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Original Research

The Effects of Tea on Cadmium Accumulation for the Emergent Accumulator Plant *Nasturtium officinale*

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Abstract

The paddy soils have been contaminated by cadmium (Cd). To remedy the Cd-contaminated paddy soils, four tea types (black, green, white, and dark tea) were added to the Cd-contaminated paddy soils and planted the emergent accumulator plants *Nasturtium officinale*, and the effects of tea the Cd accumulation of *N. officinale* were studied. The green, white, and dark tea decreased the biomass of *N. officinale*, while the black tea had no effects. Compared with the control, the green, white, and dark teas decreased the root biomass by 12.73%, 8.18%, and 17.73%, respectively, and decreased the shoot biomass by 31.50%, 26.43%, and 33.08%, respectively. All four tea types decreased the chlorophyll *a*, chlorophyll *b*, and carotenoid contents in *N. officinale*. For antioxidant enzyme activity, the four tea types decreased *N. officinale* superoxide dismutase and peroxidase activities, while only the dark tea decreased the catalase activity. The four tea types had no effect on the shoot Cd content in *N. officinale*, and decreased the shoot Cd extraction due to decreased, or zero effects on the soil exchangeable Cd concentration. Therefore, tea cannot be used to improve the phytoextraction capacity of *N. officinale* for Cd-contaminated paddy soils.

Keywords: Cadmium, growth, *Nasturtium officinale*, phytoremediation, tea

Introduction

Soil heavy metal contamination has become a serious problem in recent years, and is also one of the main problems facing the current agricultural environment [1]. This presents great potential hazards, which are concealed, easily degraded, and difficult to remedy [2]. Cadmium (Cd) is a toxic heavy metal, which has a very large contamination area in the agricultural soils, especially in the paddy field soils [1, 2]. Excessive Cd inhibits plant growth and even leads plant death. It also threatens human health by entering the human body through the food chain [3]. Therefore, Cd-contaminated soil requires urgent remediation.

Many soil heavy metal remediation methods exist, including physical, chemical, and bioremediation [4, 5]. Phytoremediation, which usually uses hyperaccumulators, is one of the bioremediation technique, and is environmentally friendly [1]. However,

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the application of hyperaccumulators is restricted by their slow growth, small biomass, and narrow distribution range [6]. To improve hyperaccumulator phytoextraction capacity, some agronomic measures are used in hyperaccumulators, including intercropping, straw return, plant growth regulators, and more [7-9]. Straw return not only improves the soil physical and chemical properties, while increasing the soil nutrient content, but also changes the soil available Cd concentration, thereby changing plant Cd uptake [10-12]. Tea is consumed on a daily basis, and is rich in inorganic matter, water-insoluble protein, fat-soluble vitamins, hydroxy acid salts, aromatic carboxylic acid esters, hydroxyls, and oxygen, and can remove heavy metals and fluoride from water [13, 14]. The main reason why tea is chosen as the adsorption material is because polyphenols are its main components. Its chemical structure contains multiple hydroxy polyphenols, which have strong chelating properties and can form stable complexes with heavy metals, thus removing them [15-17]. A previous study showed that tea applied to soil decreased the Cd uptake in common pakchoi cabbage [18]. Furthermore, no other reports exist regarding the application of tea on plant Cd uptake. Therefore, if tea is applied to either hyperaccumulator or accumulator Cd accumulation, their phytoextraction capacity can be affected.

Nasturtium officinale is a rapidly growing emergent herbal accumulator plant [19]. Compared with other hyperaccumulators or accumulators [20, 21], the phytoextraction capacity of *N. officinale* can potentially be improved. In this experiment, four tea types (black, green, white, and dark teas) were added to the Cd-contaminated soils, and their effects on *N. officinale* Cd accumulation were studied. The aim of this study was to screen the best tea type that could improve the phytoextraction capacity of *N. officinale*, and to provide a reference for the Cd-contaminated paddy soils remediation.

