*, *Solanum nigrum* var *humile*, and *Solanum nigrum*) into the Cd-contaminated soil for planting the potential Cd-hyperaccumulator *Solanum diphyllum*, and the effects of *Solanum* spp. straw on the Cd accumulation of *S. diphyllum* were studied. Compared to the control (no straw apply), only *S. alatum* straw increased the shoot biomass of *S. diphyllum*, while other straw samples decreased. The straw of *S. alatum* increased the chlorophyll *a* and total chlorophyll contents in *S. diphyllum*. In contrast, the straws of *S. alatum*, *S. photeinocarpum*, and *S. nigrum* var. *humile* increased chlorophyll *b* and carotenoid contents. Meanwhile, the straws of the four *Solanum* spp. did not affect or reduce the superoxide dismutase activity of *S. diphyllum*, while they enhanced the peroxidase and catalase activity and protein content to some extent. The straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var *humile*, and *S. nigrum* decreased the shoot Cd content in *S. diphyllum* by 20.82%, 30.90%, 17.13%, and 16.19%, respectively, compared to the control; they also decreased the Cd extraction by shoots. These observations together indicate that the straw of *Solanum* spp. reduces the phytoremediation ability of *S. diphyllum* and cannot be used for Cd-contaminated soils.

Keywords: cadmium, *Solanum diphyllum*, *Solanum* spp., straw, phytoremediation

Introduction

Heavy metal pollution caused by human activities such as mining and industrial activities is a severe soil problem worldwide. Heavy metals, especially cadmium (Cd), nickel, and copper, have mainly polluted farmland soils [1, 2]. Among the heavy metals, Cd has attracted wide attention. Due to its high mobility, the metal moves rapidly from soil to plant and causes heavy toxicity

[3]. Cadmium interferes with plant photosynthesis and various other physiological processes related to plant-water relations, ion metabolism, and mineral absorption and subsequently hinders plant growth [4]. Several studies have shown that Cd in plants affects animals and humans due to trophic transfer and bioaccumulation [5]. Therefore, remediation of Cd-contaminated farmland soil is necessary.

The methods used to remediate contaminated farmland soils typically remove heavy metals, change their form, reduce their mobility and bioavailability, and subsequently change the planting system [6, 7]. Studies have shown that straw return improves

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the soil's organic carbon content and structure, enhancing crop yield; it also changes the form of heavy metals in contaminated soils [8]. Straw also releases special allelochemicals through leaching and decomposition into soil, and regulate the plant growth [9]. The straw of Cd-hyperaccumulator *Youngia erythrocarpa* promotes the growth and Cd accumulation of the other Cd-hyperaccumulator *Galinsoga parviflora*, while the straws of Cd-hyperaccumulators *Bidens pilosa*, *Solanum photeinocarpum*, and *Siegesbeckia orientalis* have no effects [10]. The straws of the accumulator plants *Conyza canadensis* and *Cardamine hirsute* promote the Cd accumulation in *Galinsoga parviflora*, while that of *Eclipta prostrata* and *Nasturtium officinale* inhibit [11]. Among the Cd-tolerant plants such as *Ranunculus sieboldii*, *Mazus japonicus*, *Clinopodium confine*, and *Plantago asiatica*, only the straw of *M. japonicus* increases the Cd accumulation in *Galinsoga parviflora* [12]. Thus, these studies indicate that the straw of various plant species has different effects on other plants' growth and Cd accumulation [13-15], which may be related to the different allelochemicals releasing from the straw. Therefore, the best approach for increasing the Cd accumulation of plants is to use the straw of suitable plants, which needs to be screened and analyzed for their effects on Cd uptake of other plants.

