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RESEARCH ARTICLE

Ornamental hyperaccumulator *Mirabilis jalapa* L. phytoremediating combine contaminated soil enhanced by some chelators and surfactants

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Abstract

Mirabilis jalapa L. is an ornamental plant of the composite family, which was found hyperaccumulating Cd. Due to its larger biomass, developed root system, root exudation, and microbial interactions, certain organic pollutants in its rhizosphere can be effectively degraded. Thus, *M. jalapa*can be used to co-remediate heavy metal and organic pollutant co-contaminated soil. The aim of this paper is to explore the remediation capacity of *M. jalapa* for Cd-PAHs co-contaminated soil in the presence of five chelators or surfactants. The concentrations of Cd and PAHs in collected soil samples were 0.85 mg kg^{-1} Cd and 1.138 mg kg^{-1} PAHs (16 kinds of priority control polycyclic aromatic hydrocarbons by USEPA). The chelators or surfactants of EDTA, EGTA, CA, TW80, and SA were respectively spiked to the pots according to the experiment design at 1 month before the plant harvested. The results showed that the capacity of Cd in shoot of *M. jalapa* was 7.99 μg pot^{-1} without any addition (CK4, *M. jalapa* in original soil without amendment). However, Cd capacity in shoot of *M. jalapa* was increased ($p < 0.05$) by 31.7%, 181.7%, and 107.4% in treatment of R_{EGTA}, R_{CA} and $R_{\mathrm{EGTA+SA}}$, respectively. As for the degradation of PAHs in soil, there was no significant decrease ($p < 0.05$) in the treatment of CK2 (original soil spiked with 0.9 SA without *M. jalapa*), CK3 (original soil spiked with 0.3 TW80 without *M. jalapa*), and CK4 compared to the control CK1 (original soil without *M. jalapa* and amendment). When amendments were added to soils with *M. jalapa*,the PAHs concentrations in soils significantly decreased ($p < 0.05$) by 21.7%, 23.8%, 27.0%, 19.8%, 21.8%, 31.2%, and 25.5% for the treatment of $\mathrm{R_{EDTA+SA}}$, $\mathrm{R_{EDTA+T80}}$, $\mathrm{R_{EGTA+SA}}$, $\mathrm{R_{EGTA+T80}}$, $\mathrm{R_{CA+T80}}$, $\mathrm{R_{SA+T80+EDTA}}$, and $\mathrm{R_{SA+T80+CA}}$, respectively. Basically, Cd capacity in shoot of *M. jalapa* was improved by chelators. PAHs degradation was caused by the existence of surfactants in rhizosphere of *M. jalapa.* But the roles of different chelators or surfactants were quite distinct. In short, the Cd capacity in the shoot and PAHs degradation in the rhizosphere of *M. jalapa* in the treatment of $\mathrm{R_{EGTA+SA}}$ were all significantly increased ($p < 0.05$), which was more practical for *M. jalapa* phytoremediating Cd-PAHs co-contaminated soil.

Keywords *Mirabilis jalapa* L. · Phytoremediation · Cd and PAHs co-contaminated soil

Introduction

Cd-PAHs (polycyclic aromatic hydrocarbons) co-polluted soil is not quite often (Chen et al. 2015). However, some pathways such as wastewater irrigation, sludge applications, solid waste disposal, automobiles exhaust, and industrial activities are really causing this case (Yang et al. 2011; Wei et al. 2016). Phytoremediation mainly means that using hyperaccumulator or highly accumulator to extremely extract and remove heavy metal from contaminated soil, or degrade organic pollutants like PAHs in their rhizospheres (Li et al. 2015, 2017; Pan et al. 2017). There are abound resources of ornamental plant in the world. Some special ornamental plant, especially hyperaccumulators, was identified from them can not only

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remediate contaminated soil but also beautify the environment. Thus, using ornamental hyperaccumulator to remediate polluted soil has important practical significance.