Experimental

Materials

Shoot cuttings of *N. officinale* were collected from the fields surrounding the Chengdu Campus of Sichuan Agricultural University (30°71′N, 103°87′E). The four tea types were: black tea (product name: Second-level Gongfu Black Tea, produced by Leshan Yizhichun Tea Company), green tea (product name: Lvmaofeng, produced by Ya’an Southern Yejia Tea Company), white tea (Gongmei, produced by Fuding Guanzhongshan Tea Company), and dark tea (Tibetan Tea, produced by Ya’an Helong Tea Factory).

Fluvo-aquic soil was collected from the fields surrounding the Chengdu Campus of Sichuan Agricultural University. The soil physical and chemical properties were the same as described by Lin et al. (2020) [22].

Experimental Design

The experiment was conducted in a greenhouse at the Chengdu Campus of Sichuan Agricultural University from September to November 2020. In September 2020, the soil samples were treated, placed into plastic pots (25 cm diameter and 10 cm depth), and enriched with Cd (in the form of $CdCl_2 \cdot 2.5H_2O$) according to Lin et al. (2020) [22]. A total of 2.5 kg soil were weighed and placed in each pot, and the final soil Cd concentration was 5 mg kg^{-1} [23]. A total of 5 g tea was added to the Cd-contaminated soil of each pot (2 g tea per kilogram soil) [8], mixed thoroughly, and maintained in a submerged state for seven days. There were five treatments in the experiment: no application (control), and application of black, green, white, or dark tea. Each treatment was done in triplicate. In October 2020, shoot cuttings (10 cm in length) were placed into the Cd-contaminated soil. Four cuttings were planted in each pot. The plants were watered every day, and the water level was maintained at 3 cm above the soil surface until harvest.

In November 2020 (one month after transplanting), the mature *N. officinale* leaves were collected to determine the photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids) contents and antioxidant enzymes [peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT)] activities. The photosynthetic pigments contents were determined according to the acetone-ethanol extraction method [24], and the details were the same as reported by Liu et al. (2021) [25]. The nitroblue tetrazolium, guaiacol colorimetric, and potassium permanganate titration methods were used to determine the SOD, POD, and CAT activities, respectively, and the details were also the same as reported by Lin et al. (2020) [22]. Hereafter, whole plants were harvested and treated as reported by Lin et al. (2020) [22]. The biomass of root and shoot (dry weight) were measured by an electronic balance, and the root/shoot ratio (root biomass/ shoot biomass) was calculated. Dried samples were used to determine the root and shoot Cd contents using the iCAP 6300 ICP-MS spectrometer (Thermo Scientific, Waltham, MA, USA) as reported by Lin et al. (2020) [22], and the translocation factor (TF: Cd content in shoots/ Cd content in roots) was calculated [26]. Pot soils were collected to determine the soil pH (using a pH meter) and soil exchangeable Cd concentration (using the iCAP 6300 ICP-MS spectrometer) as reported by Lin et al. (2020) [22].

Statistical Analyses

Statistical analyses were performed using the SPSS 20.0 statistical software. Data were analyzed with one-way analysis of variance (ANOVA), with the Duncan’s Multiple Range Test ($P<0.05$).

