Journal of Environmental Chemical Engineering 9 (2021) 106526

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Biological response and phytoremediation of perennial ryegrass to halogenated flame retardants and Cd in contaminated soils

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ARTICLE INFO

Editor: Yunho Lee

Keywords:
Phytoextraction
Bioaccumulation
Dechlorane Plus
Tetrabromobisphenol A
Cd
Ryegrass

ABSTRACT

Two halogenated flame-retardants and cadmium in soil is harmful to human health, in order to study the biological response and bioaccumulation ability of ryegrass to pollution stress, the phytotoxicity to seedlings and the phytoremediation effect in contaminated soil were investigated. It showed that proline and malonaldehyde (MDA) contents and antioxidant enzyme activity of ryegrass seedlings were increased, cadmium was the most toxic to ryegrass seedlings. The bioconcentration factor (BCF) of roots is higher than shoot. Ryegrass could assimilate trace amount of Dechlorane Plus (DP) and Tetrabromobisphenol A (TBBPA), and its dissipation in soil mainly due to the root exudates and rhizosphere microorganisms. $T_{1/2}$ of syn-DP, anti-DP and TBBPA was 42.01, 39.38 and 74.53 d in the single pollution, $T_{1/2}$ prolonged in combined pollution. Cadmium in the roots and stems is 27.82 mg/kg and 10.25 mg/kg in the treatment of single pollution, biological accumulating coefficient (BAC) and biological transfer coefficient (BTC) were 2.16 and 0.37. Ryegrass could effectively reduce total cadmium in soil; the optimal bioremediation time was 60 days. The phytoremediation ability and phytoextraction efficiency of ryegrass in contaminated soil were clarified, which would be useful for understanding the fate of halogenated flame-retardants and Cd in phytoremediation for contaminated soil.

1. Introduction

Soil pollution causes land degradation and has a great impact on soil productivity and stability. The improper disassembly of electronic and electrical waste leads to serious pollution of heavy metal and organic pollutants in some areas of China, phytoremediation is of great significance to heavy metal and organic pollutants contaminated farmland soil [6]. Ryegrass is perennial herb, the biomass of aboveground is large and it can be mowed many times, and large numbers of microorganisms gather at their roots, and ryegrass can accumulate heavy metals and organic contaminants, and it has a great application prospect in the remediation of polluted soil [15,20,22,23,37,51].

DP is a large amount of chlorinated flame retardant applied in parallel to or as replacement product for regulated flame retardants [53] with two isomers of *syn*-DP (cis) and *anti*-DP (trans) (about 1:3) [2]. Many studies pointed out that DP is prevalent in global environmental media [12,43,44,47,50,57]. Being a contaminant labeled in list of persistent organic pollutant (POPs) [60], DP have the characteristics of persistent organic pollutants such as long-range transport [32], bioaccumulation properties [16,18,50,55] and refractory, and harmful to human health [34,58].

TBBPA is a widely used brominated flame retardant in electrical equipment and household furniture, its use amount was from 5.21 × 10^6 up to 6.43 × 10^6 t/a in 2014–2017 in China [10]. Its residue has been found in various environmental media [1,26,31]. TBBPA has potentially persistent, bioaccumulative and toxic (PBT) [49,56], and it is a potential environmental endocrine disruptor similar to POPs [11]. Some studies pointed out that TBBPA may be toxic more than other polar brominated flame-retardants (HBCD and BDEs) [19]. The research on environmental behaviors and related mechanisms such as degradation, transformation, migration and its residues is becoming popular. Contamination of TBBPA in soil has become a major environmental problem.

The main sources of TBBPA and DP in the environment are flame retardant manufacturers and electronic waste dismantling sites. Guiyu of Guangdong and Taizhou of Zhejiang in China have become the most famous electronic waste recycling areas in the world. The total number of electronic waste in China has reached 1.11 million tons per year, accounting for 5% of the total hazardous waste in China [7]. The contamination of TBBPA is very severe in air dust in southeastern China [52]. The pollution of TBBPA is the most serious in the soil of electronic

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https://doi.org/10.1016/j.jece.2021.106526
Received 6 June 2021; Received in revised form 12 September 2021; Accepted 6 October 2021
Available online 9 October 2021

waste disposal site [29,39,45]; it causes the soil quality to deteriorate, so that it threatened human health through the food chain. The level of TBBPA could reach up to 24.60 mg/kg in soil in Weifang city, Shandong Province [25]. The residual level of soil in Jiangsu Province was 1.64–7000 ng/g; Residues of TBBPA and BPA were detected in soil near waste electronic waste recycling plants in Spain and Viet Nam, and the maximum concentration is 2900 ng/g [9]. TBBPA could be easily adsorbed on particles and then deposited; TBBPA and its metabolites could be detected in the sludge of Lake Erie, the sediment of Catalan river and the sludge of sewage plant in Spanish [30].

The pollution of Cd is very severe in soil [59], while cadmium endangers human kidneys, bones and lungs, and is listed as the Category I carcinogens, it is hazardous for animals and humans even at very low concentrations [20,36]. According to the report of the National Survey of Soil Pollution (2014), the exceed rate of cadmium pollution in the soil is the highest in China, and the Cd content of cultivated land is the highest in Shandong, Gansu, Henan, Jilin and other main grain producing areas [59]. Cadmium has bioaccumulated and bioconcentration toxicity, which is harmful to animal and human health through the food chain [5]. In view of the severity of cadmium pollution in the soil in China, hyperaccumulator such as perennial ryegrass is selected. Its phytoextraction and bioaccumulation characteristics for cadmium has been studied to explore the potentiality of bioremediation for cadmium-contaminated soil. It provides theoretical basis for phytoremediation of heavy metal contaminated soil.