Solanum spp., such as *Solanum diphyllum*, *Solanum alatum*, *Solanum photeinocarpum*, *Solanum nigrum* var. *humile*, and *Solanum nigrum* have a robust ability to accumulate Cd from soil; among these, a few are Cd-hyperaccumulators (*S. nigrum*, and *S. photeinocarpum*) [16, 17]. Meanwhile, *S. diphyllum* grows fast and can tolerate high levels of Cd stress (160 μ M), and is a potential Cd-hyperaccumulator [18]. Therefore, we applied straws from four *Solanum* spp. (*S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum*) into the Cd-contaminated soil and analyzed their effects on the Cd accumulation of *S. diphyllum* grown in the soil. This study aimed to screen the best straw of *Solanum* spp. for improving the phytoremediation ability of *S. diphyllum* in Cd-contaminated soils.

Materials and Methods

Materials

The seeds of *S. diphyllum*, *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* were provided by the Institute of Pomology and Olericulture, Sichuan Agricultural University.

In March 2021, the seeds of *S. diphyllum* were sown in the fields around the Ya'an Polytechnic College (30°1'N, 103°3'E), located in Yucheng District, Ya'an City, Sichuan Province, China. The fresh shoots of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* were collected from previously planted

in the farmland fields around the Ya'an Polytechnic College in August 2020, dried at 80°C to constant weight, and cut into 1 cm pieces to obtain the straw samples.

Inceptisol soil samples (purple soil according to the Genetic Soil Classification of China) were collected from the farmland fields around the Ya'an Polytechnic College in February 2021. The basic properties of this soil were as follows: 7.71 pH value, 13.45 g/kg organic matter concentration, 102.35 mg/kg alkaline nitrogen concentration, 68.45 mg/kg available phosphorus concentration, 48.49 mg/kg available potassium concentration, and 0.03 mg/kg total Cd concentration.

Experimental Design

The pot experiment was conducted at the Ya'an Polytechnic College from February to May 2021. In February 2021, the soil samples were treated according to Lin et al. (2020) [19], and 3.0 kg of soil was put into each plastic pot (15 cm diameter and 18 cm depth). Cadmium was added to the soil in the form of CdCl₂·2.5H₂O to a final concentration of 5 mg/kg [20, 21]. The soil was mixed and watered [19]. One month later (March 2021), 6 g of *Solanum* spp. straw was added into the soil (2 g straw per kilogram soil) and mixed thoroughly. Then, four uniform *S. diphyllum* seedlings (five expanded true leaves, 10 cm plant height) were planted in each pot. The experiment was conducted in a completely randomized design using five treatments as follows: no straw (control), *S. alatum* straw, *S. photeinocarpum* straw, *S. nigrum* var. *humile* straw, and *S. nigrum* straw; triplicates were maintained per treatment (each pot was considered a replicate). The plants were watered every day to maintain the soil moisture content of 80% of the field capacity until the plants were harvested.

In May 2021 (two months after transplanting), the mature leaves of *S. diphyllum* were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) content, antioxidant enzyme [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)] activities, and soluble protein content. The photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid were extracted using a mixture of ethanol and acetone (1:1, v/v) and determined at 663, 645, 652, and 470 nm, respectively, following the methods of Liu et al. (2021) [22] and Hao et al. (2004) [23]. The SOD activity was determined by the nitroblue tetrazolium method, POD activity by the guaiacol colorimetric method, CAT activity by UV spectrophotometry, and soluble protein content by a colorimetric method using Coomassie brilliant blue [23]. After that, the whole plants were harvested and treated as reported by Lin et al. (2020) [19]. The root and shoot biomass (dry weight) was measured on an electronic balance (the precision 0.001 g), and the root/shoot ratio (root biomass/shoot biomass) was calculated. The dried samples were used

to determine the root and shoot Cd content using the iCAP 6300 ICP-MS spectrometer (Thermo Scientific, Waltham, MA, USA) as described by Lin et al. (2020) [19]. Then, the translocation factor (TF, Cd content in shoots/Cd content in roots) was calculated [24]. The Cd extraction by plants and the translocation amount factor (TAF) were calculated using the following formulas [25]:

$$\text{Cd extraction by plants} = \text{Cd content in plants} \times \text{plant biomass}$$

$$\text{TAF} = \frac{\text{Cd extraction by shoots}}{\text{Cd extraction by roots}}$$

Finally, the pot soils were collected to determine the pH value (using a pH meter) and the exchangeable Cd concentration (using the iCAP 6300 ICP-MS spectrometer), as reported by Lin et al. (2020) [19].

