Supporting Information

Degradation and Metabolism of Tetrabromobisphenol A (TBBPA) in Submerged Soil and Soil–Plant Systems

Feifei Sun[1], Boris Alexander Kolvenbach[2], Peter Nastold[2], Bingqi Jiang[3], Rong Ji [1, 4,*], Philippe Francois-Xavier Corvini[1, 2]

[1]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, 163 Xianlin Avenue, 210023 Nanjing, China

[2]Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Gründenstrasse 40, Muttenz CH-4132, Switzerland

[3]Fujian Provincial Academy of Environmental Science, No.10, Huan Bei San Cun, Fuzhou 350013, China

[4]Institute for Marine Sciences & Institute for Climate and Global Change Research, Nanjing University, 22 Hankou Road, 210093 Nanjing, China

**: Corresponding author, Tel.: +86-25-8968 0581, Fax: +86-25-8968 0559, email: ji@nju.edu.cn*

Number of pages: 19

Number of figures: 18

Number of tables: 1

HPLC analysis

HPLC was performed on a Nucleosil C18 column (250 mm×4 mm) at 40 °C using an Agilent HPLC Series 1100 (Agilent Technologies, Germany) coupled to a diode array detector and an online radio flow detector (RAMONA Star; Raytest, Straubenhardt, Germany). The mobile phase consisted of water (A) and acetonitrile (B) and flowed at 0.45 mL min^{-1}, using a gradient program as follows: 20% B with linear gradient to 40% B within 3 min, to 60% B within 27 min, 60% B isocratically for 8 min, to 100% B within 1 min, 100% B isocratically for 4 min, back to 20% B within 5 min, and 20% B for 2 min. The flow rate of the scintillation cocktail (Quicksafe Flow 2; Zinsser Analytic GmbH, Frankfurt, Germany) for the radiodetector was 2 mL min^{-1}. The UV signal was recorded at 254 nm.

Purification of metabolites

The extracts of day 66 were fractioned by HPLC by repeated injection of 100-µL aliquots. The HPLC ran under the conditions described above. The eluents were collected in seven fractions according to the retention times of the radioactive peaks (Figure S2). The fractions were concentrated to dryness under a stream of nitrogen gas and re-suspended in 200 µL of acetonitrile for gas chromatography-mass spectrometry (GC-MS) analysis.

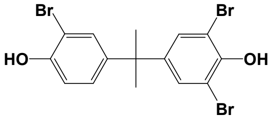
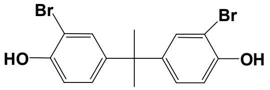
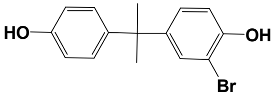
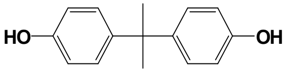
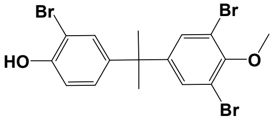
GC-MS analysis

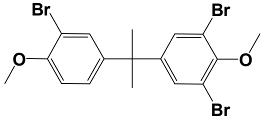
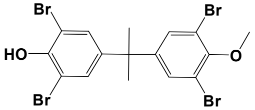
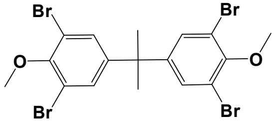
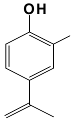
The purified metabolites in acetonitrile (90 µL) were derivatized using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (10 µL) at 75 °C for 15 min. One µL was then injected onto a ZB-5HT Inferno column (32 m × 250 µm × 0.25 µm; Phenomenex; Germany) in a gas chromatograph (Agilent 7890A series; USA) coupled to a mass spectrometer (Agilent 5975C series; USA), using a programmed temperature vaporizer inlet (60 °C for 0.16 min, then 60 °C/min to 300 °C for 10 min). The temperature gradient was 0 °C/min at 60 °C for 1 min, 30 °C/min to reach 150 °C, followed by 5 °C/min to reach 300 °C, which was held for 5 min. The transfer line was set at 280 °C, the MS ion source at 200 °C, and the quadrupole at 150 °C. Mass spectra were recorded in full scan mode at *m/z* = 60−700. The trimethylsilyl (TMS) derivatives of compounds **1**–**12** are shown in Figure S4–S15, respectively.

