frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 15 September 2022
DOI 10.3389/fenvs.2022.990174

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Toxicology, Pollution and the Environment,
a section of the journal
Frontiers in Environmental Science

RECEIVED 09 July 2022
ACCEPTED 01 August 2022
PUBLISHED 15 September 2022

CITATION
Liu R, Xie H, Xu Y and Sun F (2022), Phytotoxicity, uptake, and translocation of two halogenated flame retardants and cadmium in two rice varieties.
Front. Environ. Sci. 10:990174.
doi: 10.3389/fenvs.2022.990174

Phytotoxicity, uptake, and translocation of two halogenated flame retardants and cadmium in two rice varieties

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Dechlorane Plus (DP) and Tetrabromobisphenol A (TBBPA) are the halogenated flame retardant with the highest production volume, cadmium (Cd) is regarded as one of the hazardous heavy metals due to its bio toxicity and bioaccumulation in the environment, and it will cause environmental pollution and affect human health, so a pot experiment was conducted to investigate the phytotoxicity on seed germination and seedling of two rice varieties. The results showed that with the increasing of DP, TBBPA and Cd concentrations in soil, the germination rate, germination index and vigor index of two rice varieties decreased, and the germination of Number 7 rice was affected more than Number 1 rice. Halogenated flame retardants and Cd in soil significantly promoted root activity and proline, and significantly inhibited protein and soluble sugar contents of two rice varieties. In addition, the responses of the two rice varieties seedlings to different pollutants were obviously different. The activity of superoxide dismutase (SOD) in two kinds of rice was improved, and a large amount of malonyldialdehyde (MDA) was induced. But the activity of catalase (CAT) in Number 7 rice was increased under the action of DP and TBBPA, while the activity of Number 1 rice was increased under the action of DP and inhibited under the pollution of TBBPA. Bioaccumulation factors of DP and Cd were 0.025–0.042 and 6.59–14.20, bio transport coefficients were 0.39–0.48 and 0.034–0.087. There was no significant difference in the bioaccumulation of DP in the two rice varieties, but TBBPA and Cd were more easily bio accumulated in the Number 1 rice. These findings would provide some essential information for interpreting the ecological risks of two halogenated flame-retardants and Cd in plants.

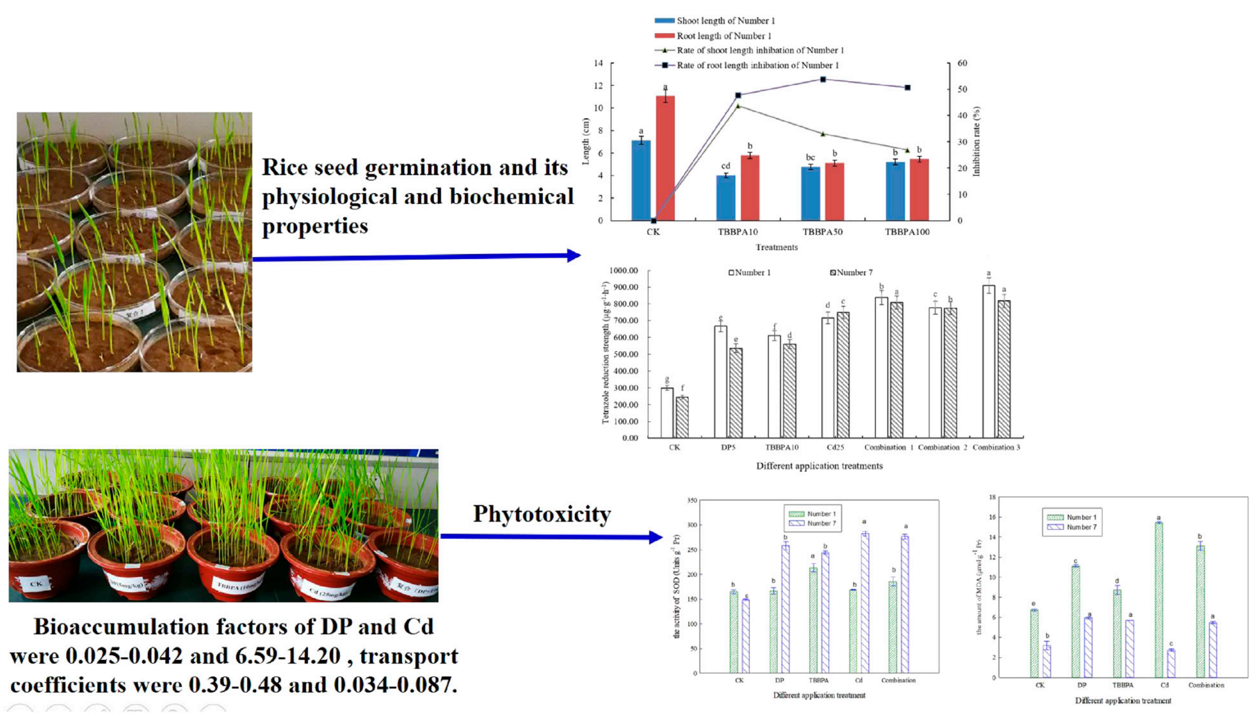
KEYWORDS

phytotoxicity, bioaccumulation, dechlorane plus, tetrabromobisphenol A, Cd

Highlights

- Two halogenated flame retardants and Cd in soil significantly promoted root activity and proline in seed germination.

10.3389/fenvs.2022.990174



Graphical Abstract

- The activity of superoxide dismutase and catalase of two rice varieties was improved, and a large amount of malonyldialdehyde was induced.
- There was no significant difference in the bioaccumulation of DP in two rice varieties, but TBBPA and Cd were more easily bio accumulated in the Number 1 rice.

1 Introduction

With the wide application of TBBPA and DP and the original extensive electronic dismantling activities for a long time, soils in typical e-waste dismantling sites in China are seriously polluted by heavy metals Cd, DP and TBBPA. The effects of its residues in soil on crops and soil ecological environment have attracted people's attention. The residual levels of DP, TBBPA and Cd in electronic waste are relatively high, so extensive electronic dismantling behavior makes the soil of electronic waste dismantling also have high concentration of residual. Therefore, it is of great significance to investigate the toxic effects of Cd, DP and TBBPA on soil organisms and reveal their potential risks to soil ecosystem. As sensitive species, terrestrial plants can be used to evaluate the ecological toxicity of contaminated soil. Under the stress of pollution, the germination of plant seeds and the response of seedlings can reflect the environmental health level of soil ecosystem comprehensively.

DP with syn-DP and anti-DP isomers, their molecular structure is shown in Figures 1A,B, are widely used in wire and cable coatings, electronic components, connectors of televisions and computers. DP has the property of environmental persistence and long-distance transport (Liu et al., 2018), with residues in both marine and polar environments (Yu et al., 2015; Wang et al., 2017; Aznar-Alemany et al., 2019; Vorkamp et al., 2019). Due to its high hydrophobicity ($\log_{KOW}$ 9.3), DP has a long half-life in the environment (Xian et al., 2011), which not only residues were in atmospheric particles (Kakimoto et al., 2014; Reche et al., 2019), sediments and water bodies (He et al., 2014; Li et al., 2021), but also it has been detected in biological substrates such as fish and shrimp (Huihui Zhang et al., 2020), human breast milk and serum (Kim et al., 2016; Pan et al., 2020). Moreover, DP can be bioaccumulated in organisms and gradually biomagnified in various trophic levels of organisms along the food chain. Gong (Gong et al., 2018) showed that the bioaccumulation of DP by algae resulted in oxidative stress and hindered the light energy utilization of algae. DP can also inhibit the photosynthetic level of plants, destroy the integrity of cell membranes, and affect the protective enzyme activity. For animals, the direct toxicity of DP exposure to earthworms is low, but it can cause oxidative damage (Yang et al., 2016). DP can damage fish intestinal epithelium, destroy the intestinal barrier, lead to intestinal bacteria disorder, thereby it affects

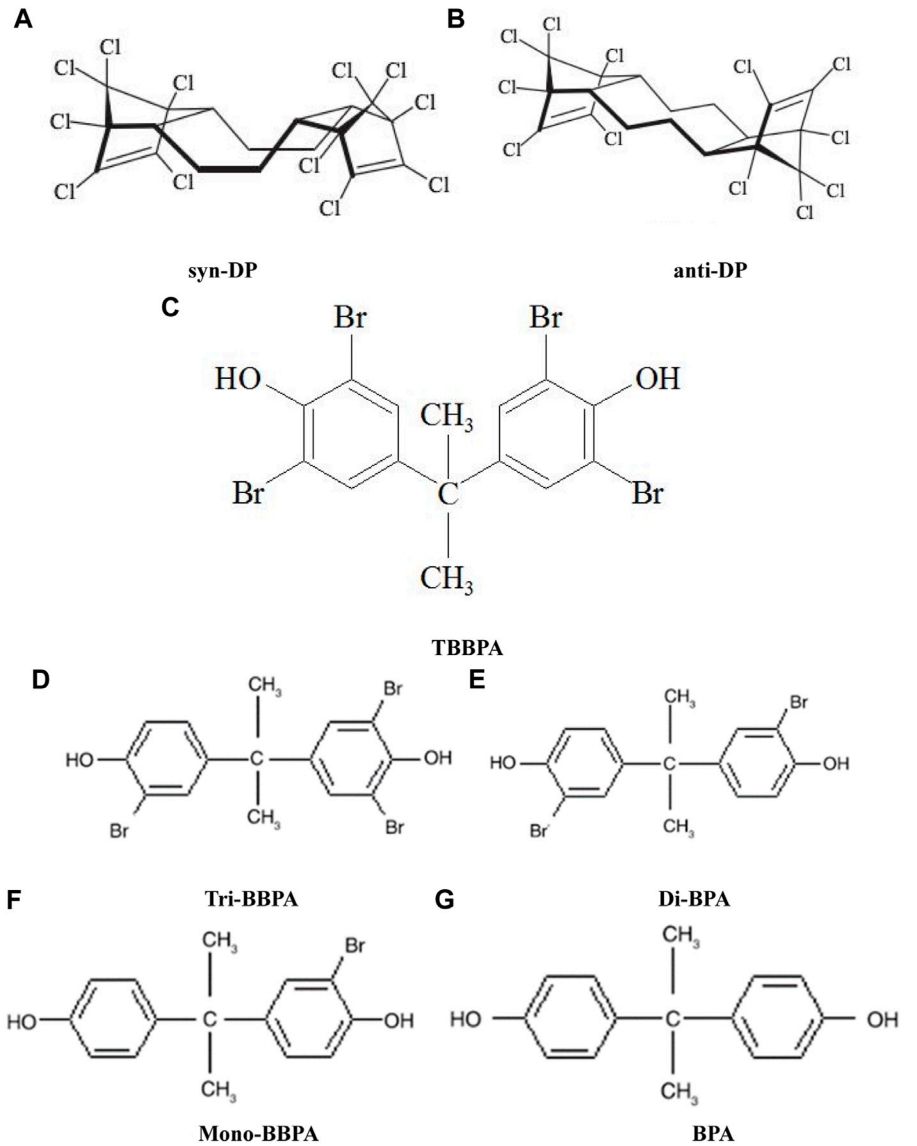


FIGURE 1
The molecular structure of syn-DP, anti-DP, and TBBPA. **(A)** syn-DP, **(B)** anti-DP, **(C)** TBBPA, **(D)** Tri-BBPA, **(E)** Di-BPA, **(F)** Mono-BBPA, **(G)** BPA.

biological health (Li et al., 2020), and it can also induce oxidative damage and apoptosis of fish (Li et al., 2019).