Mirabilis jalapa L. was a newly found Cd ornamental hyperaccumulator. The results showed that Cd concentration in shoot of *M. jalapa* was higher than 100 mg kg^{-1}, EF (enrichment factor, i.e., concentration rate of shoot to soil) higher than 1, TF (translocation factor, i.e., concentration rate of shoot to root) higher than 1, and biomass was not significantly decreased when Cd concentration was 100 mg kg^{-1} in soil (Wu 2006; Liu et al. 2007; Wang and Liu 2014). Particularly, the biomass of *M. jalapa* with developed root system is often bigger than other documented hyperaccumulators. Furthermore, *M. jalapa* showed strong tolerance and degradation capacity in its rhizosphere for petroleum-contaminated soil with higher concentration. Plot experiments showed that the degradation rate of total petroleum hydrocarbons (TPHs) was only 19.75–37.92% under natural conditions, but increased to 41.61–63.20% in the presence of *M. jalapa*. *M. jalapa* showed strong tolerance to as high as 10,000 mg kg^{-1} TPHs-contaminated soil and the rhizosphere microbial community showed enhanced adaptability (Peng et al. 2009).

Usually, the application of chelating agent in soil is an important measure to promote the enrichment of heavy metals by hyperaccumulators (Li et al. 2014). When the chelating agent was added to soil, the heavy metals in soil and chelation might form a water soluble metal chelate complex, which changed the forms of heavy metal in soil, increased the bioavailability of heavy metals, and finally, strengthened the absorption of heavy metal by hyperaccumulator. Some experiment results indicated better strengthen role of EDTA (ethylenediamine tetraacetic acid), EGTA (ethylenebis (oxyethylenetrinitrilo)-*N*,*N*,*N*′,*N*′-tetraacetic acid), and SA (salicylic acid) on hyperaccumulator accumulating Cd (Zaier et al. 2010; Yang et al. 2011; Wang and Liu 2014). PAHs are often strongly adsorbed by the soil, and its bio-degradation is restricted by its low bioavailability in the soil. Surfactants can increase solubilization and desorption of PAHs in soil, thereby improving the solubility of PAHs in soil, increasing their exposure to plants and soil microorganisms, and finally, improving the rhizosphere degradation rate (Gao et al. 2007). The study showed that adding a proper amount of TW80 (Tween 80) could increase the bioavailability of PAHs in soil, expand the PAHs degradation bacteria group and accelerate the removal of PAHs in the soil (Lai et al. 2009; Yang et al. 2011; Wei et al. 2016). SA (salicylic acid) could effectively promote the degradation of B (a) P by microorganism in soil (Rentz et al. 2005; Yang et al. 2011).

Pollutants in soil do not often exist in a single form (Liu et al. 2015). Heavy metals and organic pollutants often exist together and formed a combined pollution system. The coexistence of heavy metals may inhibit microbial degradation of organic matter, and further affect the phytoremediation of organic pollutants (Weyens et al. 2009). Furthermore, the content of heavy metals or PAHs in contaminated soil is high in some published studies (Wang and Liu 2014). The effects of these additives on phytoremediation of heavy metals or PAHs with lower concentration in soil are not yet known. Therefore, pot experiments was conducted to exlplore the roles of these chelators or surfactants on hyperaccumulator *M. jalapa* phytoremediating Cd-PAHs co-contaminated soil.

Materials and methods

Basic information of collected Cd-PAHs co-contaminated soil

The collected soil sample was meadow burozem from the top layer (0–20 cm) of a field site and the basic physic-chemical properties of soil sample were basically same with the published paper (Wei et al. 2016).

The concentration of Cd in the soil samples was 0.85 mg kg^{-1}. Compared to the Soil-Environmental Quality Standards of China classification (GB-15618-1995), the Cd pollution level was light-middle (Wei et al. 2016). The total concentration of 16 polycyclic aromatic hydrocarbons (PAHs) from the US EPA priority pollutant list was 1.138 mg kg^{-1}. The composition was mainly composed of high-molecular-weight PAHs (HMW, 4–6 rings). HMW accounted for 94.68% of total PAHs and low-molecular-weight PAHs (LMW, 2–3 rings) only for 5.32%. Compared with the IUNG grading standard for soil and crop cultivation institutions in Poland (0.245 mg kg^{-1}), this soil sample belonged to heavy PAHs-contaminated soil (Maliszewska-Kordybach et al. 2008).

The phytoremediation experiment

The soil pot experiment was conducted in a screenhouse of the Institute of Applied Ecology of CAS, Shenyang, China (Wei et al. 2016). The plastic pot was with 20-cm diameter and 18-cm height, which can contain 2.5 kg of dry soil.

Seeds of *M. jalapa* were collected from a local fieldsite at Shenyang. At the height of 5 cm, two seedlings of *M. jalapa*were transplanted to each pot. The concentrations of EDTA, EGTA, CA, SA, and TW80 used in this experiment were according to some papers, which was respectively spiked to the pots according to the detailed experiment design listed in Table 1 before 1 month of *M. jalapa* harvested at its maturity (Rentz et al. 2005; Gao et al. 2007; Lai et al. 2009; Zaier et al. 2010; Yang et al. 2011; Wang and Liu 2014; Wei et al. 2016).