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 statistical software. Data were analyzed with a one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$).

Results and Discussion

Biomass of *S. diphyllum*

Straw return is an important agricultural management measure that can be used to solve environmental pollution problems caused by stubble burning. This measure returns the nutrients in the straw to the field, improving soil nutrients and reducing soil bulk density; this also increases the effective pores and large pores to achieve a better soil environment [26-28]. Besides, straw return releases allelochemicals, which are compounds that enter the environment through volatilization, leaching, or plant decay, and directly or indirectly affect other plants [29]. Under Cd-contaminated soil conditions, mulching with the

straws of accumulator plants such as *Gnaphalium polycaulon* and *Galium aparine* promote the growth of grape seedlings, increasing its biomass, while mulching with the straws of the accumulator plants *Capsella bursa-pastoris* and *Conyza canadensis* decrease [13]. The straws of accumulator plants *Trifolium pretense*, *Eclipta prostrate*, *Stellaria media*, and *Conyza canadensis* increase the biomass of the accumulator plant *Nasturtium officinale* under Cd-contaminated soil [14]. In this study, the straws of four *Solanum* spp. had no significant ($p > 0.05$) effect on the root biomass of *S. diphyllum* compared to the control (Table 1). Meanwhile, the straws of *S. alatum*, *S. photeinocarpum*, and *S. nigrum* var. *humile* increased the shoot biomass of *S. diphyllum* by 17.95% ($p < 0.05$), 12.52% ($p < 0.05$), and 6.45% ($p < 0.05$), respectively, compared with the control, while *S. nigrum* straw had no significant ($p > 0.05$) effect. However, the straws of four *Solanum* spp. had no significant ($p > 0.05$) impact on the root/shoot ratio of *S. diphyllum*. This effect on the shoot biomass was probably via the allelochemicals released from the straw [9]. However, the straws of these species had no significant effect on the root/shoot ratio and root biomass of *S. diphyllum*. *Solanum alatum* and *S. diphyllum* are closely related species [30], and the total acid and tannin content and the effective acidity of the *S. alatum* are relatively high [31]. Therefore, the allelochemicals of *S. alatum* straw might increase the biomass of *S. diphyllum*.

Photosynthetic Pigment Content in *S. diphyllum* Leaves

Generally, under Cd stress, the Cd inhibits the root Fe (III) reductase, leading to Fe (II) deficiency of plants, which seriously affects photosynthesis and leads to leaf curl and chlorosis [32]. With the increase in Cd concentration, the chlorophyll and carotenoid contents decrease in common plants, and the influence of Cd on chlorophyll *a* is greater than that on chlorophyll *b* [33]. Under Cd-contaminated soils, the application of the straws of Cd-tolerant plants, such as *Ranunculus sieboldii*, *Digitaria sanguinalis*, *Clinopodium confine*, and *Plantago asiatica*, increase the photosynthetic pigment content in *Cyphomandra betacea* [15],

Table 1. Biomass of *S. diphyllum*.

Treatment	Root (g/plant)	Shoot (g/plant)	Root/shoot ratio
Control	0.488±0.061a	2.044±0.049d	0.238±0.024a
<i>S. alatum</i>	0.522±0.059a	2.411±0.063a	0.216±0.019a
<i>S. photeinocarpum</i>	0.517±0.015a	2.300±0.038b	0.225±0.003a
<i>S. nigrum</i> var. <i>humile</i>	0.511±0.010a	2.176±0.098c	0.235±0.006a
<i>S. nigrum</i>	0.505±0.021a	2.089±0.065d	0.242±0.003a

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$). Root/shoot ratio = root biomass/shoot biomass.

Table 2. Photosynthetic pigment content in *S. diphyllum* leaves.