TBBPA is one of the most widely used brominated flame retardants in the world, its molecular structure is shown in Figure 1C. And TBBPA is easy to lose a bromine under anaerobic conditions and generate metabolites such as tribromobisphenol A (Tri-BBPA), dibromobisphenol A (Di-BPA), monobromobisphenol A (Mono-BBPA) and bisphenol A (BPA), their molecular structure is shown in Figures 1D–G. TBBPA is not only found in environmental samples such as air, indoor dust, water, soil, sediment and sewage sludge, but also in biological substrates such as animal, plant and human blood (Sánchez-Brunete et al., 2009; Guerra et al., 2010; Yang et al., 2012; Gu et al., 2020). TBBPA has bioaccumulative and biotoxic. Its structure is similar to that of thyroid hormone, so it is an endocrine disruptor, which is toxic to the immune system, cells and nerves of aquatic organisms, mammals and amphibians (Sanders et al., 2016). TBBPA easily accumulates in organisms and spreads through the food chain and food web to the entire ecosystem, causing toxic effects. TBBPA pollution had toxic effects on seed germination and seedling growth of soybean, chickpea and maize (Dogan et al., 2010; Ge and Zhang, 2017; Wang et al., 2019). Li (Li et al., 2008) reported that TBBPA reduced the activity of antioxidant enzymes and chlorophyll content in wheat, and TBBPA also reduced the activity of soil enzyme and soil microbial biomass (Wang et al., 2019).

Cd can cause carcinogenic, teratogenetic and mutagenic effects on human body (Fan et al., 2019; Piacentini et al., 2020), and its half-life in human body is up to 20 or 30 years. Cd generally enters soil through industrial and agricultural processes (Farooq et al., 2016), and it has high mobility in soil, high water solubility and phytotoxicity (Kamran et al., 2019). Cd can enter the food chain by bioaccumulation of crops, and then it poses threat to human health (Shahid et al., 2016). If the human body is exposed to heavy metals for a long time, the balance of intestinal flora will be broken, hindering nutrient absorption, inhibiting energy metabolism, damaging immune function, and thus producing physical poisoning symptoms (Evariste et al., 2019; Kan et al., 2015). The absorption of Cd by plant roots reduces the level of photosynthesis, hinders nutrient absorption, inhibits enzyme activity, further inhibits plant growth and development (Jiang et al., 2020; Khan et al., 2020), leaf fading and dwarfing of plant, and delays in phenological period (Lux et al., 2011; Radojčić Redovniković et al., 2017). Eventually it leads to plant necrosis.

Chen (Chen et al., 2018) reported that when zebra fish are exposed to the combined pollution of brominated flame retardants and heavy metals for a long time, the adult sex ratio, sperm count/quality and egg production of zebra fish will be affected, thus reducing the reproductive success rate of their offspring. There are many kinds of pollutants in the environment, and the exposure pathways of organisms are complex. Therefore, the study on the characteristics of the toxic effects of compound pollution on organisms has important application value for the assessment of environmental risks and environmental pollution control.

Although there are studies on toxicity of flame retardants and Cd to plants, there are few reports on toxicity to rice. In this study, the toxic effects of two halogenated flame retardants in soil and Cd on rice were studied by seed germination and early seedling growth experiments of two different types of rice. Through indoor potting experiments, the effects of DP, TBBPA and Cd on antioxidant enzyme activity, MDA and chlorophyll content of rice seedlings were studied, and the absorption and bioaccumulation of halogenated flame retardants and Cd in rice seedlings were investigated, which provided a scientific basis for the assessment of environmental risks in areas contaminated by organic compounds and heavy metals.

2 Materials and methods

2.1 Experimental reagents and instruments

Rice varieties: Guose Tianxiang Number 1 and Guose Tianxiang Number 7.

DP (Accu. Standard Inc., 99.00%), 50 mg L^{-1} syn-DP and anti-DP standard sample (Accu Standard Inc., toluene solvent); $CdCl_2 \cdot H_2O$ (99.99%, Sigma-Aldrich).

Tetrabromobisphenol A (Dr, German, 99.00%) Chromatographic methanol, Methanol and acetonitrile (chromatographic purity); 50 μg ml^{-1} Tetrabromobisphenol A standard sample (Methanol, Dr, German); N-hexane (chromatographic purity); Dichloromethane (chromatographic purity); Anhydrous sodium sulfate (analytical purity).

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).

The tested soil is brown soil, which was collected from Panhe campus of Shandong Agricultural University. The topsoil (2–20 cm) was collected, mixed and screened with 20 mesh. The physical and chemical properties of the soil are as follows: organic nitrogen, available phosphorus, available potassium and organic matter are 133.3, 18.6, 123.7 and 17.8 mg kg^{-1} respectively, DP and TBBPA were not detected, and the background value of cadmium in soil is 0.25 mg kg^{-1}.

2.2 Experimental design

The concentrations of two halogenated flame retardants were designed according to the current detection concentrations of DP and TBBPA in soil, the characteristics of environmental persistence and the concealability of soil pollution. The exposure concentrations of DP were set as 5, 25 and 50 mg kg^{-1} respectively; the concentrations of TBBPA in soil were set as 10, 50 and 100 mg kg^{-1} respectively. It has been reported (Roychoudhury et al., 2012) that compared with ordinary plants, rice has the ability to tolerate Cd pollution, so the Cd concentrations were designed to be high, respectively 10, 25 mg kg^{-1} and 50 mg kg^{-1}. Then the concentration of combined pollution treatment was determined by cluster analysis. The combined pollution treatments were as follows: DP5+T50 + Cd25, DP25 + T10 + Cd25 and DP50 + T10 + Cd25, meanwhile, CK was set, and the soil was balanced for 48 h after exposure to the polluters.

2.3 Seed germination test

In this experiment, rice seeds were soaked in water for 3 days, and then put on gauze to promote germination for 24 h 50 g contaminated soil was put in 9 cm Petri dish, the water/soil ratio was adjusted to 1:5, seeds were pressed into wet soil with medical tweezers, and covered the lid, and they were put in a biochemical incubator for 10 days at (25 ± 1)°C and (75 ± 2)% humidity, 16 h 2000–6000 LX light and 8 h dark, water was added timely to maintain soil moisture.

After 10 days, the number of germinated grains was recorded, the germination rate was calculated, and the root and shoot length were measured. Seed vigor index is expressed by germination index and vigor index, which is the comprehensive reflection of seed germination rate and growth amount and the best index of seed vigor. The germination rate, germination index and vigor index of seeds were calculated according to a previous study with some modifications (He et al., 2010) and the inhibition rate of root and shoot length was calculated according to the previous research (Wang et al., 2019).

2.4 Physiological and biochemical indicators in seed germination

Seven treatments were set up for rice seed germination experiment, CK, DP (5 mg kg^{-1}), TBBPA (10 mg kg^{-1}), Cd (25 mg kg^{-1}), DP5 + T50 + Cd25, DP25 + T10 + Cd25 and DP50 + T10 + Cd25. The leaves and roots were taken to determine the content of total soluble sugar, protein, proline and seed root activity.

The proline in plants was determined by the spectrophotometry with Acid Ninhydrin, and the mixture of proline with ninhydrin reacted to form stable products with color 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 (Sun et al., 2020). The content of soluble sugar was determined by anthrone colorimetry (Yang et al., 2021). The triphenyl tetrazolium chloride (TTC) method (Wang et al., 2010) with root tip tissues was used to measure root activity.

2.5 Toxicity test of rice seedlings

A pot experiment was conducted to study the toxic effects of halogenic flame retardant and Cd in soil on rice seedlings. The treatments were CK, DP (5 mg kg^{-1}), TBBPA (10 mg kg^{-1}), Cd (25 mg kg^{-1}) and its combined pollution (DP5 + TBBPA10 + Cd25), and the soil was balanced for 48 h after being poisoned, each treatment was set with 5 parallel samples.

The seeds were soaked in clear water for 48 h, and then put on gauze for 24 h, soaked rice seeds were planted according to the experimental design, 50 rice seeds were planted in one plastic pot (diameter 50 cm), rice seeds were picked and pressed into wet soil with tweezers.

When the shoots grew to 10 cm, the soil was irrigated, and rice seedling continued to grow, and then put them in the incubator at 25°C for 20 days. The whole seedlings were taken, and the contents of SOD, CAT, MDA and chlorophyll were measured. The residual amounts of two flame retardants and Cd in the rice seedlings were determined after the seedlings were dried, and the toxic effects of flame retardant and Cd on rice seedlings were evaluated.

2.6 Determination of antioxidase activity and maldehyde aldehyde content

0.5 g fresh seedling was put into 10.0 ml homogenizer, and then 4 ml pre-cooled phosphate buffer solution was added (0.05 mol L^{-1}, pH 7.8), after that it was fully homogenized under ice bath conditions. The homogenizer was rinsed with phosphate buffer solution to obtain 10% ($m \cdot v^{-1}$) tissue homogenate, and it was transferred into 10 ml polyethylene centrifuge tube, it was centrifuged for 20 min with 9000 r min^{-1} at 4°C, and the supernatant was stored at −20°C.

The activities of CAT, peroxidase (POD) and SOD in the rice 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 rice seedlings detected by plant MDA assay kit (TBA method); all assay kit is from Jiancheng Bioengineering Institute (Nanjing, China).

2.7 Determination of chlorophyll content in the seedlings

0.1 g seedlings was weighed and cut into pieces, and then put them in a mortar, it was fully homogenized after 10 ml ethanol (95%) was added, a small amount of quartz sand was added to accelerate grinding, and 5 ml ethanol was added, then it was filtered and diluted the filtrate to 25 ml with ethanol. Ethanol was taken as the control, optical density (OD) values of sample solution were measured at 665 and 649 nm, respectively (Gao, 2006).

$$\rho_a = 13.95 \times OD_{665} - 6.88 \times OD_{649}$$
$$\rho b = 24.96 \times OD_{649} - 7.32 \times OD_{665}$$
$$\rho = \rho a + \rho b$$
$$\text{Chlorophyll content}\left(\text{mg} \cdot kg^{-1}\right) = \frac{\rho \times \text{extraction volume} \times \text{diluted multiples}}{\text{fresh weight of sample}}$$

ρ_a: chlorophyll a; ρ_b: chlorophyll b; ρ: chlorophyll.

2.8 Determination of tetrabromobisphenol A, dechlorane plus and total cadmium residue in rice seedlings

The residual amount of TBBPA, DP and Cd in rice seedlings were determined using sample pretreatment and determination methods established in literature (Jiang et al., 2020).

2.9 Data statistics and analysis

In this study, the bioaccumulation factor and transport coefficient of rice seedlings were calculated by formula. The mean values compared using the Statistical Product and Service Solutions (SPSS 22.0 for Windows) by a multiple comparison test at the 5% probability level, and ANOVA was used to evaluate the significance by the least significant difference (LSD) ($p < 0.05$). Figures were plotted by Sigma plot 12.5.

$$\text{Bioaccumulation factor} = \frac{C\,\text{root}\,(\text{mg}\cdot\text{kg}^{-1}\text{dw})}{C\,\text{soil}\,(\text{mg}\cdot\text{kg}^{-1}\text{dw})}$$

$$\text{Transport factor} = \frac{C\,\text{shoot}\,(\text{mg}\cdot\text{kg}^{-1}\text{dw})}{C\,\text{root}\,(\text{mg}\cdot\text{kg}^{-1}\text{dw})}$$

3 Results and analyses

3.1 Effect of two halogenic flame retardants and cadmium on rice seed germination and its physiological and biochemical characteristics

3.1.1 Effect of halogenic flame retardants and cadmium on rice seed germination

The seed germination stage is the most vigorous period of rice life activities, which is greatly affected by variety differences and external environment. Rice seeds are more sensitive to pollutants, so the growth of shoots and roots will be affected. Two kinds of rice were grown in contaminated soil, the results are shown in Table 1.

With the increasing of the concentrations of DP, TBBPA and Cd in the soil, the germination rate, germination index and vigor index showed decreasing trend. There was no significant difference in germination rate of Number 1 rice between 5 and 25 mg kg^{-1} DP treatments ($p > 0.05$), but there was significant difference in germination rate of Number 1 rice among other treatments. The germination rate and vigor index of Number 1 rice with 5 mg kg^{-1} DP treatment decreased 21.05% and 42.38% compared with the control, respectively. There was no significant difference in germination rate of Number 7 rice among 5, 25 and 50 mg kg^{-1} DP treatments ($p > 0.05$), but there was significant difference compared with the control. Germination rate and vigor index of Number 7 rice under 5 mg kg^{-1} DP treatment decreased 10.78% and 19.09%, respectively compared with the control, it indicated that the germination of Number 7 rice was more sensitive to DP than Number 1 rice.

