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Positive Effects and Optimal Ranges of Tea Saponins on Phytoremediation of Cadmium-Contaminated Soil

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Abstract: Confirming positive effects and offering optimal ranges of tea saponins on improving the efficiency of phytoremediation on cadmium is a prerequisite for applying tea saponins in field remediation. Existing studies qualitatively tested the feasibility of tea saponins on promoting the absorption of cadmium by hyperaccumulators in pots experiments, while this study investigated the effects of tea saponins on increasing the proportion of cadmium available fraction in contaminated soil quantitatively and confirmed tea saponins promoted the absorption by *Portulaca oleracea* in cadmium-contaminated water by independent soil experiments and hydroponic experiments. The results showed that for acquiring a higher proportion of cadmium available fraction, the concentration of tea saponins was negatively correlated with the concentration of cadmium contained in the soil, and the optimal treatment time of tea saponins was between 3–9 days depending on the cadmium concentration in contaminated soil. Using tea saponins could enhance the absorption of cadmium by *Portulaca oleracea* in a relatively short time to decrease the concentration of cadmium left in the contaminated water. The above findings help to deepen the understanding of tea saponins' effects and use ranges on phytoremediation of cadmium both in soil and water and conduce studies on phytoremediation of other heavy-metal-contaminated soil and water with the help of tea saponins.

Keywords: tea saponins; phytoremediation; cadmium; available fraction; *Portulaca oleracea*

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1. Introduction

Cadmium contamination in soil constitutes a severe situation and is a global problem. For example, cadmium ranked first among inorganic contaminants in China, where inorganic contaminants accounted for 82.8% in the total soil over-standard rate [1]. Presented in the atmosphere, rocks, sediments, and soils, cadmium is a non-essential metal with toxic effects on plants and animals [2]. Human activities, such as mining [3], have greatly broadened the distribution of cadmium in nature and increased its level. Classified as a human carcinogen, cadmium exerts toxic effects on the kidney and the skeletal and respiratory systems [4]. Cadmium is not present in the human body at birth, but it accumulates with age, mainly in the kidneys and liver [5]. Cadmium-contaminated food is the main source of cadmium exposure in the general population; smokers and workers in cadmium industries have additional exposure [6]. Therefore, it is of great importance to repair cadmium-contaminated soil.

Phytoremediation is an effective and environmentally friendly method for soil remediation, with the advantages of low cost, low energy consumption, and no secondary pollution [2,7,8]. However, phytoremediation has a disadvantage, that is, inorganic contaminants usually exist in soils in their low mobility forms, resulting in their low bioavailability. This disadvantage limits the wide application of phytoremediation.

Using surfactants, like sodium dodecyl sulfate (SDS) [9] or chelating agents [10,11], such as natural low molecular weight organic acids (NLMWOAs), ethylene diamine tetraacetic acid (EDTA), ethylene diamine disuccinate (EDD), and humic substances (HS), has been confirmed to effectively solve the problem. The secondary pollution is caused by the chemical and microbial stable complexes formed with EDTA and heavy metals in soils [12], and the persistence of EDTA-metal complexes in soils [13] plus the typical phytotoxic impacts on plants caused by EDTA [14] require strong attention from researchers, making natural surfactants a more suitable choice for increasing the bioavailability of inorganic contaminants in phytoremediation.

The application of tea saponins (TS), a kind of natural non-ionic surfactants, which are contained in the root, stem, leaf, flower, and seed of *Camellia* plants [15], has been proven to raise the bioavailability of hyperaccumulators on cadmium, pyrene, and polychlorinated biphenyls (PCBs) in contaminated soil [14,16–19]. Despite this, these studies also have their limitations. In general, the role of surfactants has two aspects on the phytoremediation of cadmium-contaminated soils: first, to promote the transformation of the chemical form of cadmium from the difficult-to-use form (bound to Fe-Mn oxides and bound to carbonates) to the available form (exchangeable) [17–19]; second, on the premise of not changing the form of cadmium in the soil, surfactants only promote the absorption of cadmium by accumulation plants or hyperaccumulators. The second role of surfactants on phytoremediation is a lack of studies, because, in existing studies, accumulation plants or hyperaccumulators were usually grown in pots, and once the surfactant had the first role in changing the form of cadmium, the effect of the second role would be covered. It is difficult to tell whether the increased absorption of cadmium from the soil by accumulation plants or hyperaccumulators is due to: (i) the increased proportion of cadmium available form in the soil; (ii) the promoted absorption by the surfactant without changing the form of cadmium; or (iii) both.