Phytoremediation technology has been proved to be a feasible method for remediation of contaminated sites with organic pollutants [46] and it is developed direction of bioremediation [14]. The aims of this study were as the following. Firstly, the phytotoxicity of two halogenated flame retardants and cadmium in contaminated soil to ryegrass seedlings was investigated by outdoor pot experiment, and then the bioremediation mechanism of ryegrass for organic pollutants and heavy metals in the contaminated soil was studied. By detecting the residual amount of flame-retardants and Cd in the shoot and root of ryegrass, it was investigated that the biological response and phytoextraction efficiency of ryegrass to halogenated flame-retardants and cadmium, and it was explored that the bioremediation mechanism of ryegrass for organic pollutants and heavy metal. In this study, the bioremediation ability and phytoextraction efficiency of ryegrass for two halogenated flame retardants and cadmium in contaminated soil were clarified, which would be useful for understanding the fate of halogenated flame retardants and Cd in phytoremediation for contaminated soil. It provides scientific and technological support for the bioremediation of organic pollutants and heavy metal.

2. Materials and methods

2.1. Experimental instruments and chemicals

Triple Class 4 Rod Mass Spectrum: TSQ 8000 GC-MS/MS (Thermo Fisher Scientific); UltiMate 3000 HPLC-TSQ Quantum Access Max Triple quaternary lever liquid binder HPLC-MS/MS (Thermo Fisher Scientific); iCAP™ TQs ICP-MS (Thermo Fisher Scientific).

DP (Accu. Standard Inc., 100%), 50 μg/mL syn-DP and anti-DP standard sample (Accu Standard Inc, toluene solvent), N-hexane and dichloromethane (chromatographic purity), Tetrabromobisphenol A (Dr, German, 99.00%), Methanol and acetonitrile (chromatographic purity), 50 μg/mL Tetrabromobisphenol A standard sample (Methanol, Dr, German), $CdCl_2 \cdot H_20$ (99.99%, Sigma-Aldrich).

10,000 μg/mL TBBPA: 2.5253 g TBBPA standard material with purity 99.00% was accurately weighed into a small beaker by 1/10,000 scales, dissolved with acetonitrile, and to volume with 250 mL volumetric flask. 400 μg/mL DP: 0.4000 g DP standard material with purity 100.00% was accurately weighed into a small beaker by 1/10,000 scales, dissolved with acetone, and to volume with 1000 mL volumetric flask.

Soil was collected from South campus farmland of Shandong Agricultural University. The soil contained 17.6 mg/kg organic matter, 57.7% silt, 31.9% sand and 10.4% clay, additional parameters of soil such as organic nitrogenous, readily available phosphorus, pH and CEC was 132.3 mg /kg, 18.4 mg/kg, 7.60 and 43.39 cmol/kg.

2.2. Experimental methods

2.2.1. Experimental design

By pot experiment, 55 flowerpots divided into 11 groups (11 treatments), each treatment set five parallels, 7 kg soil put into every flowerpot according to the following settings, and two modes of planting ryegrass and not planting ryegrass were set up, 11 treatments were shown in the Table 1.

Different treatments of soil with pollutants was as follows: after simple pretreatment, according to the conventional fertilization method, adding 2% (weight ratio) worm cast, and then the soil was divided into 5 pots, and ryegrass was planted, add the corresponding pollutants to each treatment.

2.2.2. Growing ryegrass

Sow the seeds of ryegrass according to the experimental design of Section 2.2.1. In the treatment group of ryegrass planting, 10.00 g ryegrass seeds sowed in each pot (100 g dry soil reserved before sowing, the seeds were evenly scattered in the pot and then covered with dry soil). The test pots were placed outdoors; the weather and watering date were recorded. The flowerpot has been moved under the roof to shelter in the rainstorm weather, and the pot was placed outside in the ordinary rain as usual.

2.3. Soil sample collection

Soil samples were collected by the soil drill with a length of 25 cm, inner diameter of 1.5 cm in each flowerpot at 2 h, 15d, 40d, 60d, 90d and 120d, respectively. 200 g soil samples were put into the sample bag after sieving, marked and brought back, and the concentration of pollutants and soil enzyme activity were determined. The residual concentrations of flame-retardants and Cd in soil samples has been detected.

2.4. Collection of perennial ryegrass samples

The perennial ryegrass seedlings were collected on 20th day, some physiological and biochemical indicators such as proline content, MDA and antioxidant enzyme activities of ryegrass seedlings were determined. To study phytoextraction efficiency of ryegrass to halogenated flame-retardants and Cd from the soil, the whole ryegrass was collected on 120th day to determine the concentration for *syn*-DP and *anti*-DP, TBBPA and Cd in ryegrass tissues (dry roots and shoots), and to investigate the phytoextraction capacity in ryegrass roots and shoot.

Table 1
Eleven treatments for the experimental design.

Treatments	note	Treatments	note
CK	planting ryegrass in blank soil without adding pollutants		
TBBPA	10 mg/kg	TBBPA+G	planting ryegrass
DP	5 mg/kg	DP+G	planting ryegrass
Cd	10 mg/kg	Cd+G	planting ryegrass
T + DP	10 mg/kg TBBPA and 5 mg/kg DP	T + DP+G	planting ryegrass
T + DP+Cd	10 mg/kg TBBPA, 5 mg/kg DP and 10 mg/kg Cd	T + DP+Cd+G	planting ryegrass