Each treatment was repeated for three times. All plants in pots growed in natural light and temperature conditions. Tap water was used to replenish the water loss and was maintained

Table 1 Treatment levels of amendments with or without *M. jalapa*

No.	Treatment	Detail information of treatment (mmol kg^{-1})
CK	Control 1	Original soil without *M. jalapa* and amendment
R1	Control 2	Original soil added 0.9 SA without *M. jalapa*
R2	Control 3	Original soil added 0.3 TW80 without *M. jalapa*
R3	Control 4	*M. jalapa* in original soil without amendment
R4	R_{EDTA}	*M. jalapa* in original soil added 0.1 EDTA
R5	R_{EGTA}	*M. jalapa* in original soil added 0.8 EGTA
R6	R_{CA}	*M. jalapa* in original soil added 1 citric acid (CA)
R7	TW80	*M. jalapa* in original soil added 0.3 TW80
R8	R_{SA}	*M. jalapa* in original soil added salicylic acid (SA)
R9	$R_{EDTA + SA}$	*M. jalapa* in original soil added 0.1 EDTA and 0.9 SA
R10	$R_{EDTA + TW80}$	*M. jalapa* in original soil added 0.1 EDTA and 0.3 TW80
R11	$R_{EGTA + SA}$	*M. jalapa* in original soil added 0.8 EGTA and 0.9 SA
R12	$R_{EGTA + TW80}$	*M. jalapa* in original soil added 0.8 EGTA and 0.3 TW80
R13	$R_{CA + SA}$	*M. jalapa* in original soil added 1 CA and 0.9 SA
R14	$R_{CA + TW80}$	*M. jalapa* in original soil added 1 CA and 0.3 TW80
R15	$R_{SA + TW80 + EDTA}$	*M. jalapa* in original soil added 0.9 SA, 0.3TW80, and 0.1 EDTA
R16	$R_{SA + TW80 + CA}$	*M. jalapa* in original soil added 0.9 SA, 0.3 TW80, and 1 CA

at 80% soil water-holding capacity of soil. Plant and rhizosphere soil samples were collected after *M. jalapa* mature (75 days). The rhizosphere soil was collected by shaking method (Wei et al. 2016).

Sample determination and statistical analysis

Atomic absorption spectrophotometry (AAS, WFX-120A with a 1.3-nm spectral band-width) was used to determine Cd concentration in plant and soil sample. The certified standard reference material (NIST SRM 1547, peach leaves) was used as the QA/QC (Wei et al. 2016). A pH meter and electrode (PHS-3B) was used to determine pH. The normal method was used to determine basic soil properties (Wei et al. 2016).

DuPont De Nemours & Co., USA, supplied the chelators or surfactants of EDTA, EGTA, CA, SA, and TW80. The Chem Service Inc. (West Chester, USA) supplied a standard of the 16 reference PAHs (PAH-Mixture 610/525/550). The analytical grade solvents included n-hexane, dichloromethane, and cyclohexane. HPLC grade acetonitrile was used to determine HPLC concentration. Main instrument included HPLC (Waters 1525, USA), Multi λ Fluorescence Detector and Dual λ Absorbance Detector, and an Agilent ZORBAX Eclipse PAH column (4.6 × 250 mm, 5 μm). The detail extraction and determination method concerning on PAHs concentration refered on the article (Wei et al. 2016).

Microsoft Excel was used for data processing and calculations of standard deviation. Fisher's least significant difference (LSD) was used to compare the significance among different treatments and the significant level was at $p < 0.05$(Yang et al. 2011; Wei et al. 2016).

Results

Effects of EDTA, EGTA, CA, SA, and TW80 on the biomass of *M. jalapa*

Usually, the biomass of a plant is an important indicator of its adaptability to environmental conditions (Yang et al. 2011). As shown in Fig. 1, roots and shoots biomasses of *M. jalapa* in treatments of R4, R8, R9, R10, R11, R12, R13, R14, R15, and R16 were significantly decreased ($p < 0.05$) to some degrees compared to the control R3 (CK4) without the addition of any chelators or surfactants. These results indicated that some chelators or surfactants affected the growth of *M. jalapa* (Yang et al. 2011). In particular, treatment R13, showed the largest inhibitant roles for its growth and the biomass in shoot was only equal to 30% of the control (R3).