Results and Discussion

N. officinale Biomass

Allelopathy is a common phenomenon in agricultural production, and can affect plant growth, development, and mineral element uptake [27]. Mulching with crop straw can change the soil physicochemical properties by releasing allelochemicals into the soil and affecting plant growth [28]. Under Cd-contaminated soil conditions, adding tea residues can increase the edible parts of Chinese cabbage [29]. In this experiment, the black tea application did not significantly (P>0.05) affect *N. officinale* biomass (Table 1). The green, white, and dark teas decreased the root biomass of *N. officinale* by 12.73%, 8.18%, and 17.73%, respectively, and decreased the shoot biomass by 31.50%, 26.43%, and 33.08%, respectively. These results indicate that tea application inhibited *N. officinale* growth to some extent. The reason for this may be that tea produces substances such as caffeine and phenols, which causes toxicity to other plants. When the phenolic acid concentration reaches a certain level, the cytoplasmic membrane is depolarized, thus destroying the potential difference on both membrane sides and increasing cell membrane permeability, thereby causing ion extravagation, which eventually inhibits *N. officinale* growth [30, 31]. All four tea treatments enhanced the root/shoot ratio of *N. officinale* in this experiment. This further indicates that *N. officinale* increased its root proportion to increase its resistance to tea inhibition.

Photosynthetic Pigment Content in *N. officinale*

When Cd enters plants, excess reactive oxygen species are generated and reactive oxygen radicals target the chlorophyll, thereby resulting in the destruction of chlorophyll and carotenoid structures, and eventual leaf chlorosis [32]. In this experiment, after applying the four teas, the chlorophyll *a*, chlorophyll *b*, and carotenoid contents in *N. officinale* decreased (Table 2). Compared with the control, the black, green, white, and dark teas decreased the chlorophyll *a* content by 12.41%, 56.21%, 23.02%, and 58.54%, respectively, decreased the chlorophyll *b* content by 12.95%, 58.68%, 21.76%, and 60.61%, respectively, and decreased the carotenoid content by 15.88%, 48.93%, 21.46%, and 56.65%, respectively. Therefore, tea inhibited *N. officinale* photosynthetic pigment synthesis. These results may be related to the caffeine and phenol toxicity as produced by teas [30, 31]. On the other hand, the addition of tea to the soil may cause an imbalance in the soil microbial community structure, which affects plant nutrient absorption and photosynthetic pigment synthesis [33].

Antioxidant Enzyme Activity of *N. officinale*

Under heavy metal stress, reactive oxygen species are produced inside the plant body, and this damages the cell structure and function of plants [34]. Previous studies showed that some plant straws increased the SOD, POD, and CAT activities of the Cd-hyperaccumulator

Table 1. Biomass of *N. officinale.*

Treatments	Roots (g plant^{-1})	Shoots (g plant^{-1})	Root/shoot ratio
Control	0.220±0.011a	1.578±0.070a	0.140±0.011b
Black	0.223±0.013a	1.377±0.091a	0.162±0.007a
Green	0.192±0.009c	1.081±0.015b	0.178±0.006a
White	0.202±0.007b	1.161±0.085b	0.175±0.009a
Dark	0.181±0.003c	1.056±0.079c	0.175±0.016a

Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, P<0.05). Root/shoot ratio = root biomass/ shoot biomass.

Table 2. Photosynthetic pigment content in *N. officinale.*

Treatments	Chlorophyll *a* (mg g^{-1})	Chlorophyll *b* (mg g^{-1})	Carotenoid (mg g^{-1})
Control	1.112±0.044a	0.363±0.017a	0.233±0.009a
Black	0.974±0.035b	0.316±0.007b	0.196±0.012b
Green	0.487±0.023d	0.150±0.013d	0.119±0.010c
White	0.856±0.020c	0.284±0.012c	0.183±0.012b
Dark	0.461±0.030d	0.143±0.004d	0.101±0.004c

Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, P<0.05).

Table 3. Antioxidant enzyme activity of *N. officinale*.

Treatments	SOD activity (U g^{-1})	CAT activity (mg g^{-1} min^{-1})	POD activity (U g^{-1} min^{-1})
Control	337.19±10.13a	5.584±0.053a	5222±118.82a
Black	306.69±13.10b	5.603±0.069a	4622±124.92b
Green	266.45±7.72c	5.670±0.041a	3779±106.50cd
White	278.78±12.50c	5.588±0.035a	3934±124.78c
Dark	242.81±12.44d	5.445±0.033b	3576±77.39d

Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$).