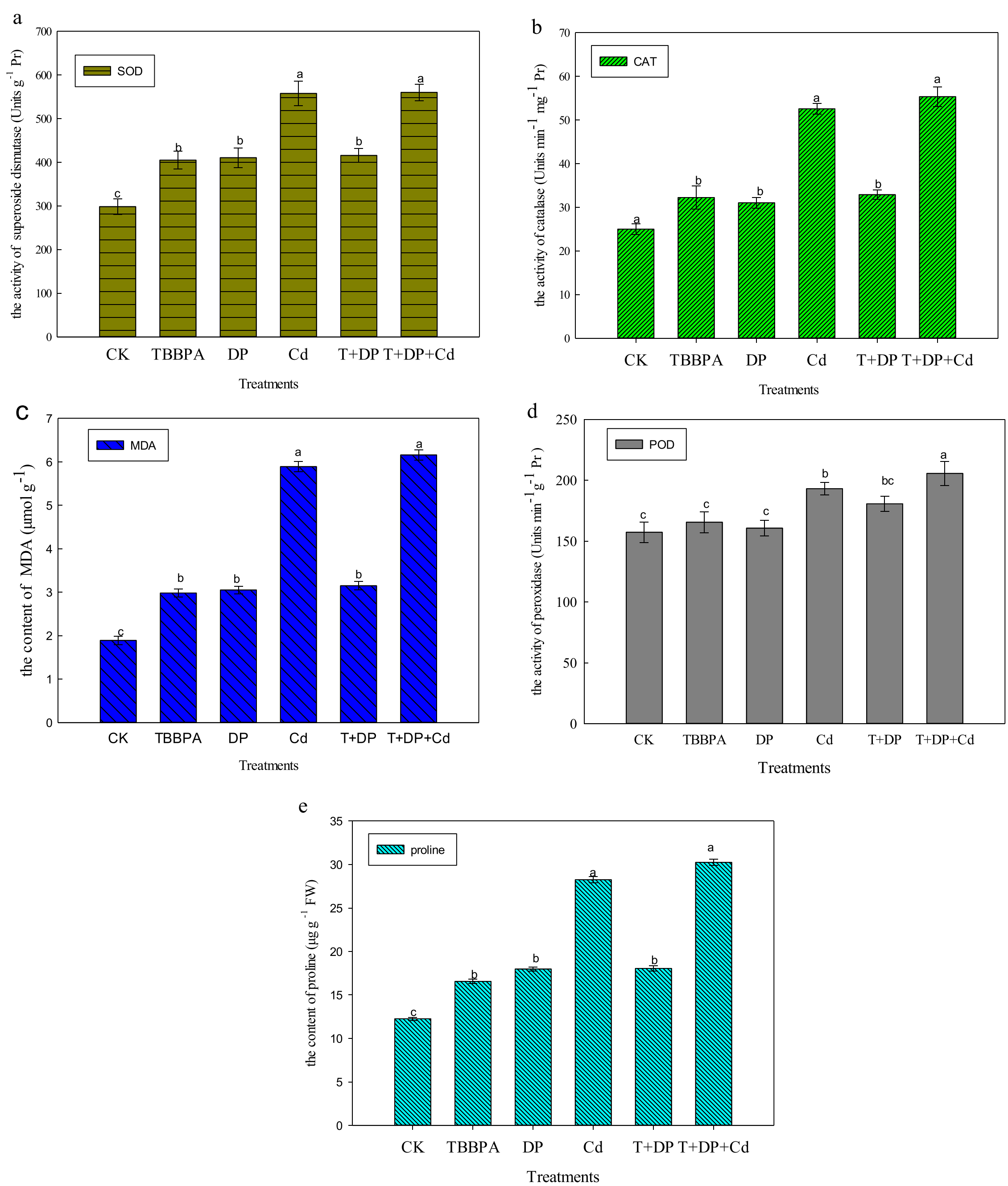

Fig. 1. Biological response of ryegrass seedlings to halogenated flame retardants and cadmium in contaminated soil.

2.5. Assays of proline content, MDA and antioxidant enzyme activities in the ryegrass seedling

The proline in plants was determined by the spectrophotometry with Acid Ninhydrin, and the mixture of proline with ninhydrin reacted to form stable colored products under acidic conditions, and the maximum absorption peak of the product was 520 nm, and the content of the product is positively correlated with the chromaticity [42].

The activities of catalase (CAT), peroxidase (POD) and superoxidedismutase (SOD) in the ryegrass seedlings were determined using CAT assay Kit (A007–1–1), POD assay Kit (A084–3–1), and SOD assay Kit of plant (A001–3–1) according to the instructions of manufacturer, respectively. MDA of ryegrass seedlings detected by plant MDA assay kit (TBA method); all assay kit is from Jiancheng Bioengineering Institute (Nanjing, China).

2.6. Determination of syn-DP, anti-DP and TBBPA residues in the soil and measurement of ryegrass accumulation

The method of determination of DP residues in soil and ryegrass was modified according to previous literature [54].

Sample pretreatment method of TBBPA: 10.00 g sample was prepared, added 3 g diatomite as dispersant, mixed evenly, put into ASE 150 sample pool, the top and bottom of the sample pool is covered with a glass fiber filter membrane (American Dionex Company) to prevent soil and diatomite from blocking the pipeline. Balance for 8 h, add 5 mL acetonitrile, V/V), to extract 5 min at 80 ℃ and 10.34 MPa pressure, add 1 mL acetonitrile after blowing dry, extraction fluid was filtered over 0.22 μm organic phase filter membrane. TBBPA residues was detected by HPLC-MS/MS.