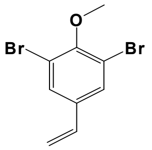
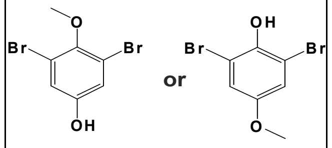
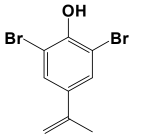
Determination of radioactivity

The radioactivity in the samples was quantitatively counted in a liquid scintillation counter (LSC; Tri-Carb 2800 TR; PerkinElmer, USA). For radioactivity in water and organic extracts, 1 mL of water or extract was mixed with 18 mL of scintillation cocktail (Hionic-Fluor, PerkinElmer). For the NaOH solution containing $^{14}CO_2$, 2 mL was mixed with 18 mL of scintillation cocktail (Ultima-Flo, PerkinElmer). For radioactivity in the plants and the soil-bound residues, dried plant material (leaves, roots, or total plants) and 150 mg of air-dried soil was combusted on a sample oxidizer (model 307; PerkinElmer). The generated CO_2 was absorbed by 18 mL of Carbo-Sorb E (PerkinElmer) and counted by LSC.

Table S1. GC-MS (after HPLC fractionation) identification of TBBPA metabolites in organic extracts prepared from ^{14}C-TBBPA-spiked soils planted or not with rice or reed seedlings and incubated for 66 days.[a]

Compound no.	Name	Structure	HPLC fraction no.[b]	*R*t on GC (min)	Identified fragments of TMS-derivatives [m/z (% relative abundance) (fragment)][c]	Soil treatment
1	2-(3-bromo-4-hydroxyphenyl)-2-(3,5-dibromo-4-hydroxyphenyl)-propane (Tribromobisphenol A, triBBPA)		3, 4	33.889	606/608/610(30)/612 (M^+) 519/593/595(100)/597 ($[M - Me]^+$) 363/365(10)/367 ($[Me - A]^+$) 285/287(18) ($[Me - B]^+$) 137/139(42) ($[Me_2SiBr]^+$)	Rice soil Reed soil Unplanted soil
2	2-bromo-4-[1-(3-bromo-4-hydroxyphenyl)-1-methylethyl]phenol (Dibromobisphenol A, diBBPA)		3, 4	30.695	528/530(22)/532 (M^+) 513/515(100)/517 ($[M - Me]^+$) 285/287(14) ($[Me - B]^+$) 137/139(20) ($[Me_2SiBr]^+$)	Rice soil Reed soil Unplanted soil
3	2-bromo-4-[2-(4-hydroxyphenyl)propan-2-yl]phenol (Monobromobisphenol A, monoBBPA)		3	27.297	450/452(19) (M^+) 435/437(100) ($[M - Me]^+$)	Rice soil Reed soil
4	4,4'-(propane-2,2-diyl)diphenol (Bisphenol A, BPA)		2	23.679	372(13) (M^+) 357 (100) ($[M - Me]^+$) 207(21) ($[M - D]^+$)	Rice soil Reed soil,
5	2-bromo-4-(2-(3,5-dibromo-4-methoxyphenyl)propan-2-yl)phenol		7	32.203	548/550/552(38)/554 (M^+) 533/535/537(100)/539 ($[M - Me]^+$) 455/457(12)/459 ($[M - Me - Br]^+$)	Rice soil Unplanted soil

