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Effect of exposure to decabromodiphenyl ether and tetrabromobisphenol A in combination with lead and cadmium on soil enzyme activity

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ABSTRACT

The two most widely used brominated flame retardants (BFRs), decabromodiphenyl ether (BDE209) and tetrabromobisphenol A (TBBPA), co-exist prevalently with heavy metals (HM), at e-waste recycling sites (EWRSs). The laboratory incubation we conducted, which 50 g of dried soil spiked with Pb, Cd, BDE209 and TBBPA, were incubated 180 d in the dark at 25 °C, and kept the soil moisture at 60%. The results demonstrated that the inhibition ratio of 4 kinds of the enzyme (catalase, dehydrogenase, polyphenol oxidase and urease) in the combined of BFRs and HMs treatments were −127%–66%, -72%–49%, -52% –76% and 54%–107% respectively, and in TBBPA or BDE209 contaminant treatments were −135%–18%, -16%–31%, -18%–15% and −17%–83% respectively. Urease was more sensitive to the combined contamination than catalase, dehydrogenase and Polyphenol oxidase. BDE209 exhibited a higher ecotoxicological potential to soil enzymatic activity than TBBPA. The co-existence of HM induces a stronger response of enzymatic activity to the artificial contamination. The availability of the residual fraction in the later stage of incubation, along with the results of principal component analysis demonstrated that there may be different mechanisms responsible for the generation of residual and extractable fractions of HM.

1. Introduction

Electronic waste (e-waste) treatment has recently become an issue of significant concern, especially in developing countries since 1990s (Chan et al., 2007; Zhang et al., 2012). It has been reported that 50%–80% of e-wastes worldwide were being exported to Asia and Africa legally or illegally, approximately 90% of which were sent to China since 2002 (Ni et al., 2013). A lack of effective recycling techniques has led to the continual release of various hazardous chemicals, including heavy metals (HM) and flame retardants (FRs) from e-waste recycling sites (EWRSs) in China. Many studies on the effects of these harmful chemicals examine only a single chemical, while very few studies have examined joint exposure to FRs and HM, such as polybrominated diphenyl esters (PBDEs) with tetrabromobisphenol A (TBBPA), PBDEs with HM (i.e., cadmium and copper), and TBBPA with HM (i.e., lead) in soils. Available information indicates that exposure to a combination of these chemicals may have different ecological effects on soil microbial communities, compared with the effects of exposure to FRs alone (Zhang et al., 2012a, b; Zhang et al., 2016; Zhang et al., 2014). It has been hypothesized that, because it contains two phenolic hydroxyl groups, the mobility and bioavailability of TBBPA in the environment would be different from that of non-ionic FRs (Xue et al., 2009), such as PBDEs. A recent study revealed insight into

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the sorption process of TBBPA and suggested that it is dependent on pH (Tong et al., 2015), which is also believed to be one of the most important factors controlling mobility and bioavailability of HM in soils. To data, the impact of HM on the ecotoxicity of TBBPA and PBDEs remains largely unknown.

Soil microorganisms respond rapidly to ecosystem perturbation and therefore are considered to be sentinels of soil pollution. Soil enzymatic activity reflects the direction and strength of biochemical processes in soil (Deng and Tabatabai, 1994; Yang et al., 2006). Hence, it is widely used as a biological indicator for estimating the adverse effects of various pollutants on soil quality (Gao et al., 2010; Matyja et al., 2016). Dehydrogenase and catalase, two common intracellular enzymes, are considered to be more sensitive to chemical toxicity than extracellular enzymes (Chu et al., 2003; García-Gil et al., 2000; Caldwell, 2005). The activity of urease is also frequently used for determining the influence of various pollutants (e.g., HM, pesticides, and crude oil) on the microbiological quality of soil (Margesin et al., 2000). Polyphenol oxidase, which could oxidize the aromatic compound (eg.PAHs) to quione, which reacts with protein, amino acids, glucide, minerals, and small organic matter compounds, plays an important role in the cycling of aromatic compounds in soil (Fang et al., 2015). The objectives of this study were: to explore the joint toxicological effects of BFRs (BDE209 and TBBPA) and two frequently studied HM (Pb and Cd) on soil enzyme activity, and to examine the impact of combined exposure to BFRs on HM speciation, which is closely related to HM bioavailability in soil. The results will provide further information about the interactions between of BFRs and HM on the ecological risk assessments of EWRSs.

2. Materials and methods

2.1. Chemicals

Decabromodiphenyl ether (commercial Deca-BDE, purity of BDE209 > 99%) and TBBPA (>98% in purity) were purchased from J&K Scientific (USA). Cadmium chloride ($CdCl_2$, purity ≥98% of A.R. grade) and lead acetate ($(CH_3COO)_2Pb$, purity >99.8% of A.R. grade) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). All organic solvents used in the study were of HPLC grade (J.T. Baker Inc., USA). BDE209 and TBBPA stock solution were prepared by dissolving the commercial product powder in toluene and methanol, respectively. Pb and Cd stock solution were prepared by dissolving $(CH_3COO)_2Pb$ and $CdCl_2$ in de-ionized water, respectively.