Consequently, this study tried to solve the above-mentioned problem. Firstly, to discover the role of TS in increasing the proportion of the cadmium available fraction in cadmium-contaminated soils by soil experiments and to investigate the quantitative effect and optimal values of TS. Secondly, with the assistance of the accumulation plant *Portulaca oleracea* L. [20,21], to discover the role of TS on accumulation plants without the transformation of the chemical form of cadmium by hydroponic experiments.

2. Materials and Methods

2.1. Soil Experiments

2.1.1. Soil Preparations

The soil for the experiments was the commercial substrate (Greenterra Ltd., Rīga, Latvia) with a pH (1:5 H_2O) of 3.5–4.5, an electrical conductivity (1:5 H_2O) of 0.1–0.3 mS/cm, and a NPK (chlorine-free) of 0. The substrates were air-dried, passed through a 2-mm stainless steel sieve, and stored dry at room temperature (25 degrees Celsius) for subsequent preparation.

Cadmium chloride solution with cadmium concentration of 100 mg/L was used as the mother liquor. According to the experimental design (seen in Table 1), a certain amount of mother liquor was added to the above-prepared substrates and mixed well. Soil samples after adding the mother liquor were air-dried, passed through a 2-mm stainless steel sieve, and stored dry for 30 days at room temperature (25 degrees Celsius) to carry out the aged procedure of soil preparations.

Table 1. Three-level Box-Behnken designs for exploring the effects of cadmium concentration in the soil, tea saponins concentration, and tea saponins treatment time on the cadmium available fraction proportion.

Run	Cadmium Concentration in the Soil (mg/kg)	Tea Saponins Concentration (%)	Tea Saponins Treatment Time (Day)
1	50	0.025	3
2	50	0.005	3
3	25	0.025	9

Table 1. *Cont.*

Run	Cadmium Concentration in the Soil (mg/kg)	Tea Saponins Concentration (%)	Tea Saponins Treatment Time (Day)
4	50	0.025	15
5	50	0.015	9
6	75	0.015	3
7	75	0.005	9
8	75	0.015	15
9	50	0.015	9
10	75	0.025	9
11	50	0.005	15
12	25	0.015	3
13	25	0.015	15
14	25	0.005	9
15	50	0.015	9

The pre-experiment investigated the sorption ability of the soil on cadmium (aged 30 days), which was extracted by the optimized Tessier sequential extraction procedure [22] and determined by atomic absorption spectrometry (AAS). The results (Table 2) indicated that the soil selected for the experiment had good sorption of cadmium, since the total cadmium content in the test soil was 80.13 $\pm$ 9.09% of the theoretical value, and the proportion of the available fraction was 13.10 $\pm$ 0.85%.

Table 2. Concentrations of each cadmium fraction in the pre-experiments *.

Fraction	Cadmium Concentration (mg/L)
Available	0.131 $\pm$ 0.009
Bound to carbonates	0.310 $\pm$ 0.042
Bound to Fe-Mn oxides	0.210 $\pm$ 0.021
Bound to organic matter	0.147 $\pm$ 0.018
Residual	0.0065 $\pm$ 0.0007

* The mass and the concentration of cadmium in the test soil were 0.2 g and 50 mg/kg, respectively.