	(Tribromobisphenol A monomethyl ether, MeO-triBBPA)				285/287(20) ([Me – B]$^{+}$) 305/307(16)/309 ([Me – C]$^{+}$)	
6	1,3-dibromo-5-(2-(3-bromo-4-methoxyphenyl)propan-2-yl)-2-methoxybenzen (Tribromobisphenol A dimethyl ether; diMeO-triBBPA)		7	32.066	490/492/494(31)/496 (M^{+}) 475/477/479/(100)481 ([M – Me]$^{+}$)	Rice soil Unplanted soil
7	2,6-dibromo-4-(2-(3,5-dibromo-4-methoxyphenyl)propan-2-yl)phenol (Tetrabromobisphenol A monomethyl ether; MeO-TBBPA)		7	35.501	626/628/630(29)/632/634 (M^{+}) 611/613/615(100)/617/619 ([M – Me]$^{+}$) 305/307(30)/309 ([M – C]$^{+}$), 363/365(23)/367 ([M – A]$^{+}$) 137/139(59) ([Me_2SiBr]$^{+}$)	Rice soil Reed soil Unplanted soil
8	2,2-bis(3,5-dibromo-4-methoxyphenyl)propane (Tetrabromobisphenol A dimethyl ether; diMeO-TBBPA)		7	34.029	568/570/572(38)/574/576 (M^{+}) 553/555/557(100)/559/561 ([M – Me]$^{+}$) 459/461/463(6)/465 ([M – Br – OMe]$^{+}$) 305/307(26)/309 ([M – C]$^{+}$),	Rice soil Unplanted soil
9	2-bromo-4(propen-2-yl)-phenol		2	12.268	284/286(69) (M^{+}) 269/271(100) ([M – Me]$^{+}$) 137/139(73) ([Me_2SiBr]$^{+}$)	Unplanted soil

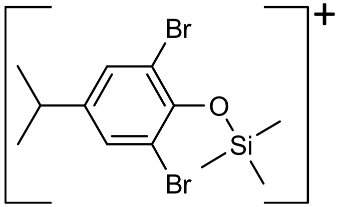
10	1,3-dibromo-2-methoxy-5-vinyl benzene		2	14.215	290/292(100)/294 (M^+) 275/277(27)/279 ($[M - Me]^+$) 196/198(12) ($[M - Me - Br]^+$)	Unplanted soil
11	3,5-dibromo-4-methoxyphenol, or 2,6-dibromo-4-methoxyphenol		2	14.259	352/354(63)/356 (M^+) 337/339(100)/341 ($[M - Me]^+$)	Unplanted soil
12	2,6-dibromo-4(propen-2-yl)-phenol		2	16.424	362/364(46)/366 (M^+) 347/349(100)/351 ($[M - Me]^+$) 268/270(34) ($[M - Me - Br]^+$) 137/139(35) ($[Me_2SiBr]^+$)	Unplanted soil

[a] Identification fragments of compounds **1–4** and **7–8** were compared with authentic compounds; compounds **5–6** were identified according to the bromine isotope patterns and the characteristic mass fragments that are similar to the methyl ethers of TBBPA (compounds **7–8**); compounds **9–12** were identified according to the bromine isotope pattern and the corresponding bromine-free counterpart molecules.

[b] Fractions on radio-HPLC; Fraction 1: 1–5 min, 2: 5–15 min, 3: 15–25 min, 4: 25–31 min, 5: 31–34 min, 6: 34–42 min, and 7: 42–50 min. For details, see Figure S2A

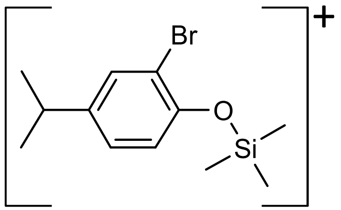
[c] $[M - A]^+$, $[M - B]^+$, $[M - C]^+$, and$[M - D]^+$ represent fragments 2,6-dibromo-4-(2-propyl)-trimethylsilyloxybenzene, 2-bromo-4-(2-propyl)-trimethylsilyloxybenzene, 2,6-dibromo-4-(2-propyl)-methoxybenzene, and 4-(2-propyl)-trimethylsilyloxybenzene, respectively.