Effects of EDTA, EGTA, CA, SA, and TW80 on *M. jalapa* accumulating Cd

Cd concentration (mg kg^{-1}) and capacity (μg kg^{-1}) in shoots or roots, and increased ratio in shoot capacity (%) of *M. jalapa* under different treatments were shown in Table 2. Cd capacity in shoot refers to the product of shoot biomass and Cd concentration in shoot of each pot. Compared to the control R3 (CK4), the percentage was

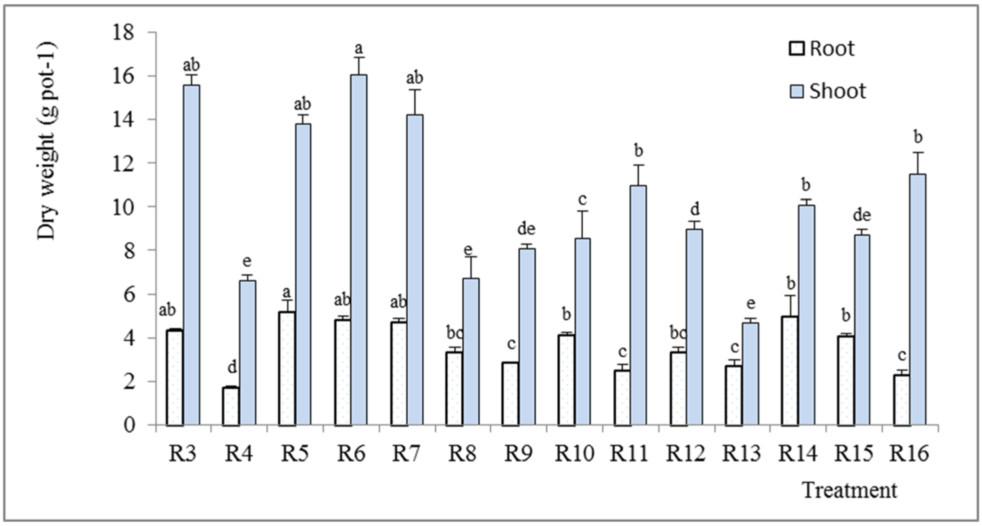

Fig. 1 Root and shoot biomass of *M. jalapa* under different treatments (in the same part of plant, data marked by the different letters are significantly different ($p < 0.05$))

used to show the effects of different additives on *M. jalapa* Cd enrichment. Basically, Cd capacity in shoot represented the potential of hyperaccumulator remediating its contaminated soil due to heavy metal was mainly translocated to shoot (Wei et al. 2016).

As shown in Table 2, Cd concentrations in roots of *M. jalapa* in treatments of R5, R9, R11, and R12 significantly increased ($p < 0.05$) compared to the control R3 (CK4). Particularly, Cd concentration in root of R9 was increased by 3.38 times of the control. As for the Cd concentration in shoot, treatments of R6, R9, and R11 were significantly increased ($p < 0.05$). However, there were significant differences among shoot Cd capacities. Cd capacities in shoots of treatments of R5 (T_{EGTA}), R6 (T_{CA}), and R11 ($T_{EGTA + SA}$) were significantly increased ($p < 0.05$) by 31.7%, 181.7%, and 107.4%, respectively. Obviously, this change was mainly caused by the low biomass in treatments of R9 and R12 (Fig. 1).

Effects of EDTA, EGTA, CA, SA, and TW80 on the degradation of PAHs in rhizosphere of *M. jalapa*

As shown in Table 3, ∑PAHs was not significantly decreaed ($p < 0.05$) when 0.9 mmol kg^{-1} SA (R1) or 0.3 mmol kg^{-1} TW80 (R2) were added to soil without *M. jalapa.* Though LMW PAHs significantly decreased ($p < 0.05$), the degradation of ∑PAHs was seldom affected because its ratio (5.32%) in ∑PAHs was quite low. Thus, natural degradation of PAHs may be omitted. Likewise, in treatment R3 (CK4), ∑PAHs in