Detecting conditions of TBBPA: column was Waters AtLantis T3C_{18} (2.1 mm × 15 cm×3 μm), and column temperature was 30 ℃; Flow speed was 0.3 mL/min, the flow phase was set to A and B, A was 0.01 moL/L ammonium formate+ 0.1% formate methanol solution, B was 0.01 moL/L ammonium formate + 0.1% formic acid aqueous solution; the modes of gradient elution were used, 0–10 min: 50%A + 50%B, 10–12 min: 100% A, 13–14 min: 50%A+ 50%B; sample size was 10 μL. SRM mode and electrospray ion source (ESI) was set. Temperature of ESI was 320 ℃, Ion spray temperature was 300 ℃, coat flow pressure is 40 arb, auxiliary gas pressure was 10 arb; Ion spray voltage (positive 3500 V, minus 2500 V), Ion discharge current was 4 μA; parent ion of TBBPA (m/Z) was 542.5, Ion (m/Z) was 417.5, energy collision of SRM was 40 ev. Retention time of TBBPA in this condition is 9.58 min

2.7. Determination of total Cd and bioavailable Cd in soils and ryegrass accumulation for Cd

The sample was prepared with the full automatic microwave digestion instrument, and then analyzed by the ICP/MS. 1.00 g soil (ryegrass sample) placed to digestion tank, added 5 mL nitric acid/perchloric acid (4:1), loaded the digester into the pressure casing, and placed the installed pressure casing in the groove of the dissolving rotor. Put the digester in the digestion instrument, and then connected the temperature sensor to the interface, the temperature was set 180 ℃, each sample is set up with three parallel samples. After digestion, the solution was cool, filtered the digestive fluid into 25 mL colorimetric tube, and flushed digestion tank multiple times with 1% nitric acid, and then volume, reagent blank and standard curve was set at the same time, ICP/MS used for sample testing.

The bioavailable Cd contents in rhizosphere soil extracted with 0.05 mol/L diethylene Triamine pentaacetic acid (DTPA), and then it determined by ICP/MS.

2.8. Biological absorption and transfer coefficient

Biological absorption coefficient (BAC), representing the pollutants absorption by ryegrass from soil, it was calculated by formula (1).

$$BAC = \frac{concentration \quad in \quad root}{concentration \quad in \quad soil} \tag{1}$$

Biological transfer coefficient (BTC), representing the transport and accumulation of pollutants from root to stem and leaf, it was calculated by formula (2).

$$BTC = \frac{concentration \quad in \quad stem \quad and \quad leaf}{concentration \quad in \quad root} \tag{2}$$

2.9. Statistical analyses

All the data was presented as $\overline{x} \pm sd$, the mean values compared using the Statistical Package for Social Sciences package (SPSS 22.0 for Windows) by a multiple comparison test at the 5% probability level. The results were analyzed by variance (ANOVA test). Figures produced using Sigmaplot 12.5.

The degradation rates of pollutants in the soil calculated as: $x = \frac{C_{initial} - C_{residue}}{C_{initial}}$.

With $C_{initial}$ being the initial concentration and $C_{residue}$ being the concentration determined at a particular time point, for each pollutant.

3. Results and discussion

3.1. Biological response of ryegrass seedlings

After 20 days of planting ryegrass, it was investigated that the biological response of ryegrass seedlings to halogenated flame-retardants and cadmium in contaminated soil by detecting the amount of proline and MDA, the activities of antioxidant enzymes, the results are shown in Fig. 1.

From Fig. 1, ryegrass seedlings were very sensitive to the two halogenated flame-retardants and cadmium. The contents of proline and MDA in seedlings increased to varying degrees with application treatments. Compared with the control, the difference was significant ($P < 0.05$). Proline content increased by 0.35, 0.48, 1.31, 0.47 and 1.47 times compared with the control with the treatment of TBBPA, DP, Cd, T + DP and T + DP+Cd, the content of MDA increased by 0.58, 0.61, 2.12, 0.67 and 2.26 times compared with the control, respectively. Cadmium pollution had the greatest effect on ryegrass and had significant difference with other treatments, and the two flame-retardants had relatively small effect. Ryegrass has different biological effects in contaminated soil, in order to protect its own cells from injury and eliminate reactive oxygen species, its antioxidant enzyme activity increased to varying degrees. The activity of SOD increased 0.36, 0.38, 0.87, 0.39 and 0.88 times compared with the control, respectively, POD increased 0.053, 0.022, 0.23, 0.15 and 0.31 times compared with the control, and the activity of CAT increased 0.29, 0.24, 1.10, 0.31 and 1.1, respectively. There was significant difference between cadmium and combined pollution treatment group and other treatments, which indicated that ryegrass had the greatest biological response to cadmium pollution and relatively small response to the two halogenated flame-retardants.

3.2. Bioaccumulation of DP, TBBPA and Cd in the ryegrass

An outdoor pot experiment was carried out; ryegrass samples were collected on the 120th day to determine the bioaccumulation of DP, TBBPA and Cd in ryegrass roots and shoot (including leaves and stems), and to explore phytoextraction amount in ryegrass roots and shoot. The results are in Table 2.

Under three treatments with ryegrass, the root and shoot of ryegrass have significant differences in the absorption of *syn*-DP and *anti*-DP. The concentrations of *syn*-DP were 63.86 ng/g and 31.66 ng/g in the roots and shoot of ryegrass, and the concentrations of *anti*-DP were 175.32 ng/g and 41.44 ng/g in DP+G treatment, respectively. The

Table 2
Phytoextraction amount in ryegrass roots and shoot of DP, TBBPA and Cadmium from soil.