[M-A]$^+$



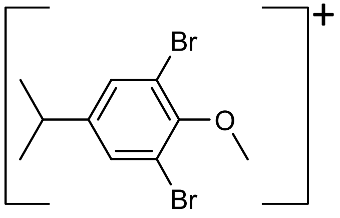
2,6-dibromo-4-(2-propyl)-trimethylsilyloxybenzene

[M-B]$^+$



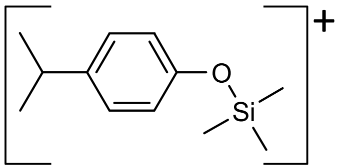
2-bromo-4-(2-propyl)-trimethylsilyloxybenzene

[M-C]$^+$



2,6-dibromo-4-(2-propyl)-methoxybenzene

[M-D]$^+$



4-(2-propyl)-trimethylsilyloxybenzene

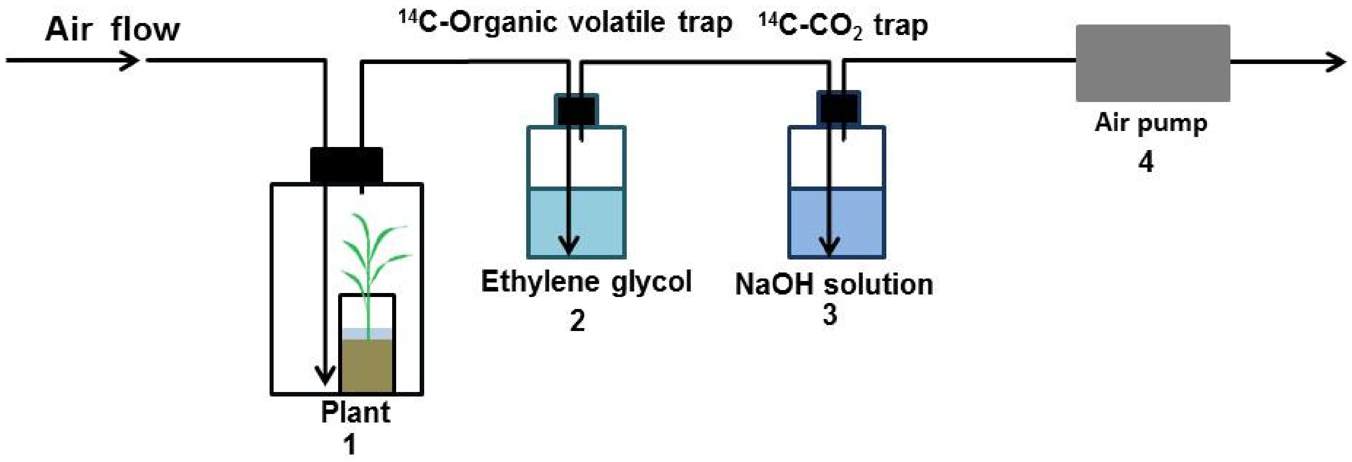


FIGURE S1. Flow-through system for the degradation of TBBPA in saturated soil with and without plant growth. Fresh air was introduced into the system at a rate of 500 mL h^{-1} by an air pump. Legend: 1, planted flask; 2, monoethylene glycol vessel for trapping volatile organic compounds; 3, NaOH solution vessel for trapping CO_2; 4, air pump.