Treatment	Chlorophyll <i>a</i> (mg/g)	Chlorophyll <i>b</i> (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
Control	1.690±0.072b	0.579±0.026c	2.269±0.098b	0.276±0.011c
<i>S. alatum</i>	2.024±0.075a	0.716±0.030a	2.740±0.104a	0.340±0.008a
<i>S. photeinocarpum</i>	1.862±0.066ab	0.663±0.011ab	2.525±0.076ab	0.310±0.010b
<i>S. nigrum</i> var <i>humile</i>	1.819±0.080b	0.644±0.018b	2.463±0.098b	0.302±0.007b
<i>S. nigrum</i>	1.776±0.078b	0.635±0.021bc	2.412±0.099b	0.295±0.008bc

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$).

and the application of the straws of hyperaccumulators *Youngia erythrocarpa*, *Bidens pilosa*, *Solanum photeinocarpum*, and *Siegesbeckia orientalis* increase the chlorophyll content in *Capsella bursa-pastoris* [34]. In this study, the straw of *S. alatum* increased the chlorophyll *a* and total chlorophyll contents in *S. diphyllum* leaves compared to the control, while the straws of *S. photeinocarpum*, *S. nigrum* var *humile*, and *S. nigrum* had no significant ($p > 0.05$) effect (Table 2). Meanwhile, the straws of *S. alatum*, *S. photeinocarpum*, and *S. nigrum* var. *humile* increased the chlorophyll *b* and carotenoid contents in the *S. diphyllum* leaves compared to the control, while that of *S. nigrum* had no significant ($p > 0.05$) effect. These results are not consistent with the previous studies [15, 34], probably due to the differences in the effects of allelochemicals on root Fe (III) reductase [9, 32], which need to be further studied.

Antioxidant Enzyme Activity and Soluble Protein Content of *S. diphyllum* Leaves

Plants have a highly efficient and complex enzymatic antioxidant defense system, including SOD, CAT, POD, and ascorbate peroxidase (APX), to avoid the toxic effects of free radicals [35]. Cadmium produces reactive oxygen species (ROS) in plants and causes oxidative stress [36]. The reactive oxygen molecules cause cell death due to lipid peroxidation, membrane damage,

and enzyme inactivation [37]. In cotton, the soluble protein contents in the roots and leaves decrease under Cd stress due to oxidative damage that inhibits protein content [38]. Studies have shown that the application of straw increases the antioxidant enzyme (SOD, POD, and CAT) activity and protein content of plants under Cd-contaminated soils; however, opposite effects have also been reported [10, 39]. In this experiment, compared to the control, the straws of *S. photeinocarpum* and *S. nigrum* var. *humile* reduced the SOD activity of *S. diphyllum* leaves, while that of *S. alatum* and *S. nigrum* had no significant ($p > 0.05$) effect (Table 3). Except for *S. nigrum*, other straws enhanced the POD activity of *S. diphyllum* leaves compared to the control. Besides, the straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var *humile*, and *S. nigrum* increased the CAT activity of *S. diphyllum* leaves by 303.07% ($p < 0.05$), 101.23% ($p < 0.05$), 60.12% ($p < 0.05$), and 57.67% ($p < 0.05$), respectively, and the soluble protein content by 99.82% ($p < 0.05$), 91.41% ($p < 0.05$), 59.32% ($p < 0.05$), and 38.20% ($p < 0.05$), respectively, compared to the control. These results are consistent with the previous studies [10, 39], which may be related to the different allelochemicals [9] released from the straw of *Solanum* spp., indicating that the straws of four *Solanum* spp. enhanced the resistance of *S. diphyllum* to Cd to some extent.

Table 3. Antioxidant enzyme activity and soluble protein content of *S. diphyllum* leaves.