There was no significant difference in germination rate of Number 1 rice between 10 mg kg^{-1} TBBPA treatment and the control, vigor index decreased by 12.72%. The vigor index decreased 30.89% and 45.80% compared with the control in the treatments of 50 and 100 mg kg^{-1} TBBPA. However, the germination rate and vigor index of Number 7 rice decreased by 32.31% and 43.80% in the treatment 10 mg kg^{-1} TBBPA comparing with the control, respectively, indicating that the germination of Number 7 rice was more sensitive to TBBPA than Number 1 rice.

TABLE 1 Effects of halogenated flame retardants and Cd on germination of two types of rice seed.

Treatment	Number 1			Number 7		
	Germination rate (%)	Germination index (%)	Vitality index (%)	Germination rate (%)	Germination index (%)	Vitality index (%)
CK	76.00 ± 6.99a	7.60 ± 0.70a	54.15 ± 4.98a	86.67 ± 7.70a	8.67 ± 0.76a	57.41 ± 5.10a
DP5	60.00 ± 3.85b	6.00 ± 0.38b	31.20 ± 2.00c	77.33 ± 2.11b	7.73 ± 0.21b	46.45 ± 1.27b
DP25	57.33 ± 2.11b	5.73 ± 0.21b	34.18 ± 1.26b	74.67 ± 3.22b	7.47 ± 0.32b	44.68 ± 1.93b
DP50	41.33 ± 1.72c	4.13 ± 0.17c	25.55 ± 1.06d	74.67 ± 3.22b	7.47 ± 0.32b	35.40 ± 1.53c
TBBPA10	74.67 ± 5.02a	7.47 ± 0.50a	47.26 ± 3.18b	58.67 ± 4.22b	5.87 ± 0.42b	32.27 ± 2.32b
TBBPA50	69.33 ± 3.44b	6.93 ± 0.34b	37.42 ± 1.86c	54.67 ± 3.22bc	5.47 ± 0.32c	29.84 ± 1.76c
TBBPA100	66.67 ± 7.20b	6.67 ± 0.72b	29.35 ± 3.17d	49.33 ± 6.44c	4.93 ± 0.64d	11.57 ± 1.51d
Cd10	76.00 ± 4.71a	8.00 ± 0.47a	38.12 ± 1.89b	66.67 ± 5.44b	6.67 ± 0.54b	28.21 ± 2.30b
Cd25	76.00 ± 4.71a	8.00 ± 0.47a	35.19 ± 2.25bc	48.00 ± 1.66c	4.80 ± 0.97c	15.01 ± 3.30c
Cd50	68.00 ± 9.58b	6.80 ± 0.96b	32.44 ± 4.99c	48.00 ± 1.72c	4.80 ± 0.17c	11.01 ± 0.40d
Combined pollution-1	64.00 ± 12.35b	6.40 ± 1.24b	33.69 ± 6.50b	53.33 ± 2.72b	5.33 ± 0.27b	33.72 ± 1.72b
Combined pollution-2	58.67 ± 1.72c	5.87 ± 0.17c	24.22 ± 0.71c	50.67 ± 3.44bc	5.07 ± 0.34bc	30.12 ± 2.05c
Combined pollution-3	16.00 ± 5.16d	1.60 ± 0.52d	6.36 ± 2.05d	46.67 ± 2.72d	4.67 ± 0.27d	13.94 ± 0.81d

Note: Mean is expressed as $\bar{x} \pm sd$; different letters (a, b, c and d) mean significant differences in the comparison among different treatments ($p < 0.05$); on the contrary, having no significant differences ($p > 0.05$).

Compared with the control, there was no significant difference for the germination rate of Number 1 rice in the treatments of 10 and 25 mg kg^{-1} Cd, but the vigor index decreased by 29.60% and 35.01%, respectively. There was significant difference in the germination rate of Number 7 rice, and its vigor index decreased by 50.86% and 73.85%, respectively, indicating that the germination of Number 7 rice was more sensitive to Cd than Number 1 rice. Compared with the control, there was significant difference in the germination rate and vigor index of the two kinds of rice under the three combined pollution treatments ($p < 0.05$), it indicated that the combined pollution had greater effect on rice germination than single pollution. In addition, the germination rate, germination index and vigor index of No. 1 rice were significantly different among the three combined treatments ($p < 0.05$), and the effect on germination was the most serious under the combined pollution-3 treatment. The germination rate and germination index of No. 7 rice were not significantly different ($p > 0.05$) between combined pollution-1 treatment and combined pollution-2 treatment, but they were significantly different from combined pollution-3 treatment ($p < 0.05$).

3.1.2 Effect of halogen flame retardants and cadmium on the root and shoot length of rice

The shoot and root length of Number 1 and Number 7 was shown in Figures 2A,B. In this study, shoot and root length of Number 1 and Number 7 were negatively correlated with DP concentration in soil, and the root and shoot length of the two kinds of rice were significantly inhibited by DP. Under 50 mg kg^{-1} DP treatment, the inhibition rates of shoot and root length of Number 1 rice were 38.22% and 64.02%, respectively, while Number 7 rice were 28.42% and 32.00%, respectively, it indicated that the roots were more sensitive to DP toxicity than the shoots, and Number 1 rice was more sensitive to DP than Number 7 rice.

The inhibition of shoot and root length of rice by TBBPA was shown in Figures 2C,D. There was no significant difference in the root length of Number 1 rice among different TBBPA concentration treatments ($p > 0.05$), and the inhibition rates ranged from 47.76% to 50.71%, the inhibition rate of Number 1 rice shoot was highest in the treatment with the 10 mg kg^{-1}, it was 43.64%. On the contrary, the inhibition rate of root and shoot length of Number 7 rice increased with the increasing of TBBPA concentration. The inhibition rates of shoot and root length of Number 1 rice were 26.86% and 50.72% in the treatment with 100 mg kg^{-1}, and those of Number 7 rice were 64.60% and 53.49%, respectively. TBBPA with low concentration had stronger inhibitory effect on rice roots (≤50 mg kg^{-1}), it is suggested that TBBPA with low concentrations in soil had higher bioavailability.

In this study, there was significant difference ($p < 0.05$) of root and shoot length between Number 1 and Number 7 rice (Figures 2E,F). The shoot length of Number 7 rice decreased with the increasing of Cd concentration in soil, and there was good dose-effect relationship between shoot length and Cd concentration. The inhibition rate of shoot length of Number 1 and Number 7 rice was 13.26% and 65.23% in the treatment with 50 mg kg^{-1}, indicating that Number 7 rice was more seriously affected by Cd. There was no significant difference in the root length of Number 1 rice among different treatments, but there was significant difference in the Number 7 rice between the treatment with 10 mg kg^{-1} and 50 mg kg^{-1}, it indicated that Number 7 rice was more sensitive to Cd than Number 1 rice.

The difference of combined pollution of DP, TBBPA and Cd on root and shoot length of rice was demonstrated in Figures 2G,H. The inhibition rates of shoot and root length were 26.13%–44.23% and 46.48%–67.59% for Number 1 rice, 4.56%–54.91% and 33.79%–45.74% for Number 7 rice, respectively. Compared with Cd treatment with 25 mg kg^{-1}, the combined pollution with the same Cd concentration had greater effect on the shoot and root length of two kinds of rice, and it significantly inhibited the shoot and root length ($p < 0.05$), the inhibition effect for root and shoot length in the treatment with 50DP + 10TBBPA + 25Cd was the greatest between the single pollutant and the combined pollution treatments. There was no significant difference in the root and shoot length of rice 1 between combined pollution-2 treatment and combined pollution-3 treatment, but they were significantly different from combined pollution-1 treatment ($p < 0.05$). However, the shoot length of No. 7 rice was not significantly different between combined pollution-1 treatment and combined pollution-2 treatment, but they were significantly different from combined pollution-3 treatment ($p < 0.05$). In addition, the root and bud length of No. 7 rice was slightly longer than that of No. 1 rice in the three combined pollution treatments, indicating that the influence of No. 1 rice was greater.

3.1.3 Effect of on the physiological and biochemical properties of tender shoot of rice germination

The effects of flame retardants and Cd on vigor of seed root system of rice germination was shown in Figure 3A. Vigor of seed root system determines the activity intensity of seeds in the germination, and it reflects the ability of roots to absorb nutrients and water. At the initial stage of tender shoot of rice germination, the vigor of seed root system of two kinds of rice seeds was significantly increased by the stimulation of pollutants, and there was significant difference among different treatments ($p < 0.05$). The vigor of seed root system of Number 1 rice was increased by 1.23, 1.04, 1.39, 1.80, 1.59 and 2.04 times in the treatments with DP, TBBPA, Cd and the combined pollution, respectively, compared with the control; the vigor of seed root system of Number 7 rice increased by 1.20, 1.30, 2.07, 2.31, 2.17 and 2.35 times, respectively. The vigor of seed root system of the two kinds of rice in the combined pollution treatments was significantly

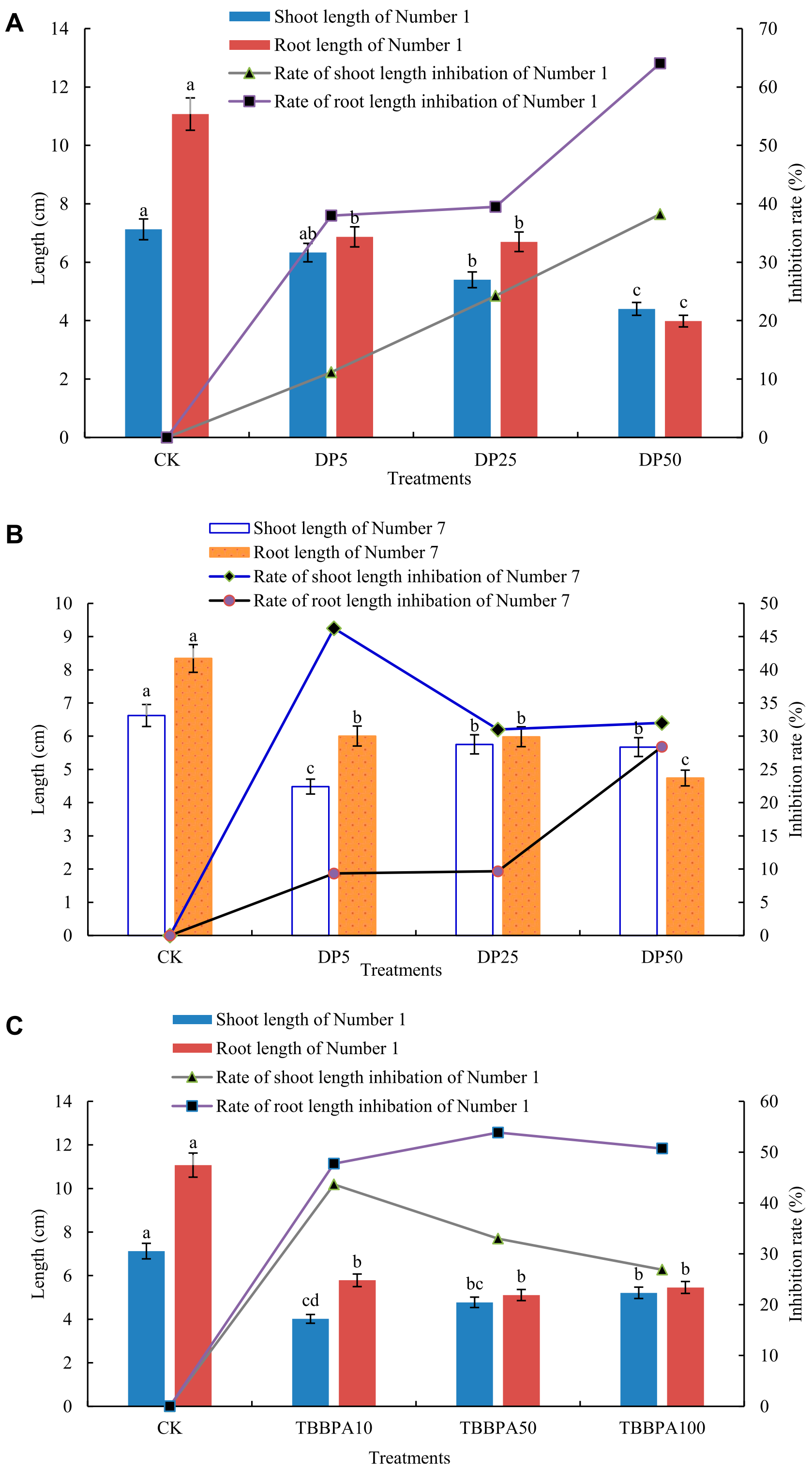


FIGURE 2
Effects of single and combined pollution of TBBPA, DP and Cd in the soil on root and shoot elongation of two rice varieties. **(A)** Effects on Number 1 rice under DP treatment. **(B)** Effects on Number 7 rice under DP treatment. **(C)** Effects on Number 1 rice under TBBPA treatment. **(D)** Effects on Number 7 rice under TBBPA treatment. **(E)** Effects on Number 1 rice under Cd treatment. **(F)** Effects on Number 7 rice under Cd treatment. **(G)** Effects on Number 1 rice under combined pollution treatment. **(H)** Effects on Number 7 rice under combined pollution treatment.