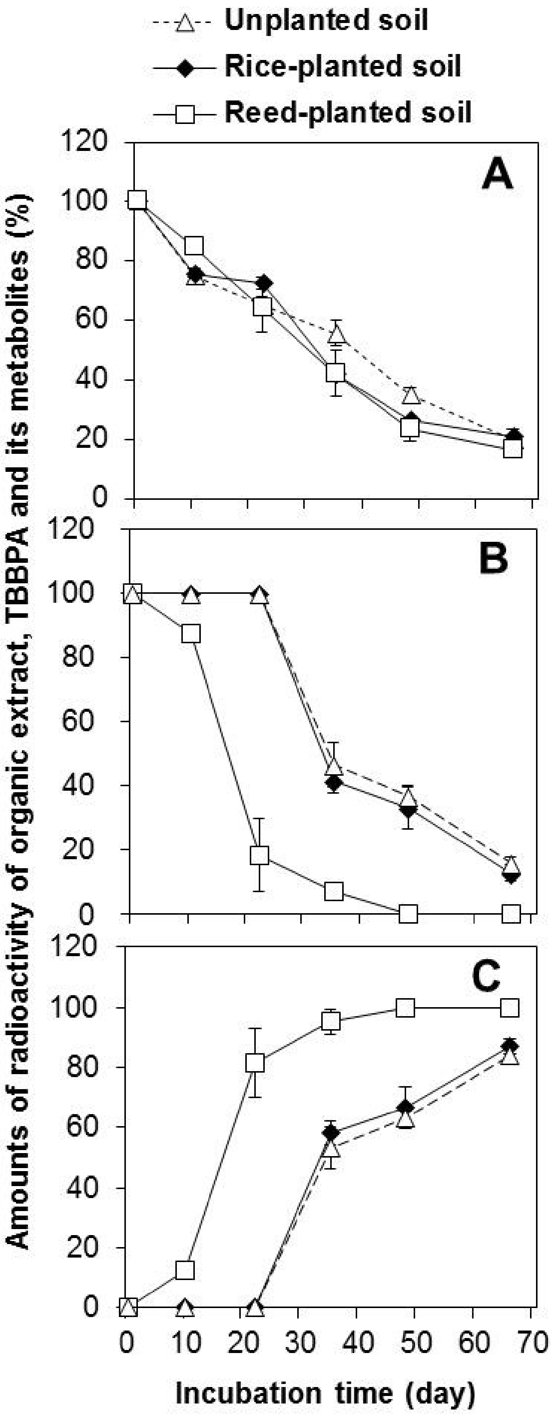


FIGURE S2. Radioactivity recovered from total organic extracts (A), TBBPA (B), and metabolites (C) in submerged soil during incubation with ^{14}C-TBBPA with or without growth of rice or reed seedlings. Amounts of radioactivity in TBBPA and its metabolites are shown as relative values to the total radioactivity in the extracts. Data are the means of three individual experiments ± one standard deviation.

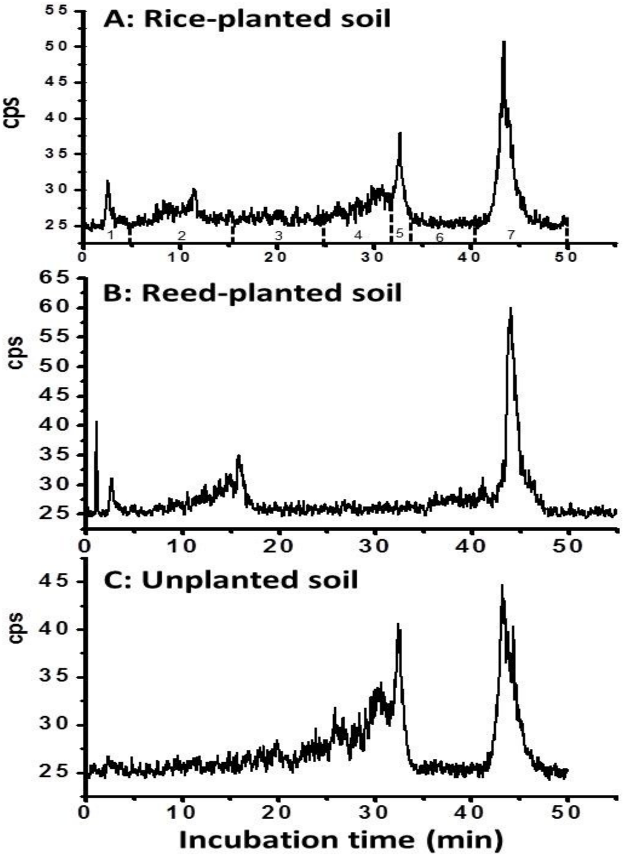


FIGURE S3. HPLC Radio-chromatograms of organic extracts of ^{14}C-TBBPA-spiked soils planted with rice (A) or reed (B) seedlings or left unplanted (C) and incubated for 66 days. The extracts were fractionated into seven parts according to their retention time on HPLC (shown in A) for further GC-MS analysis.