Treatment	SOD activity (U/g)	POD activity (U/g/min)	CAT activity (mg/g/min)	Soluble protein content (mg/g)
Control	210.55±1.39ab	2474±42.92d	0.163±0.006d	21.78±1.27d
<i>S. alatum</i>	214.27±3.12a	3197±49.98a	0.657±0.009a	43.52±1.88a
<i>S. photeinocarpum</i>	202.45±0.92cd	2757±34.82b	0.328±0.003b	41.69±1.94a
<i>S. nigrum</i> var <i>humile</i>	201.41±3.33d	2638±47.10c	0.261±0.005c	34.70±1.01b
<i>S. nigrum</i>	207.90±1.56bc	2571±35.01cd	0.257±0.011c	30.10±1.22c

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$).

Table 4. Cd content in *S. diphyllum*.

Treatment	Root (mg/kg)	Shoot (mg/kg)	TF
Control	65.45±0.64a	63.75±1.77a	0.974±0.017a
<i>S. alatum</i>	61.85±1.63a	50.48±2.15b	0.816±0.013d
<i>S. photeinocarpum</i>	47.72±0.15c	44.05±0.54c	0.923±0.008b
<i>S. nigrum</i> var <i>humile</i>	53.78±0.80b	52.83±2.00b	0.982±0.004a
<i>S. nigrum</i>	63.04±2.17a	53.43±1.81b	0.848±0.000c

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$). TF = Cd content in shoots/Cd content in roots.

Cd Content in *S. diphyllum*

Straw can directly promote or inhibit the Cd uptake of hyperaccumulators or accumulator plants by releasing the allelochemicals [10-15]. However, straw also can affect the Cd uptake of plants by changing the soil environment like soil pH value and soil available Cd concentration [13-15]. In this study, the straws of *S. photeinocarpum* and *S. nigrum* var. *humile* decreased the root Cd content in *S. diphyllum* compared to the control, while the straws of *S. alatum* and *S. nigrum* had no significant ($p > 0.05$) effect (Table 4). The straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* decreased the shoot Cd content in *S. diphyllum* by 20.82% ($p < 0.05$), 30.90% ($p < 0.05$), 17.13% ($p < 0.05$), and 16.19% ($p < 0.05$), respectively, compared to the control. The straws of *S. alatum*, *S. photeinocarpum*, and *S. nigrum* reduced the TF of *S. diphyllum* compared to the control, while that of *S. nigrum* var. *humile* had no significant ($p > 0.05$) effect. These changes, consistent with the previous studies [10-12], may be related to the allelochemicals releasing from the *Solanum* spp. straw and the higher soil pH value leading to lower soil exchangeable Cd concentration, and this need be further studied.

Cd Extraction by *S. diphyllum*

Straw can increase the plant Cd extraction by increasing the biomass and Cd content of plants. Only a few straws of plant species can increase the Cd extraction of hyperaccumulators or accumulator plants [10-15]. In this study, compared to the control, the straw of *S. photeinocarpum* decreased the Cd extraction by the roots of *S. diphyllum*, while that of *S. alatum*, *S. nigrum* var. *humile* and *S. nigrum* had no significant ($p > 0.05$) effect (Table 5). The straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* decreased the Cd extraction by shoots of *S. diphyllum* by 6.65% ($p < 0.05$), 22.24% ($p < 0.05$), 11.84% ($p < 0.05$), and 14.38% ($p < 0.05$), respectively, compared to the control. However, the straws of four *Solanum* spp. had no significant ($p > 0.05$) effect on the TAF of *S. diphyllum*. These results are consistent with the previous studies on the *Galinsoga parviflora* and *Nasturtium officina* [10-12, 14], which suggest that *Solanum* spp. could decrease the Cd uptake in *S. diphyllum* and reduce the phytoremediation ability of *S. diphyllum*.

Soil pH and Exchangeable Cd Concentration

Several factors, including soil properties and rhizosphere affect the bioavailability of heavy metals in the soil-plant system [40]. Straw return is known

Table 5. Cd extraction by *S. diphyllum*.