pollutants treatments		DP (ng/g)		TBBPA (ng/g)	Cd (μg/g)
		Syn-DP	Anti-DP		
DP+G	root	63.86 ± 2.56	175.32 ± 5.78	—	—
	shoot	31.66 ± 1.20	41.44 ± 3.21	—	—
TBBPA+G	root	—	—	289.35 ± 6.45	—
	shoot	—	—	58.01 ± 2.03	—
Cd+G	root	—	—	—	27.82 ± 1.02
	shoot	—	—	—	10.25 ± 0.25
TBBPA+DP+G	root	16.50 ± 0.72	73.32 ± 2.35	252.56 ± 6.65	—
	shoot	11.71 ± 0.26	31.88 ± 0.19	50.94 ± 1.06	—
TBBPA+DP+Cd+G	root	15.70 ± 0.21	63.75 ± 1.19	216.48 ± 8.36	28.02 ± 0.56
	shoot	11.18 ± 0.18	30.28 ± 0.78	43.86 ± 1.05	10.69 ± 0.16

Note: All samples are dry, and residual amount: $\overline{x} \pm s$

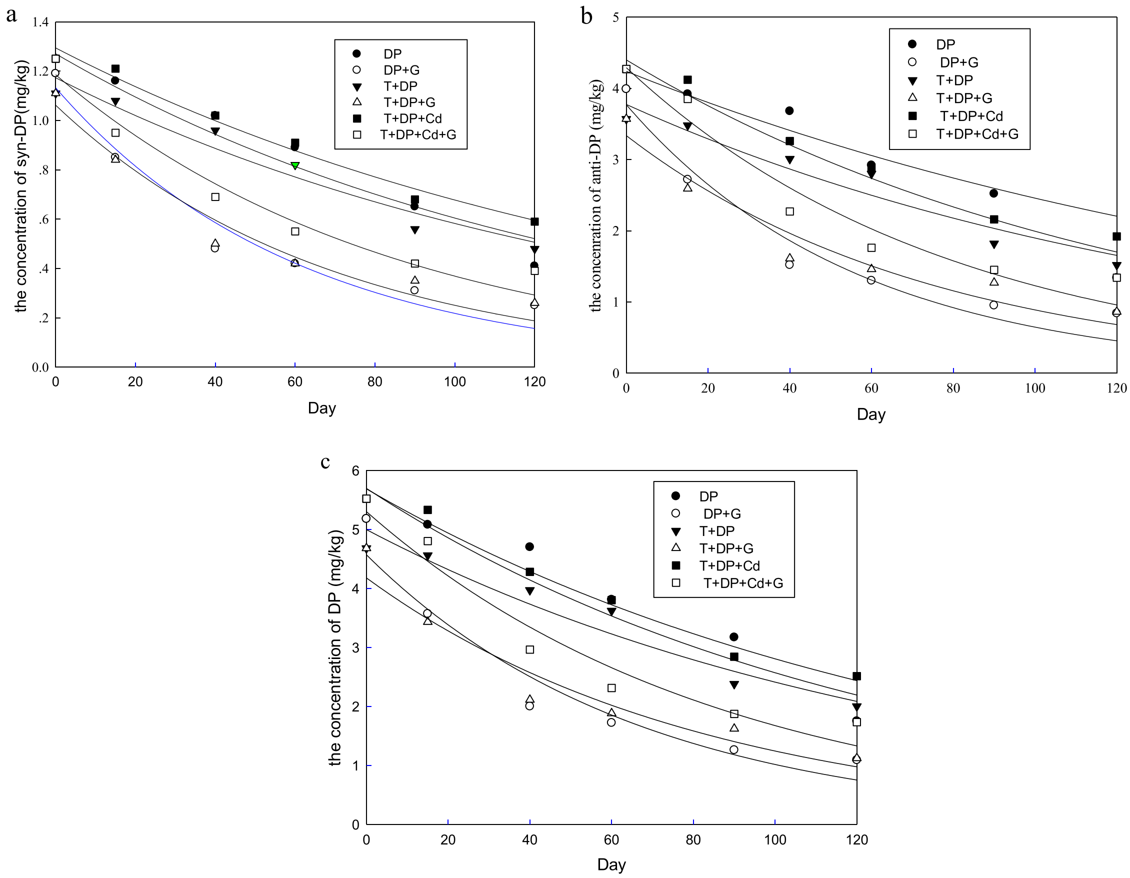

Fig. 2. Degradation dynamics of syn-DP, anti-DP and total DP in the soil with different treatment.

compositions of DP isomers is expressed by f_{anti} (the fraction of anti-isomer), the f_{anti} values in the root and shoot were 0.73 and 0.57, the values were obviously smaller than those in the corresponding rhizosphere soils (mean=0.76) as well as in the technical DP products (0.65–0.80) [8]. The absorption of ryegrass to DP is decreasing in the treatments of T + DP+G and T + DP+Cd+G. In the treatment of T + DP+G, the concentrations of *syn*-DP were 16.50 ng/g and 11.71 ng/g in the ryegrass roots and shoot, *anti*-DP were 73.32 and 31.88 ng/g, respectively. In the treatment of T + DP+Cd+G, the concentrations of *syn*-DP were 15.70 ng/g and 11.18 ng/g in ryegrass roots and shoot, *anti*-DP were 63.75 ng/g and 30.28 ng/g, respectively.

Since the molecular weight of DP is very large (653.70 g/mol), the water solubility is very weak (0.044–246 μg/L), and the absorption amount for DP was little, BAC was less, the amount of DP dissification in the soil by the absorption of ryegrass was very less. A number of literature reported that plants could absorb DP in the environment, but absorption amount was very less than ryegrass. Wang [48] reported that plants selectively absorbed DPs, the highest DPs concentrations in shoots and roots were in cabbage, the concentration of root was up to 160 ng/g, but it was very low in the shoot, other plants absorb very little. Concentrations of DP in eucalyptus foliage and pine needles at another e-waste site were 0.45–16.7 ng/g and 0.51–51.9 ng/g [3]. Sun [41]

Table 3
Parameters of syn-DP, anti-DP and DP in the Exponential Decay degradation model.