The calculated proportion of 80.13 $\pm$ 9.09% was seemingly not high enough, indicating two potential reasons: first, the cadmium sorption in soil was not strong enough, and second, the optimized Tessier sequential extraction procedure could not extract the whole content of cadmium contained in contaminated soil. Whereas, under the same experimental condition, the following results revealed the role of TS on phytoremediation of cadmium-contaminated soil.

2.1.2. Quantitative Effects of TS

Box-Behnken design (BBD) [23] is a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. Because BBD permits: (i) the estimation of the parameters of the quadratic model, (ii) the building of sequential designs, (iii) the detection of lack of fit of the model, and (iv) the use of blocks [24], it is a good design for response surface methodology and is slightly more efficient than the central composite design but much more efficient than the three-level full factorial designs. Considering the possible significant interactions between variables and the efficiency of the experimental design [24], a three-level Box-Behnken design (Table 1) was chosen for exploring the effect of cadmium concentrations in the soil (mg/L), TS concentrations (%), and TS treatment time (day) on the proportion of cadmium available fraction in the test soil.

The ranges of the above three factors were designed considering the current status of soil cadmium contamination and existing studies, whose results showed that a TS concentration around 0.02% [15] might be the upper limit without significant influences on plants. The TS treatment time was set based on the current research, whose value was 10 days [17]. The TS used in the study was of biochemical reagent grade with the purity between 10% and 25%; the actual concentration of them was determined and calculated through the standard curve built by the well-established vanillin-sulfuric acid method [25].

2.2. Plant Cultivation and Experiment

Portulaca oleracea, an annual herbaceous plant widely distributed in temperate and tropical regions of the world, is drought tolerant and waterlog drought tolerant, and demonstrates strong tolerance and a great ability of accumulation on cadmium [20,21], even cadmium concentrations in soil exceeding 50 mg/kg [26]. Thus, *Portulaca oleracea* was selected to see whether TS promoted the absorption of cadmium by accumulator plants or not and also to see whether *Portulaca oleracea* has the potential to be applied in the phytoremediation of cadmium-contaminated water.

After the wild *Portulaca oleracea* plants were harvested from the ground, the following treatments were carried out: picking several plants with consistent growth; removing soil; cutting off rotten roots, old leaves, and yellow leaves; rinsing the roots three times with deionized water; soaking the roots for 15 min with 0.1% carbendazim solution; and rinsing the roots three times with deionized water. Thereafter, *Portulaca oleracea* plants were cultured with deionized water for 1 day; then, the water was changed, and they were cultured for another 2 days, at which time the plants grew white water roots. The experiment was started after 2 days of culture in a (1/5) Hoagland aqueous solution. All hydroponic cultures were placed in a constant temperature incubator at 25 degrees Celsius with a light/dark of 16/8 h.

Supplementing the hydroponic solution with the mother liquor of cadmium chloride solution to ensure the concentration of cadmium at 50 mg/L, the nutrient solution was applied for cultivating *Portulaca oleracea* plants with or without 0.015% TS solutions. Each *Portulaca oleracea* plant was incubated in a 50 mL centrifuge tube at 25 degrees Celsius in a light/dark regime of 16/8 h for three days. Observations of the plants' growing situation and determinations of cadmium left in the hydroponic solution for three replicates were recorded in each treatment.

After the three-day cultivation, the leaves and roots of *Portulaca oleracea* plants in each treatment were harvested, rinsed with deionized water three times, dried in an oven at 70 °C until constant weight, and analyzed to determine their dry biomass.

2.3. Cadmium Concentration Determination

For soil, the concentration of the cadmium available fraction in each extraction was determined by AAS through the standard addition technique [22,27]; then, their proportions were calculated. The parameters of AAS for the determination of cadmium are listed in Table 3.

Table 3. Parameters of atomic absorption spectroscopy for the determination of cadmium.

Parameters		Values
Lamp current (mA)		4.0
Wavelength (nm)		228.8
Slit width (nm)		0.5
Range of the standard curve (mg/L)		0–2.0
Volume flow rate (L/min)	Air	13.5
	Acetylene	2.0

The concentrations of cadmium left in each solution after the three-day cultivation of *Portulaca oleracea* plants were determined by AAS in the unit of mg/L to see whether TS promoted the absorption of cadmium by *Portulaca oleracea* plants or not.