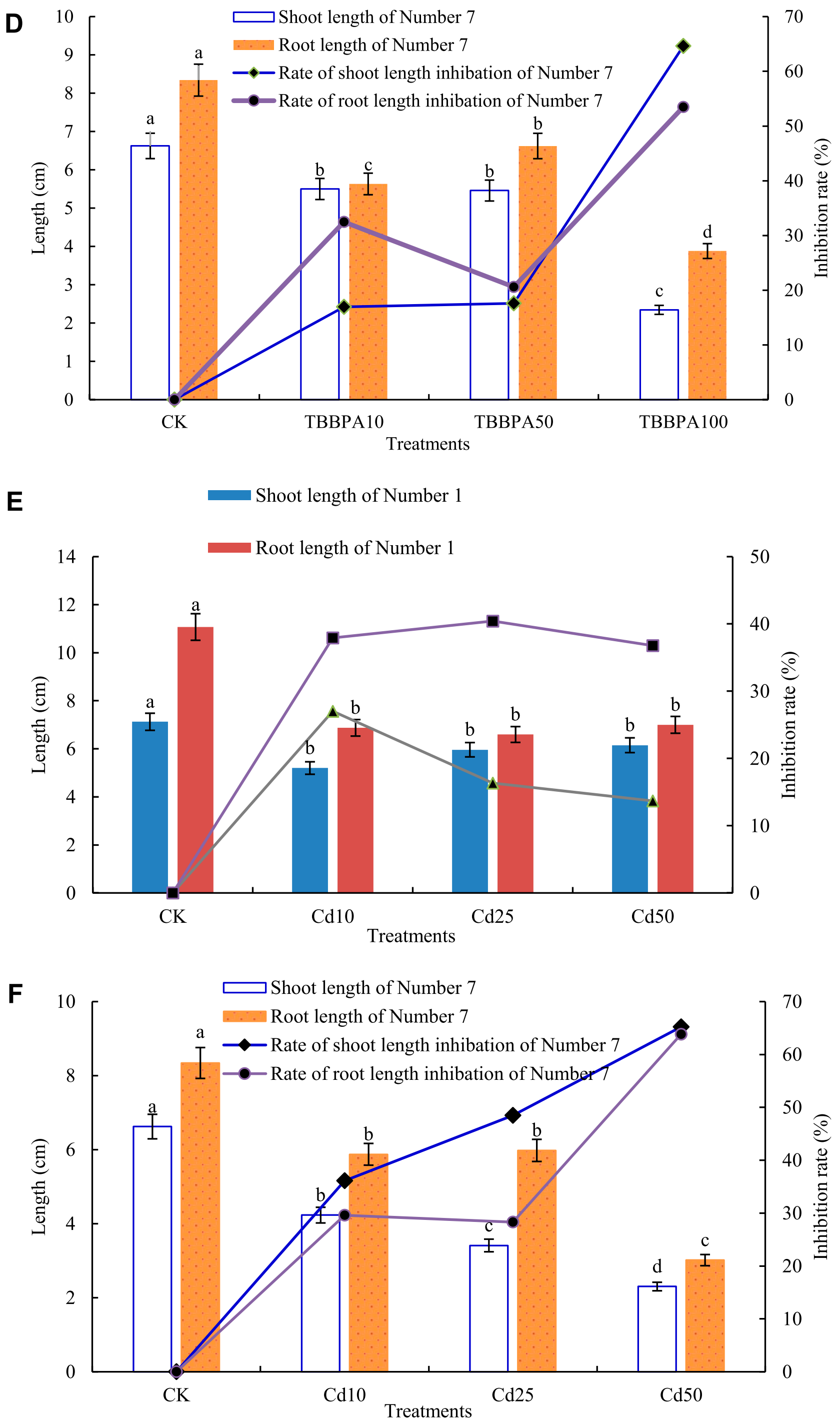


FIGURE 2
(Continued).

higher than single pollution treatments ($p < 0.05$). In conclusion, the halogenated flame retardants and Cd in soil significantly promoted the vigor of seed root system, the main reason is that the pollutants stimulated the synthesis of substances in the root system and enhanced the root metabolism. It is a kind of stress resistance mechanism for the tender shoot of rice germination to the stress of halogenated flame retardants and Cd.

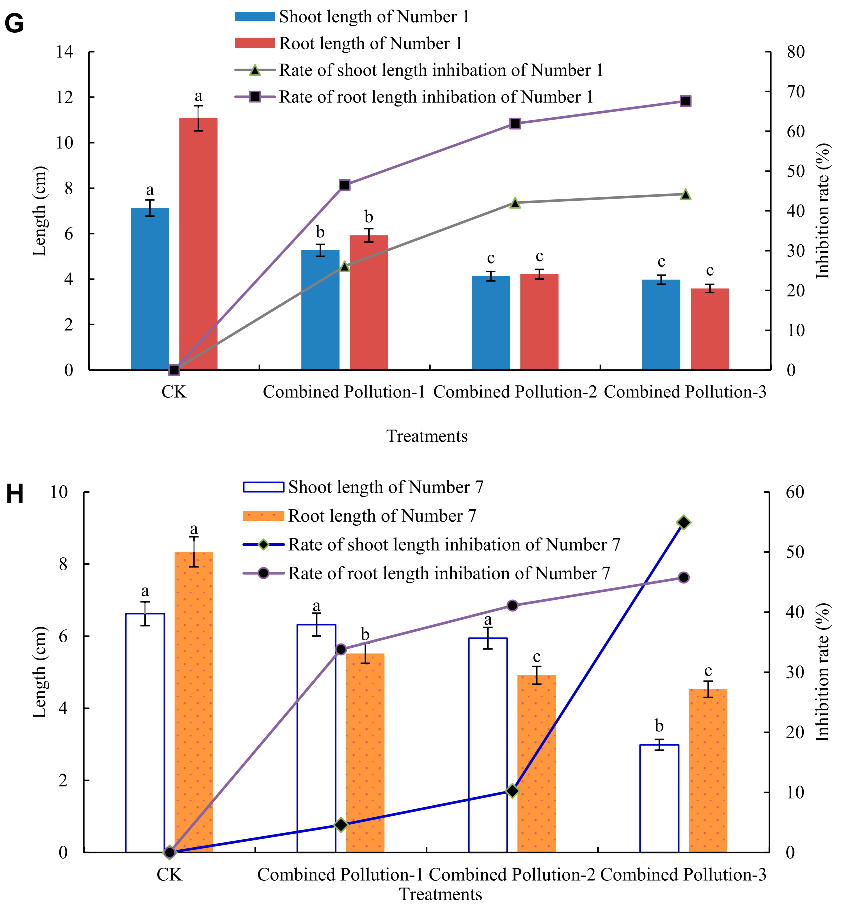


FIGURE 2
(Continued).

The proline content in two kinds of rice under different pollutant stress was shown in Figure 3B. Proline is the protective substance of plant cell membrane and enzyme, which has the functions of scavenging reactive oxygen species, regulating cytoplasmic osmotic potential and maintaining protein stability. Under the stress of pollution, proline accumulates rapidly in plants, which improves the stress resistance of plants. Compared with the control, the proline content of Number 1 rice was increased by 0.46, 1.24, 0.64, 1.80, 1.12 and 3.93 times, and that of Number 7 rice was increased by 1.08, 0.78, 0.24, 1.71, 1.13 and 2.10 times in the treatments with DP, TBBPA, Cd and combined pollution, respectively. Under the treatments of combined pollution 1 and combined pollution 3, the proline content of two kinds of rice was significantly higher than that of single pollution ($p < 0.05$), indicating that the combined pollution treatments had stronger toxicity effect on rice, and rice produced more proline to resist the toxicity of pollutants. Therefore, the accumulation of proline can reflect the stress degree of pollutants during rice germination.

The effects of flame retardants and Cd on protein content was shown in Figure 3C. Compared with the control, the protein content of Number 1 rice in the treatment with Cd decreased significantly ($p < 0.05$), the inhibition rate was 19.67%, and there was no significant difference among other treatments. However protein content in Number 7 rice decreased in all treatments compared with the control, and the strongest inhibitory effect was in the treatment of TBBPA and combined pollution 3, with inhibition rates of 59.97% and 54.69%, respectively. Proteins in rice can enhance plant tolerance to pollution stress by inducing antioxidant enzymes, detoxifying enzymes, metabolic enzymes and eliminating plant free radicals. The stress of soil pollutants induced rice to produce more antioxidant enzymes, so protein consumption increased (Lee et al., 2010). The total metabolic level and the degree of plant damage under pollution stress can be indicated by the changes of protein content. The protein content of Number 1 rice did not change significantly in the treatment with TBBPA and various combined pollution, because the antioxidant enzyme system of rice was inhibited and no more soluble protein was needed, indicating that the responses of the

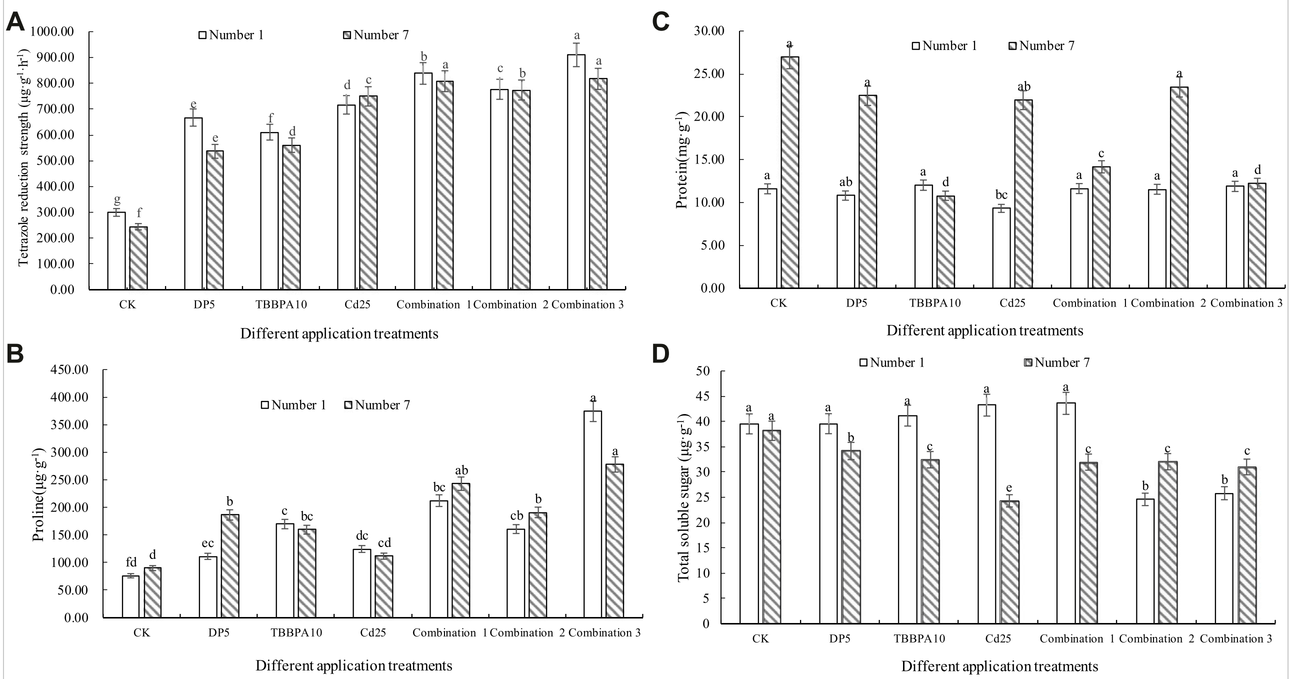


FIGURE 3
Effects of two halogenated flame retardants and cadmium on physiological and biochemical indexes of two kinds of rice seed germination. **(A)** The tetrazole reduction strength ($\mu g \cdot g^{-1} \cdot h^{-1}$) in different application treatments. **(B)** The amount of proline ($\mu g \cdot g^{-1}$) in different application treatments. **(C)** The amount of protein ($mg \cdot g^{-1}$) in different application treatments. **(D)** The amount of Total soluble sugar ($\mu g \cdot g^{-1}$) in different application treatments.

two kinds of rice seedlings to the halogenated flame retardants in soil were obviously different.