DP	treatment	degradation model $C=a\cdot e^{-bt}$	*P* (a)	*P* (b)	r	$T_{1/2}$ (d)
Syn-DP	DP	$C = 1.2704e^{-0.0074\ t}$	<0.0001	0.0027	0.9663	93.67
	DP+G	$C = 1.1326e^{-0.0165\ t}$	<0.0001	0.0015	0.9793	42.01
	T + DP	$C = 1.1732e^{-0.0070\ t}$	<0.0001	0.0014	0.9753	99.02
	T + DP+G	$C = 1.0619e^{-0.0144\ t}$	<0.0001	0.0012	0.9799	48.14
	T + DP+Cd	$C = 1.2941e^{-0.0065\ t}$	<0.0001	0.0002	0.9910	106.64
	T + DP+Cd+G	$C = 1.18546e^{-0.0117\ t}$	<0.0001	0.0008	0.9819	59.24
Anti-DP	DP	$C = 4.2388e^{-0.0054\ t}$	0.0010	0.0697	0.8059	128.36
	DP+G	$C = 3.7695e^{-0.0176\ t}$	0.0001	0.0023	0.9748	39.38
	T + DP	$C = 3.7738e^{-0.0069\ t}$	<0.0001	0.0021	0.9690	100.46
	T + DP+G	$C = 3.3358e^{-0.0132\ t}$	0.0001	0.0025	0.9695	52.51
	T + DP+Cd	$C= 4.3897e^{-0.0072\ t}$	<0.0001	0.0001	0.9923	96.27
	T + DP+Cd+G	$C = 4.2905e^{-0.0125\ t}$	<0.0001	0.0019	0.9736	55.45
DP	DP	$C = 5.6943e^{-0.0080\ t}$	0.0010	0.0497	0.9517	86.64
	DP+G	$C = 4.5689e^{-0.0150\ t}$	0.0001	0.0033	0.9568	46.21
	T + DP	$C = 4.9957e^{-0.0073\ t}$	<0.0001	0.0025	0.9750	94.95
	T + DP+G	$C = 4.1776e^{-0.0121\ t}$	0.0001	0.0026	0.9627	57.28
	T + DP+Cd	$C = 5.6859e^{-0.0071\ t}$	<0.0001	0.0001	0.9930	97.63
	T + DP+Cd+G	$C = 5.3009e^{-0.0115\ t}$	<0.0001	0.0018	0.9656	60.27

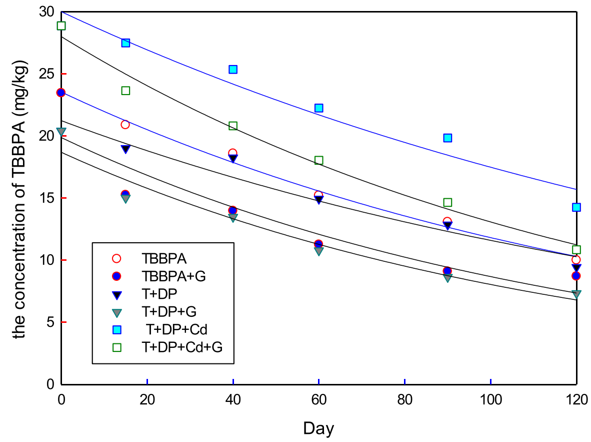

Fig. 3. Degradation dynamics of TBBPA in the soil with different treatment.

reported that DP concentrations of root in tomato were 63–168 ng/g and 42–88 ng/g in soil containing fresh DP in the greenhouse and the conventional open field, and concentrations of root in cucumber were 41–129 ng/g and 37–82 ng/g. the uptake potential indexes in greenhouse vegetables were generally higher than those in conventional vegetables. Uptake of DP into peanut and corn was very less [8]. Although a variety of vegetables and grain can absorb DP, but the absorption amount is very little, ryegrass with its strong absorption capacity and biomass would be the best plant for phytoremediation.

Since the molecular weight of TBBPA is very large (543.87 g/mol), the water solubility is weak (<0.1 g/100 mL), ryegrass could bioaccumulate much TBBPA, and the absorption amount of ryegrass for TBBPA is more than DP. The concentrations of TBBPA were 289.35, 252.56 and 216.48 ng/g in ryegrass roots, TBBPA in shoot were 58.01, 50.94 and 43.86 ng/g in TBBPA+G, T + DP+G and T + DP+Cd+G treatments respectively. BACs were 0.012, 0.012 and 0.0075 respectively, and BTCs were all about 0.20. The absorption amount of the root is more than shoot.

Previous studies reported that the roots of radish and cabbage could accumulate higher concentrations of TBBPA than the shoots [24], higher concentrations of TBBPA and its metabolites accumulated in the roots of rice and reed seedlings, similarly [40]. Two mangrove species accumulated TBBPA and its hydroxylated and debrominated metabolites in the roots [17]. It indicated that a large amount of TBBPA was absorbed by the root of ryegrass and then translocated to stem and leaf in the present study, this study is consistent with the findings reported in the literature. The bioaccumulation factor (BAF) and translocation factor (TF) are useful parameters to evaluate the ability of plants to accumulate and translocate TBBPA [21]. From the present study, BCF of ryegrass for TBBPA was low, and TF was relatively higher.

The uptake of ryegrass roots for Cd was different from shoot. The concentrations of cadmium were 27.82 mg/kg and 10.25 mg/kg in the ryegrass root and shoot (dry sample), BAC and BTC were 2.16 and 0.37 in the treatment with cadmium, respectively. The concentration of ryegrass in the roots and shoot was 28.02 mg/kg and 10.69 mg/kg, BAC and BTC were 2.15 and 0.38 in combined pollution treatment, respectively. For roots and shoot, there was no significant difference between the two treatments; the results proved that ryegrass did not reduce the absorption amount for cadmium in the treatment of combined pollution.