The experimental flow chart is shown in Figure 1.

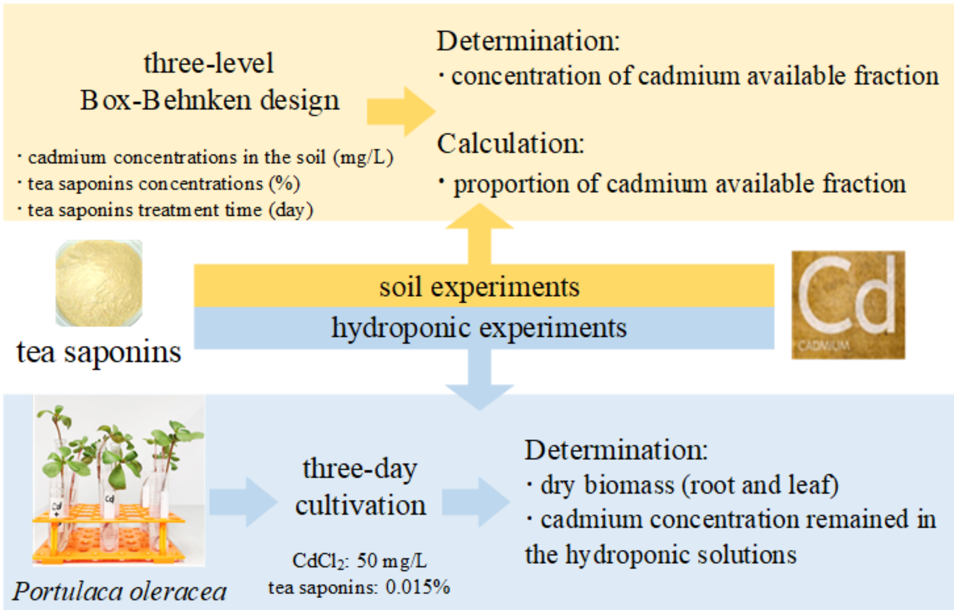

Figure 1. Experimental flow chart.

2.4. Statistical Analysis

Soil experiments were performed three times with three replicates of *Portulaca oleracea* plants for each treatment in plant hydroponic experiments. The precision of the data was calculated and expressed as a standard error (SE). The MATLAB statistical software (Version R2019a, MathWorks, Natick, MA, USA) was used, and the one-way ANOVA [28,29] test was conducted to assess statistically significant differences with 0.05 as the significant level.

3. Results and Discussion

3.1. Effects of Cadmium Concentration in the Soil, TS Concentration, and TS Treatment Time

Figure 2 presents the quantitative effects of three influential factors on the proportion of the cadmium available fraction in the test soil, and, among all factors, the interaction between cadmium concentration in the soil and TS concentration was significant ($p < 0.05$), suggesting that the concentration of TS was significantly correlated with the concentration of cadmium in the soil and that the correlation was negative. In detail, when the concentration of cadmium in the soil was low, the concentration of TS needed to be higher, while the concentration of TS could be moderately reduced when the concentration of cadmium in the soil was higher, to acquire a higher proportion of the cadmium available fraction.

For the TS treatment time, its effect was a parabolic type with an open downward, which indicated that too long or too short of a TS treatment time reduced the proportion of the cadmium available fraction, attributed to the fact that TS may not have had enough time to work and that TS would be decomposed when the treatment time was too long. In comparison, the optimal treatment time of TS was between 3–9 days, depending on the concentration of cadmium in the soil.