2.2. Incubation experiments

A silty clay soil (pH 6.0, organic matter content of 3.2%, and cation exchange capacity 10.9 cmol kg^{-1}) was sampled from a forest field located in Qingyuan, Guangdong province, China. The soil was air-dried, separated from plants residues and stones, and then sieved through a 2 mm sieve before use. The background levels of the investigated contaminants in pristine soil were determined: 3.3 μg g^{-1} for Pb, 0.14 μg g^{-1} for Cd, 0.029 ng g^{-1} for BDE209 and not detectable for TBBPA, respectively. The background concentrations of the chemicals were far below the spiked levels. Incubation experiments were carried out as follows: about 50 g of dried soil was spiked with BDE209 and TBBPA in dichloromethane, Pb^{2+} and Cd^{2+} in water. Before joined Pb and Cd, it must be removed dichloromethane by evaporation in a fume hood for 30 min. The mixture was thoroughly combined with the submerged soil, resulting in a final BDE209 and TBBPA concentration of 10 mg kg^{-1} soil (dry weight). Table 1 presents the contaminant of the treatments. The control received the same amounts of distilled water instead of the contaminant solution. Total of 15 beakers were incubated in the dark at 25 °C. Deionized water was added to keep the soil moisture at 60% of field capacity every 3 days. Soil samples were collected for further analysis on day 0, 5, 10, 30, 60, 120 and 180 after incubation.

Table 1
Experimental design of PBDEs, TBBPA, Pb and Cd at different concentrations used in this study.

Treatments	Concentrations (μg/g dw, 25 °C, 60% water-holding capacity)			
	PBDEs	TBBPA	Pb	Cd
CK	0	0	0	0
P	10	0	0	0
T	0	10	0	0
PT	10	10	0	0
CA	10	10	100	10
CB	10	10	1000	100
CC	10	10	5000	500

2.3. Enzymatic activity assay

Catalase activity was determined using a titrimetric method (Roberge, 1978). Two gram of the soil was put into a 150 mL Erlenmeyer flask, and then 40 mL of distilled water and 5 mL of 0.3% H_2O_2 were added into the flask. The flask was sealed and shaken, and then gently placed in a water bath at 37 °C for 30 min. Then 5 mL of 1.5 mol L^{-1} H_2SO_4 was added to terminate the reaction. And the light absorbance was measured using the spectrophotometer at 240 nm. The amount of surplus H_2O_2 from 25 mL of the filtrate was determined by titration using 0.02 mol L^{-1} $KMnO_4$.

Dehydrogenase activity was determined using the measurement of triphenyl formazan (TPF) produced from the microbial reduction of 2, 3, 5-triphenyltretrazolium (TTC) (Chaperon and Sauve, 2007). Samples of 2 g soil were placed in 50 mL EP tubes. To each tube, 2 mL of 1% TTC, 2 mL of 0.1 mol L^{-1} glucose solution and 2 mL of Tris-HCl buffer (pH7.4) were added. After being mixed on a vortex, the tubes were stoppered and incubated in the dark for 24 h at 37 °C. After incubation, 0.5 mL of methanal was added and additional 5 mL of methylbenzene were added. Then the samples were shaken in a water bath for 30 min and stood for 30 min. The samples were separated with a centrifuge for 5 min in 4000 $r \cdot min^{-1}$. The red-colored complexes were measured within 1 h using the spectrophotometer at 485 nm.

Urease activity was determined using the method of measurement of mg NH_4^+-N produced from dry soil g^{-1} d^{-1} (May and Douglas, 1976). Two grams of the soil were incubated with 5 mL 10% urea and 10 mL citrate buffer at 37 °C for 24 h, then diluted with38 °C distilled water to 50 mL. One mL filtered liquor was put into a 50 mL volumetric flask, and then diluted with distilled water to 10 mL. Then 4 mL of sodium phenate and 3 mL sodium hypochlorite were added. Finally the flask was sealed and shaken, and the light absorbance was measured using the spectrophotometer at 578 nm 20 min later.

Polyphenol oxidase activity was determined using the method of measurement of mg gallnut produced form dry soil g^{-1} d^{-1} (Vepsäläinen et al., 2001). One gram of the soil was incubated with 10 mL of 1% pyrogallol at 30 °C for 2 h and the samples were shaken for 30 min. Then 4.5 mL of citric acid-phosphate buffer and 35 mL of diethyl ether were added into the flask. Finally the flask was sealed and shaken, and the light absorbance was measured using the spectrophotometer at 430 nm.