The soluble sugar content in two kinds of rice under different pollutant stress was shown in Figure 3D, it is an indicator of plant stress tolerance. The soluble sugar content of Number 1 rice in the treatments with combination 2 and combination 3 was significantly inhibited by 37.80% and 34.72%, respectively, and there was no significant difference between the other treatments and the control. Compared with the control, the soluble sugar content of Number 7 rice was significantly decreased ($p < 0.05$), the strongest inhibitory effect in Cd treatment was showed, and the inhibition rate was 35.42%. The effect of combined pollution on shoot of rice germination was greater than that of single pollution, the strongest toxic effect in the treatment with combined pollution 2 was showed.

3.2 The toxicity of dechlorane plus, tetrabromobisphenol A and cadmium in the soil to rice seedlings

The responses of the two kinds of rice seedlings to DP in soil were obviously different (Figure 4). DP promoted the protein content of rice Number 1 (10.32%), but inhibited the protein content of rice Number 7 (38.51%). The damage degree of plants under pollution stress can be indicated by the change of chlorophyll content in plants. Due to the action of DP, chlorophyll A content in Number 1 rice increased significantly (10.59%), but chlorophyll B content decreased significantly (22.04%). Although total chlorophyll content decreased slightly, there was no significant difference compared with the control ($p > 0.05$). However, the chlorophyll A content, chlorophyll B content and total chlorophyll content of Number 7 rice seedlings were significantly inhibited by DP, with the inhibition rates of 35.77%, 19.37% and 32.26%, respectively. The SOD activity of two kinds of rice seedlings was induced by DP, and the activation rates were 0.94% and 73.14%, respectively, which had significant activation effect on Number 7 rice ($p < 0.05$). And the CAT activity of two kinds of rice seedlings was induced by DP, the activation rates were 6.83% and 1.89 times, respectively, and the CAT activity of Number 7 rice was significantly activated ($p < 0.05$). On the contrary, the POD enzyme activity of Number 1 seedlings in DP treatment was increased (11.50%), but it was inhibited in Number 7 seedling (13.59%). MDA is one of the products of membrane lipid peroxidization, which can reflect the degree of membrane damage. When the oxidation rate of membrane tissue increases, the accumulation of MDA increases, and the protective ability of membrane tissue decreases. Under the pollution stress of the DP, a large amount of MDA were produced in the two kinds of rice seedlings, which was significantly increased by 65.29% and 85.86% (p < 0.05).

The toxicity of TBBPA in soil to two kinds of rice seedlings was obviously different. Protein content in two rice seedlings with TBBPA treatment was inhibited significantly (p < 0.05), the inhibition rates were 17.36% and 41.48%, respectively. On the contrary, SOD activity in two rice seedlings was activated significantly (p < 0.05), and the activation rates were 29.49% and 63.77%, respectively. The CAT activities was inhibited significantly in Number 1 rice (17.89%), but activated in Number 7 rice (145%). The POD activities was activated significantly in Number 1 rice (46.04%), but inhibited

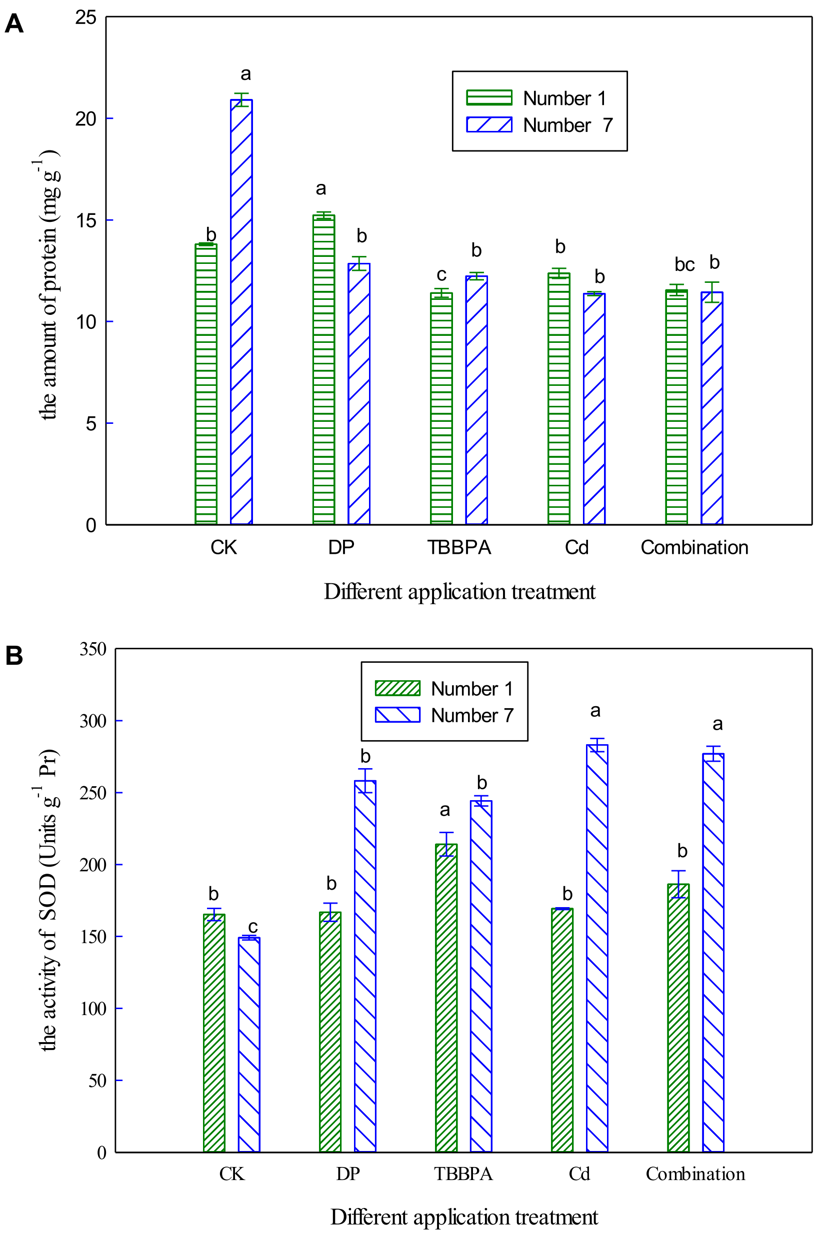


FIGURE 4
Phytotoxicity of two halogenated flame retardants and cadmium in the soil on the seedlings of two rice varieties. **(A)** The amount of protein ($mg \cdot g^{-1}$) of two rice varieties in different application treatments. **(B)** The activity of SOD ($Units \cdot g^{-1}Pr$) of two rice varieties in different application treatments. **(C)** The activity of CAT ($Units \cdot mg^{-1}Pr$) of two rice varieties in different application treatments. **(D)** The activity of POD ($U \cdot g^{-1} \cdot min^{-1}Pr$) of two rice varieties in different application treatments. **(E)** The amount of MDA ($\mu mol \cdot g^{-1}Pr$) of two rice varieties in different application treatments. **(F)** The amount of Chlorophyll a ($\mu g \cdot g^{-1}$) of two rice varieties in different application treatments. **(G)** The amount of Chlorophyll b ($\mu g \cdot g^{-1}$) of two rice varieties in different application treatments. **(H)** The amount of Chlorophyll ($\mu g \cdot g^{-1}$) of two rice varieties in different application treatments.

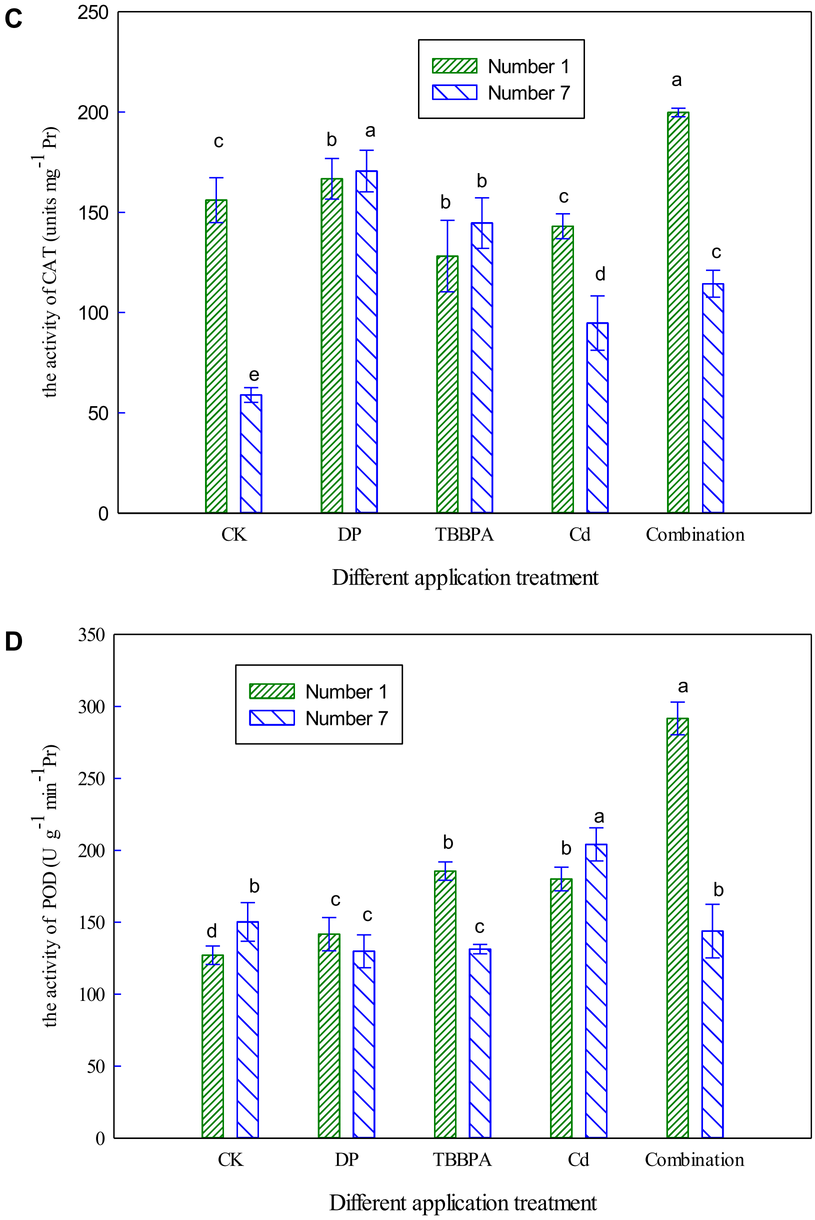


FIGURE 4
(Continued).

significantly in Number 7 rice (12.58%). Under the pollution stress of TBBPA, a large amount of MDA were produced in two kinds of rice seedlings, which was significantly increased by 29.9% and 77.85% compared with the control ($p < 0.05$). Chlorophyll A production was inhibited by 15.27% and 43.89% in TBBPA treatment, respectively; while chlorophyll B production was promoted by 24.48% and 8.49%, respectively. It had little effect on chlorophyll content of Number 1 rice seedlings in TBBPA treatment, but chlorophyll content of Number 7 rice seedlings was inhibited significantly ($p < 0.05$).