When the cadmium concentration in the soil was 50 mg/kg, the increasing rate of the proportion of cadmium available fraction in the soil reached 121.37% by adding TS (available cadmium concentration of 0.290 mg/L acquired from Run 2 in the Box-Behnken designs, compared with the available cadmium concentration of 0.131 mg/L in Table 2), confirming that TS increased the available fraction of cadmium. The above result was consistent with the current research, in which TS took an active part in enhancing the exchangeable fraction of cadmium [17–19], and the addition of TS at 40 mg/L showed a great advantage of solubilization capability than other surfactants in enhancing the accessible fraction of cadmium [18]. Nevertheless, the concentration of cadmium in the soil was fixed in the above-mentioned studies, and the interaction between cadmium concentration in the soil and TS concentration was not studied. However, the results of this study showed that there was a significant negative interaction between the concentration of cadmium in the soil and the concentration of TS. At the same time, the treatment time of TS in the above-mentioned studies was also fixed, without the quantitative effects and optimal ranges of TS treatment time discussed and given. In the above-mentioned studies, the quantitative effect of TS concentration was not investigated thoroughly; the effects of TS on the available cadmium concentration were studied at a fixed concentration of 40 mg/L [17]; three values of 20, 40, and 80 mg/L [18]; and four values of 1, 3, 5, and 10 mmol/L [19], respectively.

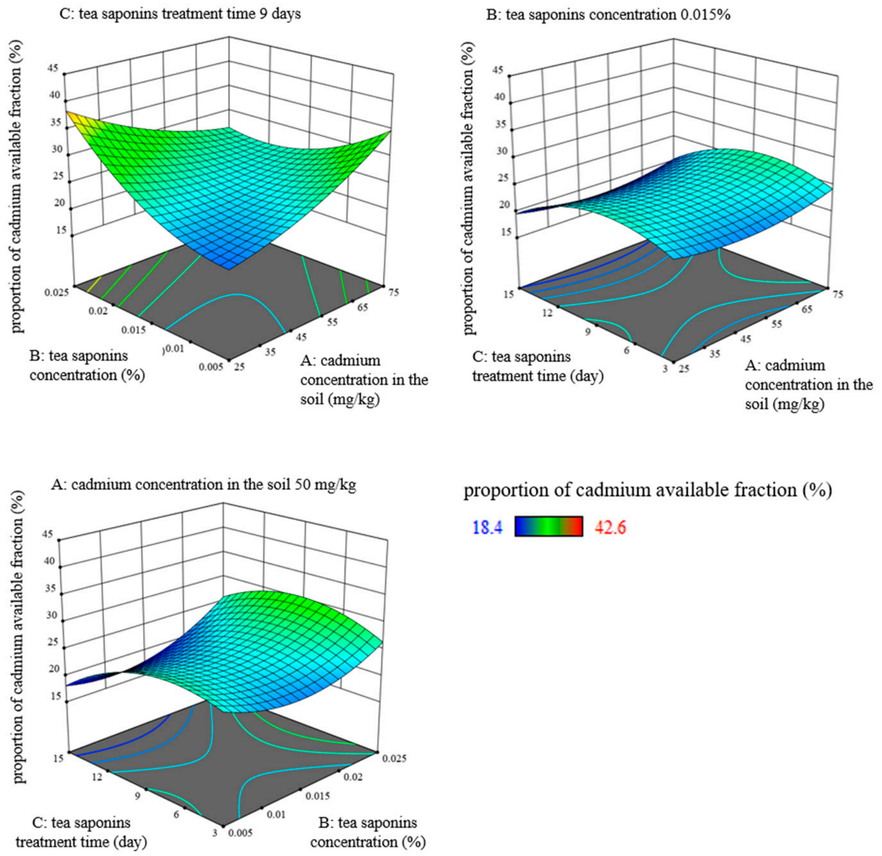

Figure 2. Effects of the cadmium concentration in the soil, tea saponins concentration, and tea saponins treatment time on the cadmium available fraction proportion in cadmium-contaminated soils by Box-Behnken designs.

3.2. Effects of TS on Accumulator Plants

TS also promoted the absorption of cadmium by *Portulaca oleracea* in hydroponic experiments, as did the decreasing rates of cadmium concentrations—presented in Figure 3.