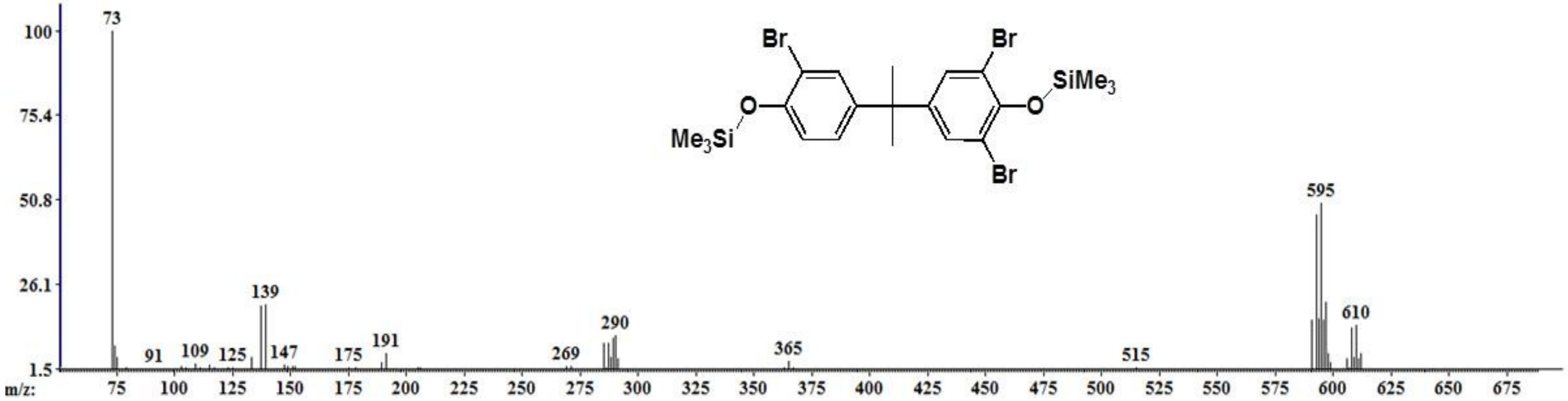


FIGURE S4. Mass spectra of the TMS derivative of tribromobisphenol A (triBBPA) (compound **1** in Table S1)

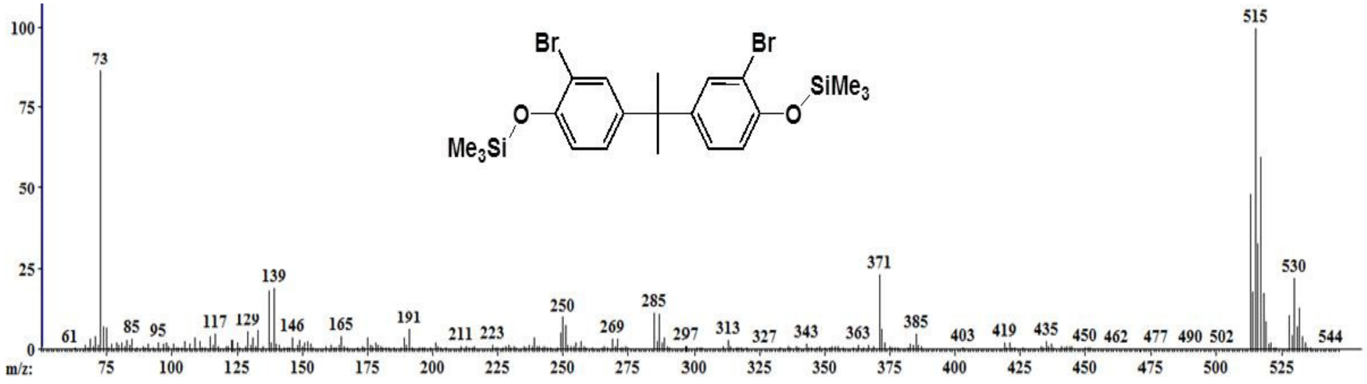


FIGURE S5. Mass spectrum of the TMS derivative of dibromobisphenol A (diBBPA) (compound **2** in Table S1)