Cd has great toxicity to two rice seedlings varieties, and the degree of influence varies with different varieties, Number 7 rice is more sensitive to Cd than Number 1. Protein content in rice seedlings was inhibited significantly ($p < 0.05$), and the inhibition rates were 10.43% and 45.62%, respectively. The SOD activity of Number 7 rice seedling was significantly higher than that of the control, increasing by 89.77%. Compared with the control, there was no significant difference in the CAT activity of Number 1 rice seedling in Cd treatment, but CAT activity of Number 7 rice seedling increased by 60.85%. The POD activity of the two kinds

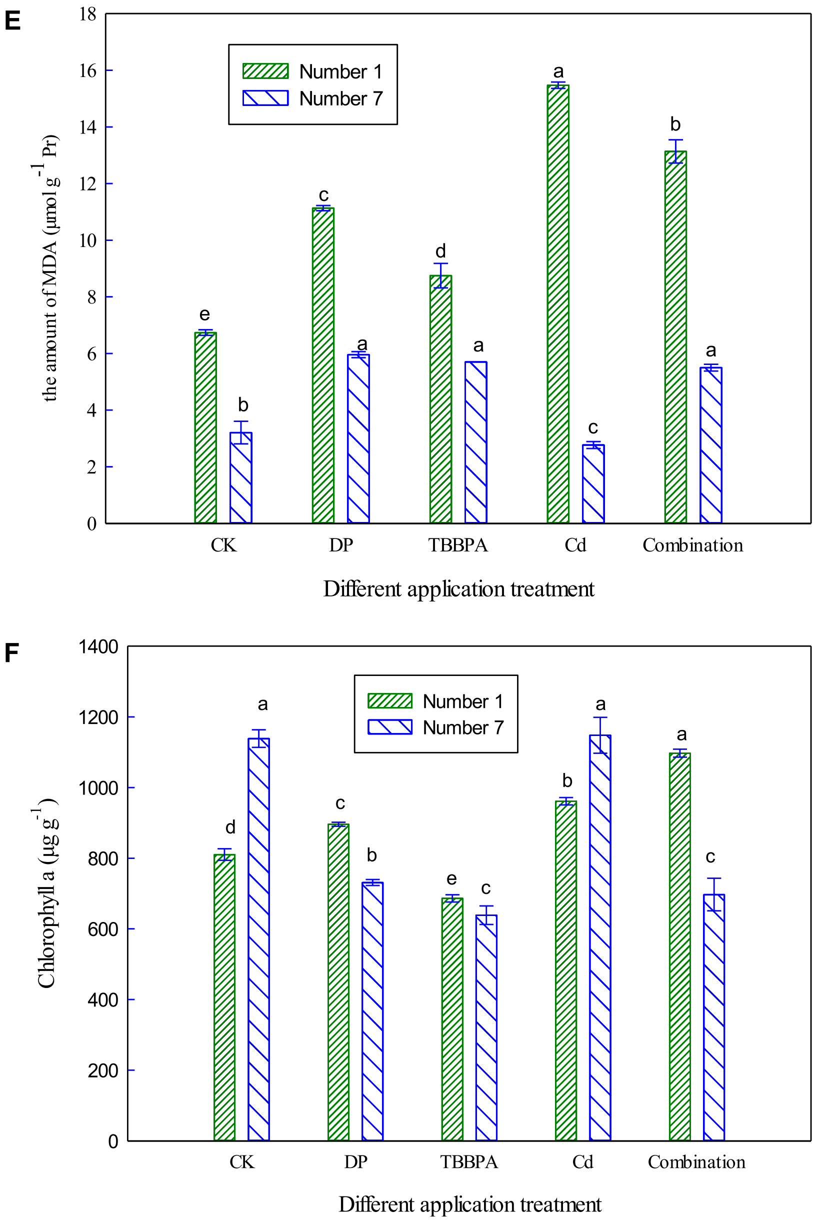


FIGURE 4
(Continued).

of rice seedlings in Cd treatment was significantly higher than that of the control in order to remove reactive oxygen species *in vivo* ($p < 0.05$), the activation rates were 41.72% and 35.90%, respectively. The MDA content of the two kinds of rice seedlings was significantly higher than that of the control, and the induction rate was 129.53% and 143.53%, respectively. A large amount of MDA were accumulated in the two kinds of rice seedlings, indicating that Cd was more toxic to rice seedlings. Chlorophyll A content and total chlorophyll content in Number 1 rice in Cd treatment were increased by 18.64% and 5.34%, respectively, but chlorophyll B content was inhibited by 13.55%. However, the contents of chlorophyll A, chlorophyll B and total chlorophyll in Number 7 rice in Cd treatment increased by 0.82%, 14.31% and 3.70%, respectively.

The effects of combined pollution on the two kinds of rice were significantly different. The inhibition rates of protein content in two kinds of rice seedlings were 16.29% and 45.29%, respectively ($p < 0.05$); but SOD activity increased significantly ($p < 0.05$), the activation rates were 12.71% and 85.73%, respectively; and the activation rates of CAT were

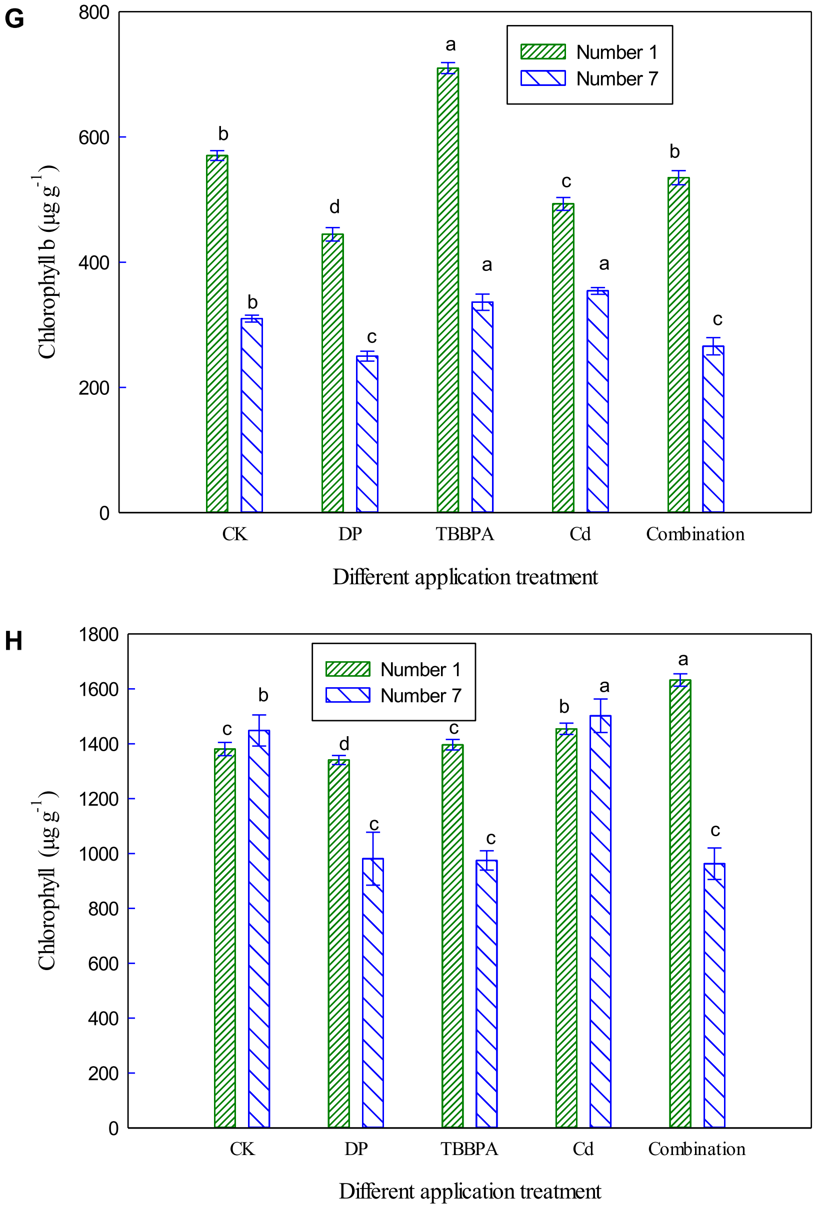


FIGURE 4
(Continued).

28.01% and 94.20% ($p < 0.05$). The POD activity of Number 1 rice was activated (129.49%), but there was no significant difference between combined pollution and the control in Number 7 rice seeding ($p > 0.05$). The MDA content of two kinds of rice was significantly increased by 95.02% and 71.58%, respectively, which was significantly different from that of single pollution ($p < 0.05$). Under combined treatment, chlorophyll A content of Number 1 rice seedling increased by 35.14%, chlorophyll B content decreased by 6.19%, and total chlorophyll content increased by 18.22%; and the content of chlorophyll A, chlorophyll B and total chlorophyll of Number 7 rice seedling decreased by 38.75%, 14.24% and 33.51%, respectively.

3.3 Bioaccumulation of two flame retardants and cadmium in rice seedlings

In the treatment of 5 $mg\,kg^{-1}$ DP, bioaccumulation and biological transport of DP in rice seedlings were shown in Table 2. Bioaccumulation factors of syn-DP and anti-DP were 0.040–0.042 and 0.24–0.25, transport coefficients of syn-DP and anti-DP were 0.39–0.41 and 0.46–0.48. The bioaccumulation capacities of syn-DP and anti-DP in roots were significantly higher than those in shoot during the growth stage of the rice seedlings, but there was no significant difference between Number 1 rice and Number 7 rice ($p > 0.05$).

In the treatment of 10 mg kg^{-1} TBBPA, residual concentrations of TBBPA and its metabolites in rice seedlings were shown in Table 3. TBBPA and its metabolites are more likely to be bioaccumulated in Number 1 rice seedlings, the accumulation amounts of TBBPA and BPA are more, and Tri-BBPA is relatively the lowest, which corresponds to the changes in concentrations of TBBPA and its metabolites in soil. In the soil, TBBPA debrominated to Tri-BBPA, Tri-BBPA is unstable, and it continues to debrominated to Di-BBPA and more stable product BPA, and then BPA continues to degrade into other substances. The accumulation of TBBPA and its metabolites in roots was much more than that in shoot, it shows that TBBPA and its metabolites have a small upward transport effect, rice seedlings were more likely to accumulate the pollutant under the treatment of combined pollution.

In the treatment of 25 mg kg^{-1} Cd, there was significant difference in the response level of the two kinds of rice to cadmium. Bioconcentration factor of Cd in roots of Number 1 and Number 7 rice was 9.39 and 6.84, and transport coefficient was 0.071 and 0.087, respectively. Bioconcentration factor of Cd were 14.20 and 6.59 in roots, and transport coefficient was 0.034 and 0.038 in the treatment of combined pollution, respectively. Two kinds of rice had a certain bioaccumulation for Cd, and the bioaccumulation ability of Number 1 rice was greater than Number 7 rice; there was no significant difference in transport coefficient between two kinds of rice. After combined action with halogenated flame retardants, the bioaccumulation ability of rice Number 1 was enhanced, while rice Number 7 was weakened.

4 Discussion

4.1 Effects of flame retardants and cadmium on plant seed germination

In this study, the effects of single pollution of DP, TBBPA and Cd and combined pollution of flame retardants and Cd on the growth of two kinds of rice were analyzed mainly from seed germination and root length and shoot length, and the effects of pollution stress on seed germination were analyzed by germination rate, germination index and vigor index.

DP significantly inhibited seed germination rate, root length and shoot length of the two kinds of rice, and a dose-response relationship was showed, which might be because oxidative stress in rice was induced in DP treatment and cell division and growth was inhibited. The reports showed that TBBPA significantly inhibited the germination rate and root elongation of soybean, chickpea and maize seeds, and the inhibition increased with the increase of TBBPA concentration (Dogan et al., 2010; Ge and

TABLE 2 Bioaccumulation and biological transport of DP in rice seedlings.