Treatments	Root ($\mu\text{g/plant}$)	Shoot ($\mu\text{g/plant}$)	TAF
Control	31.90±3.66a	130.29±0.51a	4.113±0.488a
<i>S. alatum</i>	32.22±2.81a	121.63±2.01b	3.792±0.393a
<i>S. photeinocarpum</i>	24.68±0.66b	101.32±0.42e	4.106±0.093a
<i>S. nigrum</i> var <i>humile</i>	27.50±0.37ab	114.87±0.81c	4.178±0.085a
<i>S. nigrum</i>	31.83±0.26a	111.55±0.30d	3.505±0.038a

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$). Cd extraction by plants = Cd content in plants \times plant biomass. TAF = Cd extraction by shoots/ Cd extraction by roots.

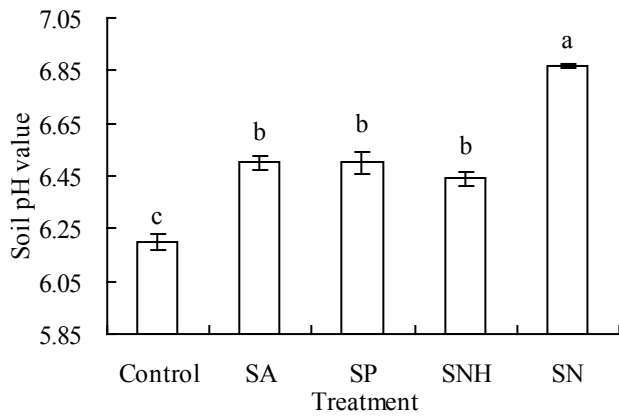


Fig. 1. Soil pH value. Different lowercase letters indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$).

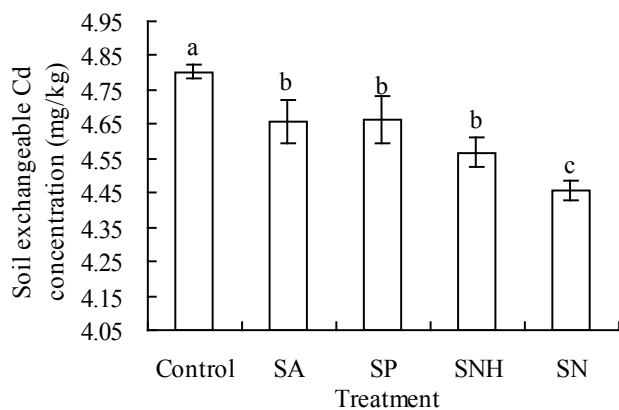


Fig. 2. Soil exchangeable Cd concentration. Different lowercase letters indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference ($p < 0.05$).

to affect the bioavailability of heavy metals in the soil in various ways, including the release of nutrients and allelochemicals into the soil through microbial decomposition. These effects change the soil pH, thereby changing the effective soil cadmium content [41]. During the process of straw decomposition, various biochemical reactions may reduce the bioavailability of heavy metals in the soil [10-12]. Studies have confirmed that straw return increases the bioavailability of heavy metals in the soil [42]. In this experiment, the pH values of the soils treated with the straws of four *Solanum* spp. were higher than the control, with the maximum under *S. nigrum* straw treatment (Fig. 1). The exchangeable Cd concentrations of the soils treated with the straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* were 2.99% ($p < 0.05$), 2.85% ($p < 0.05$), 4.86% ($p < 0.05$), and 7.19% ($p < 0.05$), respectively, lower than the control (Fig. 2). These results are consistent with the previous studies [10, 40, 41].

Conclusions

In the present study, the straw of *S. alatum* promoted *S. diphyllum* growth by increasing the shoot biomass, while the straws of *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* inhibited. The straws of *S. alatum*, *S. photeinocarpum*, *S. nigrum* var. *humile*, and *S. nigrum* decreased the Cd uptake in the shoots of *S. diphyllum* and the Cd extraction by the shoots of *S. diphyllum*. These observations conclude that the straw of *Solanum* spp. can not improve the phytoremediation ability of *S. diphyllum* and cannot be used in Cd-contaminated soils.

Conflict of Interests

The authors declare no conflicts of interest.

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