Table 2 Cd concentration and capacity in root and shoot of *M. jalapa*

Treatment	Concentration (mg kg^{1})		Capacity (μg pot^{-1})		Increased ratio in shoot capacity (%)
	Root	Shoot	Root	Shoot	
R3	0.32 ± 0.03d	0.51 ± 0.01e	1.39 ± 0.09bc	7.99 ± 0.01d	—
R4	0.36 ± 0.02d	0.55 ± 0.05de	0.62 ± 0.02c	3.64 ± 0.22e	—
R5	0.86 ± 0.04ab	0.77 ± 0.06c	4.41 ± 0.26a	10.52 ± 0.52c	31.7
R6	0.35 ± 0.08d	1.40 ± 0.07a	1.68 ± 0.34bc	22.51 ± 2.24a	181.7
R9	1.08 ± 0.09a	1.04 ± 0.06b	3.05 ± 0.30ab	8.38 ± 0.30cd	—
R10	0.36 ± 0.02d	0.58 ± 0.06de	1.50 ± 0.06bc	4.84 ± 0.22e	—
R11	0.72 ± 0.09b	1.52 ± 0.09a	1.81 ± 0.18bc	16.57 ± 1.37b	107.4
R12	0.89 ± 0.08ab	0.76 ± 0.09c	2.99 ± 0.51ab	6.71 ± 0.34de	—
R13	0.43 ± 0.01c	0.74 ± 0.08c	1.14 ± 0.13bc	3.43 ± 0.04 e	—
R14	0.49 ± 0.09c	0.60 ± 0.01de	2.60 ± 0.37b	6.08 ± 0.25de	—
R15	0.35 ± 0.06d	0.60 ± 0.05de	1.41 ± 0.18bc	5.19 ± 0.03e	—
R16	0.49 ± 0.06c	0.62 ± 0.06d	1.13 ± 0.25bc	7.04 ± 0.58de	—

Note: in the same column, data marked by the different letters are significantly different ($p < 0.05$). "—" means that there was no significant increase ($p < 0.05$) compared to T3

Table 3 Degradation of PAHs in rhizosphere of *M. jalapa* under different treatments

Treatment	PAHs concentration (mg kg^{-1})			Degradation ratio		
	LMW	HMW	∑PAHs	LMW (%)	HMW (%)	∑PAHs (%)
CK	0.061 ± 0.014	1.045 ± 0.157	1.106 ± 0.167			
R1	0.031 ± 0.012	1.017 ± 0.064	1.048 ± 0.075	48.2	—	—
R2	0.027 ± 0.003	1.087 ± 0.073	1.115 ± 0.076	54.9	—	—
R3	0.047 ± 0.003	1.041 ± 0.075	1.087 ± 0.078	22.6	—	—
R7	0.036 ± 0.017	0.96 ± 0.018	0.996 ± 0.001	40.3	—	—
R8	0.041 ± 0.002	1.077 ± 0.010	1.118 ± 0.012	31.8	—	—
R9	0.043 ± 0.003	0.824 ± 0.062	0.866 ± 0.065	29.6	21.2	21.7
R10	0.025 ± 0.003	0.79 ± 0.076	0.843 ± 0.079	58.6	24.4	23.8
R11	0.028 ± 0.004	0.779 ± 0.059	0.807 ± 0.063	53.4	25.5	27.0
R12	0.033 ± 0.004	0.853 ± 0.057	0.887 ± 0.053	45.3	18.3	19.8
R13	0.04 ± 0.013	0.944 ± 0.107	1.091 ± 0.120	34.0	—	—
R14	0.027 ± 0.010	0.837 ± 0.054	0.865 ± 0.064	54.7	19.9	21.8
R15	0.034 ± 0.007	0.727 ± 0.005	0.761 ± 0.013	43.8	30.5	31.2
R16	0.026 ± 0.010	0.798 ± 0.016	0.824 ± 0.116	57.8	23.7	25.5

Note: degradation ratio showed as percentage means significant increase ($p < 0.05$) compared to the control (CK). “—” means that there was no significant increase ($p < 0.05$) compared to the control (CK)

rhizosphere of *M. jalapa* grown in original soil without amendment were not significantly decreased ($p < 0.05$) either. In fact, the contribution of *M. jalapa* extracted capacity (μg pot^{-1}) to the removal of ∑PAHs in soil was only 0.35%. Thus, the extraction capacity of *M. jalapa* for ∑PAHs was omitted from Table 3.