Plant absorption has two ways, including root absorption and leaf surface absorption. Halogenated flame-retardants with high molecular weight and hydrophobicity are easily concentrated in soil, it mainly was plant root absorption [4]. Nonionic halogenated flame-retardants are passively absorbed into plants, solubility of halogenated flame-retardants in lipids and water affects the ability of halogenated flame-retardants absorbed by plants to transport from roots to stems and shoot. BAFs of ryegrass roots to flame retardants in soil is higher than that shoot, the absorption amount related to the concentration of pollutants in soil, and BAF of combined pollution is less than that of single pollution. Ryegrass have higher tolerance and accumulation ability to Cd, it has been widely used in phytoremediation of soils contaminated with Cd because it could accumulate high amount of Cd in shoots and roots.

3.3. Degradation mechanism of DP in soil

Two treatments with ryegrass and without ryegrass were set, the concentration of syn-DP and anti-DP in the soil of 2 h, 15d, 40d, 60d, 90d and 120 d were determined, respectively, and to explore its degradation dynamics in the two treatments of soil. The degradation dynamics shows in Fig. 2, and the degradation models predicted by Sigmaplot 12.5 software as shown in Table 3.

The result showed that degradation half-life of *syn*-DP and anti-DP in the soil have significant difference in the soil with ryegrass and unplanted ryegrass. The degradation half-lives of *syn*-DP and *anti*-DP in the soils without ryegrass were 93.67–106.64 d and 96.27–128.36 d, respectively. The concentrations of *syn*-DP and *anti*-DP in soils significantly decreased in the treatment of ryegrass cultivation (Fig. 2). The loss of DP was induced by wash out, volatilization, degradation, ryegrass uptake and the cooperative effect of rhizosphere microorganisms, the most important thing is that root exudates of ryegrass plays an important role in the degradation of DP in soil. It reported that root exudates of plant comprised amino acids, sugars, fatty acids and a series of secondary metabolites, all of which are as an energy source for the

Table 4
Parameters of TBBPA in the Exponential Decay degradation model.

treatment	degradation model C=a·e^{-bt}	*P* (a)	*P* (b)	r	$T_{1/2}$ (d)
TBBPA	$C = 23.4887e^{-0.0068\,t}$	<0.0001	<0.0001	0.9960	101.93
TBBPA+G	$C = 20.8626e^{-0.0093\,t}$	0.0003	0.0086	0.9338	74.53
T + DP	$C = 21.0663e^{-0.0058\,t}$	<0.0001	0.0012	0.9758	119.51
T + DP+G	$C = 19.0718e^{-0.0089\,t}$	<0.0001	0.0012	0.9762	77.88
T + DP+Cd	$C = 29.7573e^{-0.0051\,t}$	<0.0001	0.0010	0.9770	135.91
T + DP+Cd+G	$C = 28.0317e^{-0.0076\,t}$	<0.0001	0.0001	0.9925	91.20

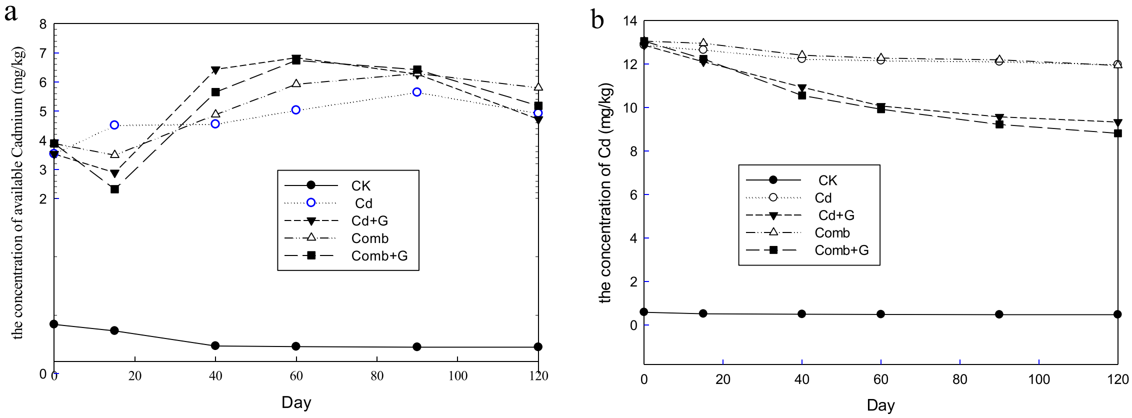

Fig. 4. Trends of bioavailable cadmium and total cadmium in the soil with different treatment.

amplification and aggregation of microorganisms [27,35,38]. The degradation of DP was faster in the soils with ryegrass. The half-lives of *syn*-DP and *anti*-DP were 42.01–59.24 d and 39.38–55.45 d, respectively, and $T_{1/2}$ of *syn*-DP was more than *anti*-DP, $T_{1/2}$ of DP was 46.21–60.27d.

The degradation half-lives of DP in the soil of single and combined contaminations are different ($P < 0.05$), and the $T_{1/2}$ of DP in the soils of single contamination is significantly less than that in the soil of combined contamination. Because cadmium pollution can lead to the enzyme activity decrease in the soil and enzyme plays an important role in the degradation of soil organic polluters, which leads to the degradation of organic pollutants slow in soil, and $T_{1/2}$ was prolonged [13].

3.4. Degradation mechanism of TBBPA in soil

The degradation dynamics showed in Fig. 3, and the degradation model predicted by Sigmaplot 12.5 software and it showed in Table 4.