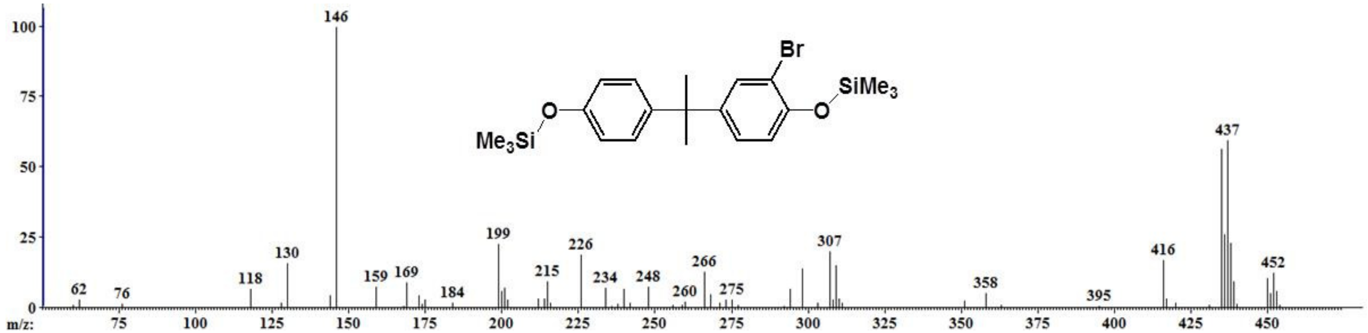


FIGURE S6. Mass spectrum of the TMS derivative of monobromobisphenol A (monoBBPA) (compound **3** in Table S1)

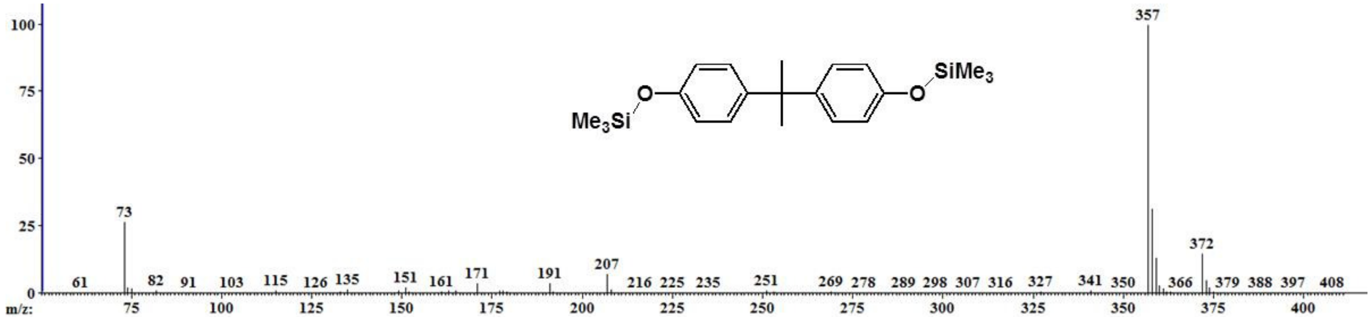


FIGURE S7. Mass spectrum of the TMS derivative of bisphenol A (BPA) (compound **4** in Table S1)