Rice type	Parts	Treatment	Pollutants	Residual concentrations	Bioaccumulation factor	Transport coefficient
Number 1 rice	Root	DP	Syn-DP	52.58 ± 1.71	0.042	—
			Anti-DP	95.18 ± 2.78	0.025	
		Combined pollution	Syn-DP	50.19 ± 2.05	0.040	
			Anti-DP	92.67 ± 2.65	0.025	
	Shoot	DP	Syn-DP	21.46 ± 0.29	—	0.41
			Anti-DP	45.25 ± 1.25		0.48
		Combined pollution	Syn-DP	19.85 ± 0.32		0.39
			Anti-DP	43.56 ± 1.28		0.47
Number 7 rice	Root	DP	Syn-DP	50.24 ± 1.06	0.040	—
			Anti-DP	93.18 ± 1.42	0.025	
		Combined pollution	Syn-DP	51.05 ± 2.08	0.041	
			Anti-DP	91.43 ± 1.98	0.024	
	Shoot	DP	Syn-DP	19.89 ± 0.25	—	0.39
			Anti-DP	43.29 ± 1.29		0.46
		Combined pollution	Syn-DP	20.85 ± 0.27		0.41
			Anti-DP	42.89 ± 1.06		0.47

Note: Results are expressed as $\bar{x} \pm sd$, the unit of DP residual amount is ng/g.

TABLE 3 Residual concentrations and bioaccumulation factor of TBBPA and its metabolites in rice seedlings.

Rice type	Parts	Treatment	Pollutants	Residual concentrations	Bioaccumulation factor
Number 1 rice	Root	TBBPA	TBBPA	1207.66 ± 18.62	7.96
			Tri-BBPA	29.08 ± 0.56	0.076
			Di-BBPA	178.69 ± 1.25	0.47
			Mono-BBPA	249.65 ± 3.68	0.65
			BPA	364.37 ± 4.89	0.95
		Combined pollution	TBBPA	1676.03 ± 22.56	8.53
			Tri-BBPA	32.56 ± 2.12	0.078
			Di-BBPA	180.03 ± 8.12	0.43
			Mono-BBPA	247.89 ± 9.62	0.59
			BPA	360.56 ± 10.12	0.86
	Shoot	TBBPA	TBBPA	81.63 ± 2.56	—
			Tri-BBPA	5.74 ± 0.09	
			Di-BBPA	39.73 ± 0.56	
			Mono-BBPA	99.69 ± 0.58	
			BPA	101.06 ± 1.25	
		Combined pollution	TBBPA	610.05 ± 3.68	
			Tri-BBPA	35.16 ± 0.28	
			Di-BBPA	160.20 ± 2.35	
			Mono-BBPA	427.84 ± 2.76	
			BPA	320.36 ± 2.26	
Number 7 rice	Root	TBBPA	TBBPA	674.25 ± 7.35	2.80
			Tri-BBPA	17.06 ± 0.68	0.027
			Di-BBPA	225.11 ± 5.89	0.36
			Mono-BBPA	171.13 ± 3.56	0.27
			BPA	278.60 ± 5.52	0.45
		Combined pollution	TBBPA	831.11 ± 8.61	3.44
			Tri-BBPA	18.82 ± 1.62	0.034
			Di-BBPA	229.90 ± 8.68	0.42
			Mono-BBPA	187.42 ± 8.62	0.34
			BPA	280.59 ± 9.68	0.51
	Shoot	TBBPA	TBBPA	53.92 ± 0.45	—
			Tri-BBPA	5.39 ± 0.09	
			Di-BBPA	50.67 ± 0.12	
			Mono-BBPA	85.22 ± 1.06	
			BPA	72.47 ± 0.96	
		Combined pollution	TBBPA	129.91 ± 1.25	
			Tri-BBPA	16.72 ± 0.08	
			Di-BBPA	118.01 ± 1.25	
			Mono-BBPA	152.92 ± 1.36	
			BPA	239.67 ± 1.28	

Note: Results are expressed as $\bar{x}$ ± sd, the unit of residual amount is ng/g.

Zhang, 2017; Wang et al., 2019), the results of this experiment are consistent with them. There was no effect on seed germination and root length of Trifolium pratense in TBBPA treatment, which may be due to species differences in TBBPA stress, and different plants have different responses to TBBPA (Sverdrup et al., 2006).

Under Cd treatments, Number 7 rice was more seriously affected by Cd than Number 1 rice. The above ground part and

TABLE 4 Bioaccumulation and biological transport of cadmium in rice seedlings.

Rice type	Parts	Treatment	Residual concentrations ($mg \cdot kg^{-1}$)	Bioaccumulation factor	Transport coefficient
Number 1 rice	Root	Cd	234.78 ± 5.25	9.39	—
		Combined pollution	355.03 ± 4.78	14.20	
	Shoot	Cd	16.69 ± 1.09	—	0.071
		Combined pollution	12.13 ± 0.78		0.034
Number 7 rice	Root	Cd	171.14 ± 3.25	6.84	—
		Combined pollution	164.75 ± 2.97	6.59	
	Shoot	Cd	14.96 ± 1.06	—	0.087
		Combined pollution	6.32 ± 0.36		0.038

Note: Results are expressed as $\bar{x}$ ± sd, and the unit of Cd residual amount is mg/kg.

root length of the two kinds of rice was significantly inhibited in Cd stress (Dabral et al., 2019), which was consistent with the results of this study. Most plants are sensitive to Cd stress. For example, the root length of pea plants is inhibited in Cd stress (RodrÍGuez-Serrano et al., 2006) and the growth of both root and above ground parts of tobacco plants is decreased (Hui et al., 2015).

Compared with the control, seed germination and root and shoot length were significantly inhibited under the three combined pollution treatments. The results of different proportions of compound pollution showed that DP had a greater inhibitory effect on rice than TBBPA at the same concentration of Cd. Mousavi (Mousavi et al., 2021) studied the effects of single contamination of Cd and combined application of Ethylene Diamine Tetraacetic Acid (EDTA) and Maleic Acid (MA) on the growth of okra. The results showed that Cd inhibited the root length and dry weight of the above ground part of okra, while the tolerance of okra to Cd was improved due to the application of EDTA and MA.

The ability of roots to absorb nutrients and water is reflected by root vigor. Under different treatments, the root vigor of the two kinds of rice was significantly increased and the metabolism was more active. In these treatments, the root vigor of the combined treatment was significantly higher than that of the single pollution, which may be due to the stimulation of the synthesis of substances in the root, which may be a stress resistance mechanism of rice seedlings to flame retardants and heavy metal pollution.

Maintenance of osmotic pressure is the main defense mechanism in response to stress conditions. Osmotic regulators are water-soluble compounds with low molecular weight that help maintain osmotic pressure by maintaining cell water potential under environmental stress, providing protection for macromolecules and enzymes from the destructive effects of reactive oxygen species in oxidative stress (Osman, 2015). The main osmotic regulators include proline, soluble sugar, soluble protein and total flavonoids. In plants, the accumulation of proline can cope with different stresses. Proline not only acts as osmotic regulator, but also acts as reactive oxygen scavengers to improve the stress resistance of plants (Trovato et al., 2008). Soluble sugar and protein, as osmotic regulatory substances, can enhance resistance to the environment by regulating cell permeability and inducing antioxidant enzyme activity under abiotic stress (Cha-um et al., 2009; Naeem et al., 2011). Soluble sugars are also involved in scavenging reactive oxygen species and are part of the defense system that controls the balance of reactive oxygen species (Couée et al., 2006; Moustakas et al., 2011). Soluble protein not only induces the production of antioxidant enzymes in plants, but also induces the production of several scavenging reactive oxygen species as a functional factor (Naeem et al., 2011), and the tolerance of plants to pollution stress can be indicated by the content changes.

In this study, under 5 $mg\ kg^{-1}$ DP treatment, the consistency of the two kinds of rice in resisting pollution stress was also reflected by the increase of proline content. The contents of soluble proteins in rice were inhibited, and the proteins in rice could improve the tolerance of plants to environmental stress by inducing the production of antioxidant enzymes, detoxifying enzymes, metabolic enzymes and functional factors that eliminate free radicals (Naeem et al., 2011). More antioxidant enzymes in rice are induced to product by stress from soil pollutants, leading to increased protein consumption. However, the soluble sugar content of Number 7 rice decreased significantly, and no significant change was observed in Number 1 rice.

The soluble sugar and protein contents of soybean seedlings were reduced in high concentrations of TBBPA, and the stress resistance was weakened (Ge and Liu, 2020), which was consistent with the physiological response of soybean to TBBPA stress (Ge and Zhang, 2017). The response of Number 7 rice was consistent with the above results, while no significant change was observed in Number 1 rice, which may be due to inhibition of antioxidant enzyme system and no need for more soluble protein.

The cell osmotic potential of all types of rice was maintained by accumulating different amounts of proline (Yang et al., 2021), but their sensitivity to Cd were different. The results of this experiment showed that under the stress of Cd, the vigor of seed root system was significantly increased, a large amount of proline was produced, and a large amount of Cd was also bio accumulated in rice. Under the stress of pollution, soluble sugar accumulation was promoted to maintain the osmotic potential of cells and maintain the water required for normal growth, thus enhancing the tolerance to Cd (Deng and Cao, 2016). Under the effect of less toxic pollution, soluble sugar in rice can be produced to improve its stress resistance, but with the aggravation of toxic degree, rice's ability to synthesize soluble sugar is inhibited. The ability of proteolytic enzymes was promoted to hydrolyze existing proteins or have toxic effects on the organelles associated with protein synthesis was affected to inhibit soluble protein content by the toxicity of Cd (Hall, 2002; John et al., 2009). There was no significant difference in soluble sugar content between the two kinds of rice of the control. However, under Cd treatment, the soluble sugar content in Number 7 rice was significantly inhibited, while the soluble sugar content in Number 1 rice had no significant change, indicating that Number 1 rice had stronger tolerance to Cd.

The contents of soluble sugar and soluble protein of the two kinds of rice decreased significantly in the combined pollution treatment, and the inhibition degree was significantly greater than that of single pollution, indicating that the toxicity of rice in the combined pollution treatment was larger.

4.2 Toxic effects of flame retardants and cadmium on plants

Plants normally produce reactive oxygen species (ROS), but excess ROS are produced under different levels of biological and abiotic stress, such as hydroxyl radical (·OH), superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) (Amari et al., 2017). High levels of ROS can lead to decreased photosynthetic activity (Shahid et al., 2012), oxidative damage and lipid peroxidization (Jumrani and Bhatia, 2019). Under environmental stress, plants can be induced to product a variety of proteins, including antioxidant enzymes, SOD, POD and CAT (Song et al., 2012), which maintain the balance of ROS in plants. Cui (Cui et al., 2015) believed that the ability to remove intracellular ROS is the result of plant evolution triggered by environmental stress.

SOD is the first line of defense to remove ROS. It mainly removes O^{2-} from the endogenous antioxidant system of plants and converts them into H_2O_2 and O_2 through enzymatic reactions (Kováčik et al., 2020). Since H_2O_2 is highly hazardous, CAT and POD are required to further decompose it into H_2O and O_2 (Ahmad et al., 2008). However, their reaction mechanism is different. POD has a higher affinity with H_2O_2 than CAT, and can participate in the biosynthesis of lignin to convert H_2O_2 into H_2O. CAT can remove H_2O_2 by dismutation without substrate, but the effect is obvious when H_2O_2 concentration is high (Li et al., 2008). MDA is a sensitive biological indicator of oxidative damage in plant cells (Kováčik et al., 2020). The accumulation of H_2O_2 and O^{2-} can lead to lipid peroxidization, while the content of MDA can indicate the degree of lipid peroxidization (Siddiqui et al., 2019).

In this study, the antioxidant enzyme activities of two kinds of rice were affected by DP. The POD activity of Number 1 rice was activated and that of Number 7 rice was inhibited in DP treatment. The decrease of POD activity would weaken the ability of scavenging ROS, but SOD and CAT kept relatively high activities in Number 7 rice, indicating that SOD and CAT mainly were used in Number 7 rice as the main enzyme system to cope with oxidative damage. Studies have reported that SOD and CAT activities of large marine juvenile algae can be activated by DP, suggesting that DP can induce oxidative stress of algae. When the exposure concentration of DP is too high, SOD cannot remove the excessive ROS in time, leading to the increase of MDA content and lipid peroxidization (Gong et al., 2018).

The activity of antioxidant enzymes and MDA content of maize were increased significantly in TBBPA stress (Wang et al., 2019), but the antioxidant enzyme activity of soybean increased firstly and then decreased with the increase of TBBPA concentration (Ge and Zhang, 2017). The reason is that a large number of ROS were produced in TBBPA stress, which activated the activities of SOD, CAT and POD to remove excessive ROS and improved the stress resistance of plants. However, when the concentration of TBBPA exceeds the tolerance threshold of plants, excessive ROS cannot be removed, resulting in the decrease of antioxidant enzyme activity. While the content of MDA increased with the increase of TBBPA concentration, indicating that lipid peroxidization was positively correlated with the concentration.