Galinsoga parviflora, while other plant straws decreased these activities [8, 35, 36]. In this experiment, the SOD activity of *N. officinale* treated with teas was lower than the control (Table 3). The black, green, white, and dark teas reduced the SOD activity by 9.05%, 20.98%, 17.32%, and 27.99%, respectively, compared with the control. The black, green, and white teas did not significantly ($P>0.05$) affect the CAT activity of *N. officinale*, while the dark tea reduced the CAT activity. The POD activity of *N. officinale* treated with the teas was also lower than the control. The black, green, white, and dark teas reduced the POD activity of *N. officinale* by 11.49%, 27.63%, 24.66%, and 31.52%, respectively. These results are consistent with previous studies [8, 35, 36], indicating that the tea treatments reduced *N. officinale* Cd-resistance.

Cd Content in *N. officinale*

Under heavy metal contaminated soil, it is important to adjust plant heavy metal absorption by changing the external environment, which is important for alleviating the heavy metal stress on the plants [37]. Certain studies showed that adding straws from different crops to soil reduced the heavy metal uptake of plants [37, 38]. For hyperaccumulators or accumulators, certain plant straws (such as *Youngia erythrocarpa* and *Cardamine hirsuta*) promoted the Cd uptake of the Cd-hyperaccumulator *Galinsoga parviflora* and the Cd-accumulator *Capsella bursa-pastoris*, while other plant straws inhibited these properties [8, 35, 36, 39-41]. In this experiment, the black, green, and white teas did not significantly ($P>0.05$) affect the Cd content in *N. officinale* roots, while the dark tea increased this root Cd content (Table 4). None of the four teas significantly ($P>0.05$) affected the shoot Cd content in *N. officinale*. Therefore, tea had no effect on *N. officinale* Cd uptake, which differs from other plant straws [8, 35, 36, 39-41]. These results may be related to the soil available Cd concentration. There are many hydroxyl and carboxyl functional groups in tea residues, and these functional groups can form relatively stable complexes with the heavy metal ions [42]. Tea can also effectively remove heavy metals from water, and the amount of tea is significantly positively correlated with the heavy metal ion removal rate [43]. These factors directly decrease the soil available Cd concentration, and thus decrease *N. officinale* Cd uptake. The black and green teas in this experiment reduced the TF of *N. officinale*, while the white and dark teas had no significant ($P>0.05$) effects. This indicates that tea treatment cannot promote the Cd transport from the roots to shoots of *N. officinale*.

Cd Extraction by *N. officinale*

The black tea did not significantly ($P>0.05$) affect the root Cd extraction of *N. officinale*, while the green, white, and dark teas decreased the root Cd extraction (Fig. 1). All four teas decreased the shoot Cd extraction of *N. officinale* (Fig. 2). Therefore, tea treatment decreased the phytoremediation capacity

Table 4. Cd content in *N. officinale*.

Treatments	Roots (mg kg^{-1})	Shoots (mg kg^{-1})	TF
Control	26.02±0.56b	36.49±0.74ab	1.403±0.049a
Black	26.70±0.66ab	35.49±0.49b	1.330±0.023b
Green	26.34±0.17ab	35.25±0.66b	1.338±0.032b
White	25.96±0.47b	35.31±1.10b	1.360±0.018ab
Dark	27.10±0.38a	37.35±0.66a	1.378±0.028ab

Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$). Translocation factor (TF) = Cd content in shoots/Cd content in roots.

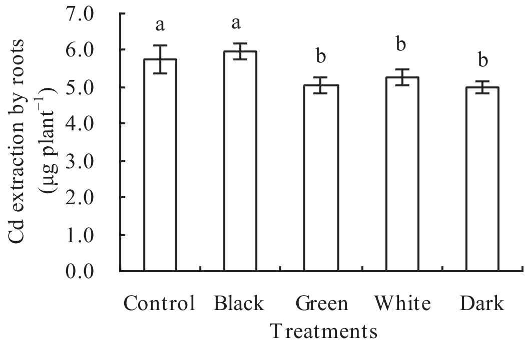

Fig. 1. Cd extraction by roots of *N. officinale*. Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$).