After the three-day cultivation, cadmium concentrations left in the hydroponic solutions of each treatment showed significant differences; significant differences also existed between the decreasing rate of cadmium concentrations in each treatment. In the cadmium treatment group without the addition of TS, the residual cadmium concentration decreased from the 50.0 mg/L before the hydroponic experiment to 5.10 $\pm$ 0.05 mg/L, and the decreasing rate of cadmium reached 89.80 $\pm$ 0.10%, showing that *Portulaca oleracea* plant has good cadmium absorption capacity and the potential to be applied to phytoremediation of cadmium-contaminated water. On this basis, the addition of 0.015% TS further significantly increased the absorption of cadmium by *Portulaca oleracea*. After three days of hydroponic cultivation, in the cadmium + TS treatment group, the residual cadmium concentration in the hydroponic solution dropped to 4.32 $\pm$ 0.33 mg/L, and the decreasing rate of cadmium reached 91.73 $\pm$ 0.66%.

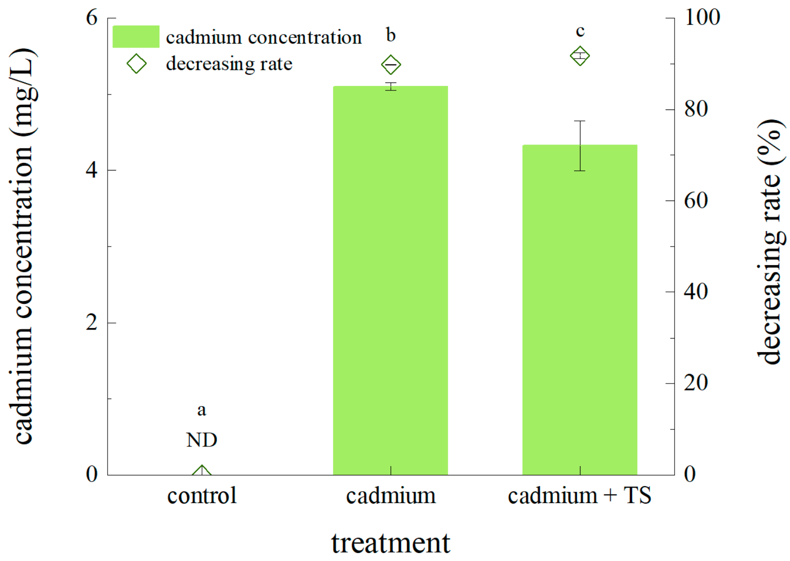

Figure 3. Concentrations of cadmium remained in the hydroponic solutions and decreasing rates of cadmium concentrations after the three-day cultivation of *Portulaca oleracea* with or without 0.015% tea saponins solutions in the presence or absence of 50 mg/L $CdCl_2$. Different letters indicate significant differences ($p < 0.05$).

Portulaca oleracea grew well after the three-day hydroponic experiments. Figure 4 gives the dry biomass of leaves and roots of *Portulaca oleracea* plants in each treatment, and the measurements showed that the addition of cadmium significantly reduced the dry leaf biomass of *Portulaca oleracea* plants, while TS not only alleviated the inhibitory effect of cadmium on the dry leaf biomass of *Portulaca oleracea* plants but also promoted the growth of leaves to some extent. As for roots, the addition of cadmium plus the addition of cadmium and TS both enhanced the dry biomass of root significantly, which were consistent with current studies, though the hyperaccumulators used were *Lolium multiflourm* [14,17] and *Sedum plumbizincicola* [19]. Growing situation observations and dry biomass measurements revealed that a TS concentration of 0.015% and a treatment time of 3 days were relatively safe for the accumulator plant *Portulaca oleracea*.