The result showed that, the half-lives of TBBPA dissipation in soil without ryegrass were 101.93–135.91 d, but in the soil with ryegrass, the concentration of TBBPA in soil decreased rapidly. One reason was the uptake of ryegrass; the other reason was cooperative action between soil and rhizosphere microorganisms. Ryegrass could remove pollutants in soils, relieve their stress to soil bacteria and promote biomasses and activities of soil bacteria. Root exudates contributed to biological amplification of rhizospheric microorganism, which was significantly abundant in near rhizosphere soil with ryegrass treatment, and it was a powerful contribution to TBBPA reduction in soil, so that the half-lives of TBBPA significantly shortened to 74.53–91.20 d. The half-lives of TBBPA dissipation in single and compound contaminated soil were different ($P < 0.05$), the $T_{1/2}$ of TBBPA in single contaminated soil was significantly less than that in combined contaminated soil. The degradation half-lives of TBBPA in the soil with T + DP+Cd treatment were the longest, and $T_{1/2}$ were 135.91 d and 91.20 d in the soil with and without ryegrass, respectively.

3.5. The migration and transformation dynamics of total and bioavailable cadmium in soil

Fig. 4a showed the change trend of bioavailable cadmium at different times, and Fig. 4b showed the migration and transformation dynamics of total cadmium in different treated soils.

The results showed that ryegrass could effectively reduce the residual concentration of total cadmium in soil, with the extension of time, the total amount of cadmium in soil decreased gradually, in the Cd+G treatment, the concentration of total cadmium in soil decreased by 22.28% and 27.99% at 60 and 120 days. The results showed that the total cadmium decreased very slowly from 60 to 120 days, the probable reason was that the first 60 days of the trial, the germination and growth of ryegrass absorbed large amounts of nutrients from the soil, simultaneously it absorbed lots of cadmium in the soil, the concentration of cadmium in soil decreased rapidly. However, the total concentration of cadmium in the soil without ryegrass changed less. The concentration of total cadmium decreased by 23.44% and 32.00% in the soil with treatment of T + DP+Cd+G on the 60th and 120th days, respectively. The results showed that ryegrass could bio accumulate lots of cadmium in soil, it grew fast and tillered strongly, and ryegrass would be recommend as hyperaccumulators for cadmium in soil, a highly developed root system contributed to accumulate Cd. In view of the absorption characteristics of ryegrass to cadmium, the period of bioremediation cpuld be set to 60 days every time.

The change of bioavailable cadmium in soil was different from the total cadmium, both single pollution and combined pollution had the same trend, and there were significant differences in the soil with and without ryegrass ($P < 0.05$). With the extension of time, firstly, the concentration of bioavailable cadmium in the soil without ryegrass increased and then decreased, while bioavailable cadmium in the soil with ryegrass decreased at the beginning, and then increased, and it decreased in the end. The possible reason was that in the initial stage (the first 15 days), ryegrass seeds were in the germination stage, and it absorbed many nutrients as well as a large amount of bioavailable cadmium in the soil, which leaded to the decrease of bioavailable cadmium concentration in the soil. Ryegrass grew vigorously, the root system developed on 40th day, the root of ryegrass could secrete carbohydrate, phytochelatins, organic acid and amino acid to acidize the soil [28], and soil pH was a critical factor to heavy metal bioavailability, which was beneficial to the existence of available cadmium, and the concentration of available cadmium increased gradually. Root exudate promoted the nutrient absorption, and it interfered with the migration of heavy metals in soils. It could be an effective strategy for the improvement of phytoextraction because of relative lower soil pH [33]. After 90 days, the total cadmium concentration decreased and the bioavailable cadmium concentration decreased gradually. From the 40th day, the concentration of bioavailable cadmium in the soil with combined contamination without ryegrass was significantly more than that of single contamination. Because the main forms of interaction between organic pollutants and heavy metals in soil were as follows: interaction of adsorption behavior, interaction of chemical process and interaction of microbial process, while the interaction was mainly reflected in the competition, complexation and oxidation-reduction of adsorption sites. Organic pollutants would affect the biological effective state of heavy metals, and the entry of organic pollutants in soil would affect the concentration of bioavailable state of heavy metals [20].

4. Conclusion

Proline, MDA contents and the activity of antioxidant enzyme of ryegrass seedlings were increased under the action of two flame-retardants and Cd, cadmium had a great influence on ryegrass seedlings. BAFs of ryegrass roots was higher than shoot. For the absorption of *syn*-DP and *anti*-DP, the root and shoot had significant differences. Ryegrass could assimilate trace amount of DP and TBBPA, and ryegrass had the potential of phytoremediation for contaminated soil. Ryegrass could promote two flame-retardants degradation in the mechanism of the root exudates and rhizosphere microorganisms. $T_{1/2}$ of DP and TBBPA prolonged in the treatments with combined pollution. Ryegrass roots could assimilate cadmium from the soil and it accumulated in different parts of ryegrass, the optimal bioremediation time was set to 60 days. The change trend of bioavailable cadmium in soil is different from total cadmium due to the presence of ryegrass. Organic pollutants and heavy metals dissipated in soil under the rhizosphere microorganism and root exudates of ryegrass, and the mechanism of phytoremediation needed further study.

Ethical approval

This work does not contain any study with humans or animals.

CRediT authorship contribution statement

Yuhan Ma: Conceptualization, Methodology, Formal analysis. **Yuying Wang:** Conceptualization, Methodology, Formal analysis. **Ruiyuan Liu:** Formal analysis, Data curation, Software. **Xin Liu:** Formal analysis, Data curation, Software. **Fengxia Sun:** Investigation, Resources, Supervision. **Yuxin Xu:** Investigation, Resources, Supervision. **Hui Xie:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data, associated metadata, and calculation tools are available from the corresponding author (huixie@sdau.edu.cn).

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China [Nos. 41671321].

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