In this experiment, SOD activity of two kinds of rice was activated, CAT activity was inhibited significantly and POD activity was activated of Number 1 rice was activated significantly, but CAT activity was activated and POD activity was inhibited of Number 7 rice in TBBPA treatment. These changes reflected the complex interactions between plant antioxidant enzymes, indicating that the response of the two kinds of rice to TBBPA pollution stress was different. Both kinds of rice produce a large amount of MDA, indicating that TBBPA can cause lipid peroxidization in rice and damage the cell membrane to a certain extent. Kovačik (Kováčik et al., 2020) revealed that TBBPA can cause oxidative stress in mangroves through the formation of MDA.

The sensitivity of the two kinds of rice species to Cd was different, and the tolerance of Number 7 rice to Cd was stronger than that of Number 1 rice. SOD, CAT and POD contents were activated significantly in Number 7 rice by Cd, while MDA content did not change significantly, which was consistent with the results of the study on the adaptive response mechanism of onion and pea seedlings induced by Cd stress (Chakrabarti and Mukherjee, 2021). These changes indicate that plants produce excessive ROS under Cd stress, and the activity of antioxidant enzymes is activated to remove excess ROS. The POD activity of Number 1 rice was significantly increased, while the activities of SOD and CAT were not significantly changed, and the content of MDA was significantly higher than that of the control group. Excessive MDA reflected the large toxic effect of Cd on Number 1 rice, indicating that the concentration of 25 mg kg^{-1} Cd exceeded the tolerance threshold of Number 1 rice to Cd, leading to the decrease of SOD and CAT activities, and the production of large amounts of MDA. It has been reported that other Cd-tolerant plants such as mulberry, brassica juncea and sedum alfredii hance can cope with Cd pollution by regulating the activities of antioxidant enzymes (Liu et al., 2019b; Du et al., 2020; Zaiwang Zhang et al., 2020).

The combined pollution had significant effect on both kinds of rice. The activities of SOD and CAT in both kinds of rice were activated significantly, and the POD activity was activated of Number 1 rice, but no significant difference was found in POD activity of Number 7 rice ($p > 0.05$). In addition, MDA content in both kinds of rice was significantly increased compared with that of single pollution ($p < 0.05$). There were few studies on the combined pollution effects of flame retardants and Cd, but Kovačik et al. (2020) studied the combined effects of glyphosate and Cd on matricaria recutita. The effects of combined pollution on plants were reflected by the contents of antioxidant enzymes (SOD, CAT and APX), and the results showed that Cd had significant toxic effects and the combined effect of Cd and glyphosate on root non-protein mercaptan was synergistic.

4.3 Effects of flame retardants and cadmium on plant chlorophyll

Chlorophyll content is a vital index to observe the damage of pollution stress on plant growth and development (Kováčik et al., 2018). Chlorophyll is an important pigment for plant photosynthesis. Chlorophyll A and chlorophyll B can capture light energy and convert it into chemical energy (Liang et al., 2019). Therefore, the contents of chlorophyll A and chlorophyll B are critical characteristics for monitoring plant tolerance to abiotic stress (Chakhchar et al., 2017). It was reported that the decrease of chlorophyll content and photosynthetic efficiency of algae may be caused by oxidative damage of photosynthetic membrane in DP stress (Gong et al., 2018). Under 5 mg kg^{-1} DP treatment, the chlorophyll A content of Number 1 rice increased, chlorophyll B content decreased, and total chlorophyll content decreased slightly, but there was no significant difference with the control, indicating that DP had little effect on chlorophyll of Number 1 rice. However, the contents of chlorophyll A, chlorophyll B and total chlorophyll in Number 7 rice were significantly inhibited by DP and a large amount of MDA was produced, indicating that the cell membrane of rice was damaged and the content decline of photosynthetic pigment, which also revealed the relationship between the production of excessive ROS and photosynthesis.

In this experiment, under 10 mg kg^{-1} TBBPA treatment, chlorophyll A content of two rice seedlings was inhibited, but chlorophyll B content was promoted. And the total chlorophyll content of Number 1 rice seedling was slightly affected, but the total chlorophyll content Number 7 rice seedling was significantly inhibited. However, the results showed that with the increase of TBBPA concentration, the chlorophyll content of corn, wheat and soybean was significantly inhibited (Wang et al., 2019), which was slightly different from the above results, which might be caused by the difference in tolerance of different species to TBBPA.

The oxidative stress, impaired chlorophyll and impaired nutrient uptake can be caused by the accumulation of Cd in soil (Hafeez et al., 2019; Hussain et al., 2019). A number of studies have shown that chlorophyll content decreased by Cd accumulation in plants such as rice (Pramanik et al., 2018) and corn (Anjum et al., 2016). Yang (Yang et al., 2021) showed that the contents of chlorophyll A, chlorophyll B and total chlorophyll of the two kinds of rice decreased significantly under Cd stress, but there were significant differences in their contents between the two kinds of rice. In this experiment, under 25 mg kg^{-1} Cd treatment, chlorophyll A and total chlorophyll content of Number 1 rice were significantly increased, but chlorophyll B content was inhibited. However, chlorophyll A, chlorophyll B and total chlorophyll contents of Number 7 rice were significantly increased. These changes are different from the above results, perhaps it is because Cd stimulates the biosynthetic enzymes that make photosynthetic pigments, thus maintaining strong photosynthesis to enhance the tolerance to Cd, indicating that the response mechanism of the two kinds of rice to Cd is different.

The combined pollution had different effects on chlorophyll content of the two kinds of rice, chlorophyll A and total chlorophyll content was increased and the chlorophyll B content of Number 1 rice seedling decreased. The variation trend of chlorophyll A and total chlorophyll is consistent, which may be because chlorophyll B is more sensitive than chlorophyll A under TBBPA stress, and the increase of chlorophyll content may be the response of plants to improve stress resistance. However, chlorophyll A, chlorophyll B and total chlorophyll contents of Number 7 rice seedlings were significantly inhibited by the combined pollution, which may be due to the inhibition of chlorophyll biosynthesis by the light-trapping complex formed (Li et al., 2008), or the excessive production of reactive oxygen species by the pollutants in plants, resulting in oxidative damage (Xue et al., 2009).

4.4 Bioaccumulation of flame retardants and cadmium in plant

DP residue analysis mainly focused on environmental media such as air, soil, water and sediment (Sverko et al., 2011; Wu et al., 2017; Xu et al., 2017; Hong et al., 2018; Zhong et al., 2020) as well as some aquatic biological samples (Liang et al., 2014; Barón et al., 2016). DP residue was also found in human hair and serum (Qiao et al., 2018; Yin et al., 2020), but there were relatively few studies on plants. Salamova (Salamova and Hites, 2013) reported that DP residues in birch bark from all over the world ranged from 0.89 to 48 ng/g. Gong et al. (2013) reported that DP residues in Marine green macroalgae were 4.84 ng/g dw. In this study, there was no significant difference in DP accumulation capacity between the two kinds of rice, but the accumulation capacity of syn-DP and anti-DP in roots was significantly higher than that in stems and leaves. The $\log_{Kow}$ of syn-DP is higher than that of anti-DP, and the hydrophobicity is stronger. Therefore, syn-DP is more easily absorbed by particles (Fang et al., 2014), and less easily absorbed by plants, resulting in a larger residual level in the soil. In this study, the residual amount of anti-DP in plants was significantly higher than that of syn-DP, which may be related to the strong hydrophobic nature of syn-DP (Table 2).

TBBPA is easy to lose a bromine under anaerobic conditions and generate metabolites such as Tri-BBPA, Di-BPA, Mono-BBPA and BPA (Liu et al., 2019a). Intermediate metabolites are unstable and easy to continue to lose a bromine and produce the final product BPA (Huang et al., 2020). TBBPA and its metabolites were more easily bioaccumulated in Number 1 rice seedlings, and TBBPA and BPA were more bioaccumulated, while Tri-BBPA was relatively lowest (Table 3), which was consistent with the environmental behavior and concentration change of TBBPA in soil. The combined pollution promoted the absorption of TBBPA in rice, but the mechanism needed to be further discussed.

In this experiment, the bioaccumulation of Cd in plants was analyzed by enrichment coefficient, and the residues of TBBPA and DP in roots, stems and leaves of rice seedlings were also determined. This study showed that the two kinds of rice had a bioaccumulation effect on Cd (Table 4), and the accumulation ability of root was greater than shoot, which was consistent with the results (Hegedüs et al., 2001), the bioaccumulation effect of Cd was studied in barley seedlings, root was the main accumulation organ of cadmium. The response of the two kinds of rice to Cd stress was also different, and the accumulation level of cadmium in rice Number 1 was higher than that in rice Number 7. Under the combined pollution stress, the bioaccumulation capacity of Number 1 rice root was enhanced, while that of Number 7 rice root was weakened, because the two kinds of rice had different response mechanisms to different pollutants and different damage degrees to the root, thus affecting the accumulation capacity of Cd.

5 Conclusion

The germination of Number 7 rice was more influenced than Number 1 rice in single and combined pollution. The root of rice was more sensitive to DP than the shoot, and Number 1 rice was more sensitive to DP than Number 7 rice. The root and shoot length of Number 7 rice were more affected by TBBPA than Number 1 rice, and Number 7 rice was more sensitive to Cd than Number 1 rice. In addition, the inhibition effect of combined pollution treatment on root and shoot length was greater than single pollutant treatment. Among the three combined pollution treatments, the impact was the most serious on germination rate, germination index and vitality index under combined pollution-3 treatment of the two kinds of rice.

The root activity and proline content of two kinds of rice seeds were significantly promoted, The root activity of the two kinds of rice was significantly higher under the combined pollution treatment than that under the single pollution treatment. And the proline content of the two rice varieties was significantly different among the three combined pollution treatments. And the content of protein and soluble sugar were significantly reduced by two halogenated flame retardants and Cd in soil. As well as, the protein content of No. 7 rice was significantly different among the three combined pollution treatments, while No. 1 rice was not significantly different. However, the soluble sugar content of No. 1 rice was significantly decreased under combined pollution-2 treatment and combined pollution-3 treatment compared with single pollution.

The responses of the two rice varieties seedlings to different pollutants were obviously different, the activity of SOD in two kinds of rice was improved, and a large amount of MDA was induced. But the activity of CAT in Number 7 rice was increase under the action of DP and TBBPA, while the activity of Number 1 rice was activated under the action of DP and inhibited under the pollution of TBBPA. The SOD activity of No. 7 rice was significantly increased than that of the control under the combined pollution treatment, but no significant difference was found in No. 1 rice. However, the CAT activities of the two rice varieties were significantly activated under the combined pollution treatment compared with the control. The POD activity and chlorophyll contents of No. 1 rice were significantly higher than those of the single pollution treatment under the combined pollution treatment, while the POD content of No. 7 rice was not significantly different from that of the control, and the chlorophyll content was significantly decreased. However, the MDA content of No. 7 rice was significantly higher than that of the control under the compound pollution treatment.

There was no significant difference in DP accumulation capacity between the two rice varieties, and the bioaccumulation capacity of syn-DP and anti-DP in roots was significantly higher than that in shoots. TBBPA and BPA were more easily bio accumulated in Number 1 rice seedlings, and the bioaccumulation amount was larger. Two rice varieties had certain bioaccumulation to Cd in the root, and the bioaccumulation capacity of Number 1 rice was greater than Number 7 rice. There was no significant difference in the bioaccumulation capacity of DP and TBBPA in the two rice varieties between the combined and single contamination treatments. However, the bioaccumulation capacity of Cd in No. 1 rice under the combined contamination treatment was greater than that of single contamination treatment, while there was no significant difference in the bioaccumulation capacity of Cd in rice No. 7.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

HX, FS, and YX contributed to conception and design of the study. HX organized the database. RL performed the statistical analysis. RL wrote the first draft of the manuscript. HX wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This study was supported by grants from the National Natural Science Foundation of China (No. 41671321).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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