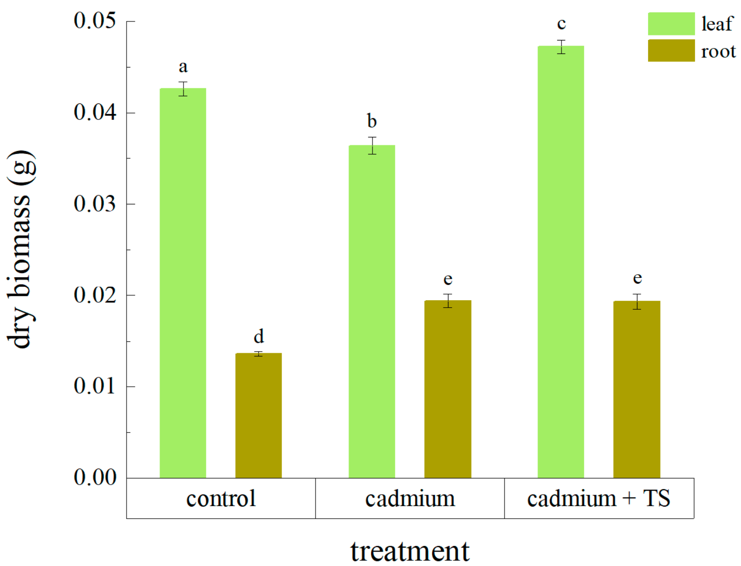

Figure 4. Dry biomass of leaves and roots of *Portulaca oleracea* plants in control, cadmium (50 mg/L $CdCl_2$), and cadmium + tea saponins (50 mg/L $CdCl_2$ + 0.015% tea saponins solutions) treatment. Different letters indicate significant differences ($p < 0.05$).

The effective promotion of TS on the accumulator plant *Portulaca oleracea* indicated that TS could be used for the phytoremediation of cadmium-contaminated water, promoting accumulator plants or hyperaccumulators to absorb more cadmium ions within a relatively short time, therefore reducing the concentration of cadmium ions in the sewage.

4. Conclusions

This study found that, in phytoremediation of cadmium-contaminated soil, TS not only increased the proportion of cadmium available fraction but also promoted the absorption of cadmium by *Portulaca oleracea*; therefore, TS improved the bioavailability of accumulator plants or hyperaccumulators on cadmium through the above two aspects. Meanwhile, the feasibility of using TS in the phytoremediation of cadmium-contaminated water was confirmed. Quantitative effects and optimal ranges of TS concentrations and treatment time for increasing the proportion of cadmium available fraction were investigated and proposed for the first time: TS concentration was negatively correlated with the cadmium concentration in contaminated soil, and the effective TS treatment time was between 3–9 days when the cadmium concentration in the soil ranged between 25–75 mg/kg. By adding 0.005% TS to cadmium-contaminated soil with the cadmium concentration of 50 mg/kg for 3 days, the increasing rate of the proportion of cadmium available fraction in the soil reached 121.37%.

The next step in the study of TS on phytoremediation would be concerned with the use of TS and the accumulator plant *Portulaca oleracea* for practical remediation of cadmium-contaminated soil and water. Further studies also need to focus on exploring how TS promotes the morphological transformation of heavy metals in the contaminated soil and how to promote the absorption of cadmium from soil or water by accumulator plants or hyperaccumulators.

Besides phytoremediation, biomass-derived biochar [30] and biomass-derived carbon nanotubes [31] are utilized for removing heavy metals present in water as a greener environmental remediation. This suggests that TS-rich tea waste, such as tea seed husks, could also be used as a biomass-derived biochar source for the removal of cadmium ions from cadmium-contaminated water, which has acquired attention and study.

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Abbreviations

AAS	Atomic absorption spectrometry
ANOVA	Analysis of variance
BBD	Box-Behnken design
EDD	Ethylene diamine disuccinate
EDTA	Ethylene diamine tetraacetic acid
HS	Humic substances
NLMWOAs	Natural low molecular weight organic acids
PCBs	Polychlorinated biphenyls
SDS	Sodium dodecyl sulfate
SE	Standard error
TS	Tea saponins

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