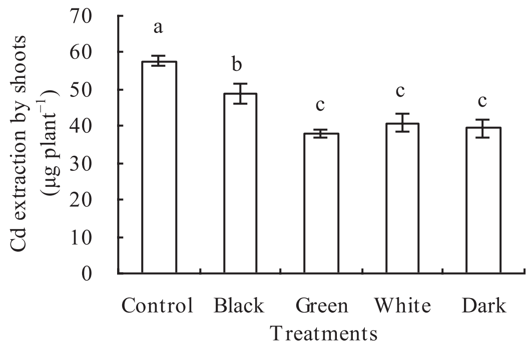

Fig. 2. Cd extraction by shoots of *N. officinale*. Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$).

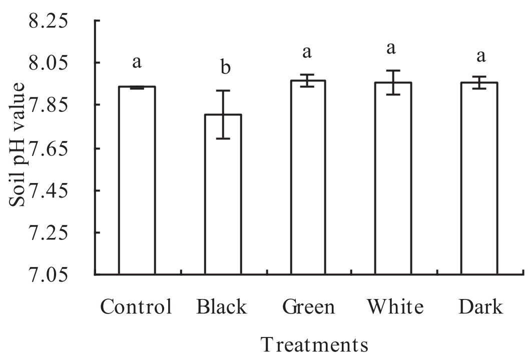

Fig. 3. Soil pH value. Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$).

of *N. officinale*, and cannot be used for Cd-contaminated paddy soils.

Soil pH value and Exchangeable Cd Concentration

Soil available Cd concentration is directly affected by soil pH value, and soil pH value is in turn affected by the organic acid levels generated from straw [44,

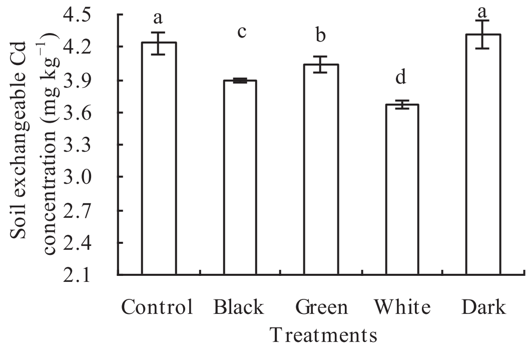

Fig. 4. Soil exchangeable Cd concentration. Values are means (±SD) of triplicates. Different letters indicate significant differences among the treatments (Duncan's Multiple Range Test, $P<0.05$).

45]. Therefore, if the soil pH value is low, then the soil available Cd concentration becomes high, and more Cd can be absorbed by plants. In this experiment, only the black tea reduced the soil pH value, while the other three teas had no significant ($P>0.05$) effects (Fig. 3). The black, green, and white teas decreased the soil exchangeable Cd concentration, while the dark tea had no significant ($P>0.05$) effects (Fig. 4). These results indicate that tea may fix or adsorb Cd in the soil [42, 43], and further suggest that tea treatment decreased the soil available Cd concentration to some extent, thereby leading to zero effects or decreased Cd uptake by *N. officinale*.

Conclusions

The four teas in this study could not promote *N. officinale* growth. However, they decreased the biomass, photosynthetic pigment content, and antioxidant enzyme activity of *N. officinale* to some extent. Generally, the four teas had no effect on Cd content in *N. officinale*, but reduced the Cd transport from the roots to shoots, and decreased the Cd extraction ability of *N. officinale*. Therefore, tea is not suitable for improving the phytoextraction capacity of *N. officinale*.

Conflict of Interest

The authors declare no conflict of interest.

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