As shown in Table 3, the degradation ratios of LMW PAHs were all higher than that of HMW PAHs, indicating the former was easier to be degraded. However, the HMW PAHs were the main component of this soil sample. There were similar trends of the effects of different treatments on the degradation of HMW and ∑PAHs, i.e., their degradation ratios were significantly increased ($p < 0.05$) by 21.2%, 24.4%, 25.5%, 18.3%, 19.9%, 30.5%, 23.7%, and 21.7%, 23.8%, 27.0%, 19.8%, 21.8%, 31.2%, and 25.5% in the treatments of R9 ($R_{EDTA + SA}$), R10 ($R_{EDTA + T80}$), R11($R_{EGTA + SA}$), R12 ($R_{EGTA + T80}$), R14 ($R_{CA + T80}$), R15 ($R_{SA + T80 + EDTA}$), and R16 ($R_{SA + T80 + CA}$), respectively.

Discussion and conclusion

Wang and Liu (2014) studied the effects of EDTA and EGTA on *M. jalapa* hyperaccumulating Cd. When Cd concentration added to soil was 25 mg kg^{-1}, the biomass of *M. jalapa* did not significantly decrease ($p < 0.05$) compared to the control (clean soil), indicating its strong tolerance to Cd. However, the biomass of shoot treated with spiked EDTA significantly decreased ($p < 0.05$). In this experiment, Cd concentration was only 0.85 mg kg^{-1}. But the biomasses of *M. jalapa* in treatments of R4, R8, R9, R10, R11, R12, R13, R14, R15, and R16 significantly decreased ($p < 0.05$), indicating the effects of added chelators and surfactants. Thus, the addition of chelators and surfactants with negligible effects on plants is very important. By contrast, EGTA was better than that of EDTA. The treatment of R11 ($T_{EGTA + SA}$) is acceptable from the point of view of increasing the plant extraction rate.

Gao et al. (2005) studied the effects of ryegrass on phenanthrene- and pyrene-contaminated soil. The results showed that the contribution of plant absorption and accumulation on the removal of phenanthrene and pyrene in soil was less than 0.54%.This indicates that the direct absorption and accumulation of plants is not the main mechanism of PAHs removal in soil. The existence of plants changes the microbial community structure in rhizosphere soil, and increases the number and activity of microorganism, which promotes the removal of PAHs in soil. When organic pollutants enter the soil, they tend to be strongly adsorbed by the soil, and their degradation will be normally restricted by low bioavailability. Surfactant can increase solubilization and desorption of hydrophobic organic pollutants in soil, thereby improving the solubility of hydrophobic organic pollutants in soil and increasing their exposure to plants and soil microbes. For example, TW80 can promote the degradation of phenanthrene and pyrene in rhizosphere of the plant (Gao et al. 2007). SA is an intermediate product of PAHs degradation like naphthalene and phenanthrene. The results showed that the mineralization rate of *Pseudomonas saccharophila* P15 to B (a) P was 20% under the induction of SA (Pinyakong et al. 2003). Rentz et al. (2005) studied the co-metabolism mechanism of *Sphingomonas yanoikuyae* JAR02 on B (a) P by using SA as the inducer. The results showed that less toxic water-soluble SA could serve as a potential substrate for PAHs, especially for the common metabolite HMW PAHs. Because SA is the intermediate product of naphthalene degradation, SA addition to the source should be appropriate. If the SA content is too high, it will block the degradation of naphthalene, which will inhibit the degradation of naphthalene by microorganisms (Ogunseitan and Olson 1993). Obviously, the role of SA in the degradation of PAHs may be better than that of TW80 based on their mechanism. Thus, the treatment of R11 ($R_{EGTA + SA}$) is acceptable for the strengthening plant on the degradation of PAHs.

The results of this experiment showed that the Cd capacities in shoots of treatments of R_{EGTA}, R_{CA}, and $R_{EGTA + SA}$ were significantly increased ($p < 0.05$) by 31.7%, 181.7%, and 107.4%, respectively. The degradation ratios of ∑PAHs in the treatments of $R_{EDTA + SA}$, $R_{EDTA + T80}$, $R_{EGTA + SA}$, $R_{EGTA + T80}$, $R_{CA + T80}$, $R_{SA + T80 + EDTA}$, and $R_{SA + T80 + CA}$ were significantly increased ($p < 0.05$) by 21.7%, 23.8%, 27.0%, 19.8%, 21.8%, 31.2%, and 25.5%, respectively. In general, the treatment $R_{EGTA + SA}$ did not only significantly promote the accumulation of Cd in *M. jalapa* but significantly improved the degradation of PAHs in its rhizosphere due to increased concentration of available Cd and PAHs, which is of practical significance for the phytoremediation of Cd-PAHs co-contaminated soil.

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