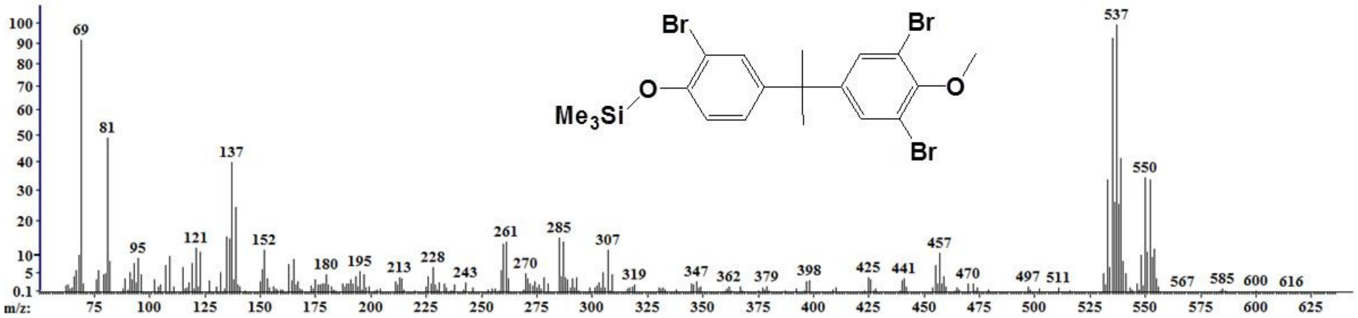


FIGURE S8. Mass spectrum of the TMS derivative of tribromobisphenol A monoethyl ether (MeO-triBBPA) (compound **5** in Table S1)

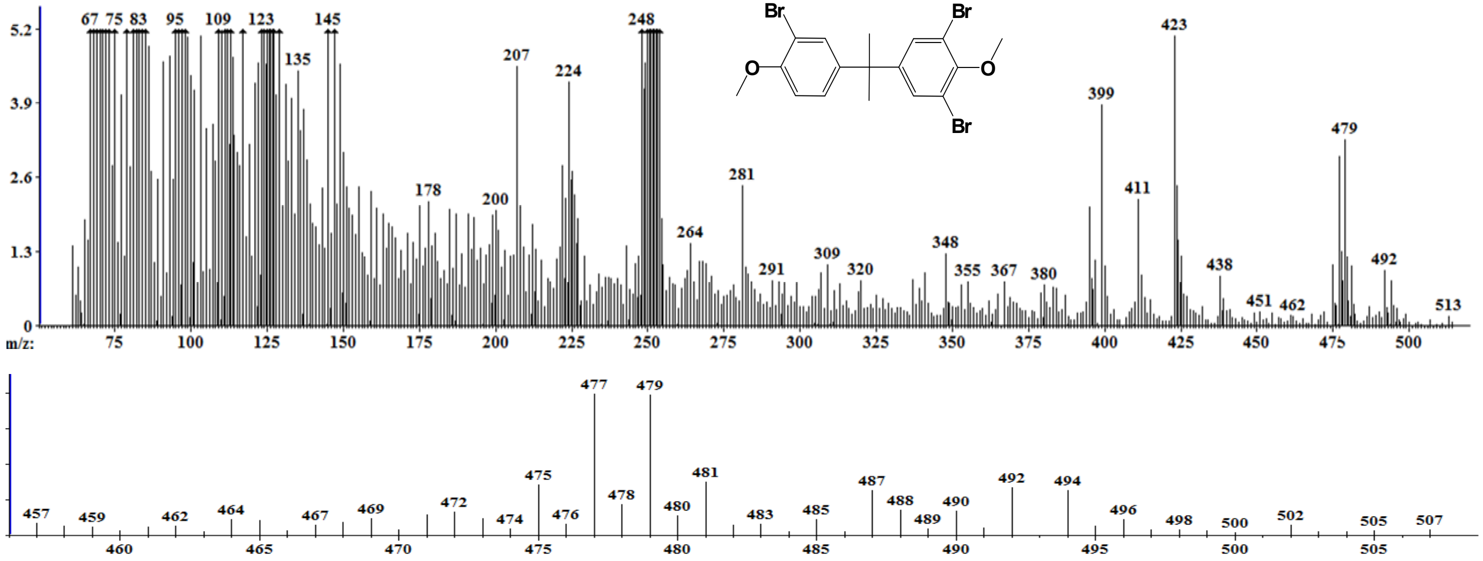


FIGURE S9. Total mass spectra (top) and bromine isotope patters (bottom) of tribromobisphenol A dimethyl ether (diMeO-triBBPA) (compound **6** in Table S1)

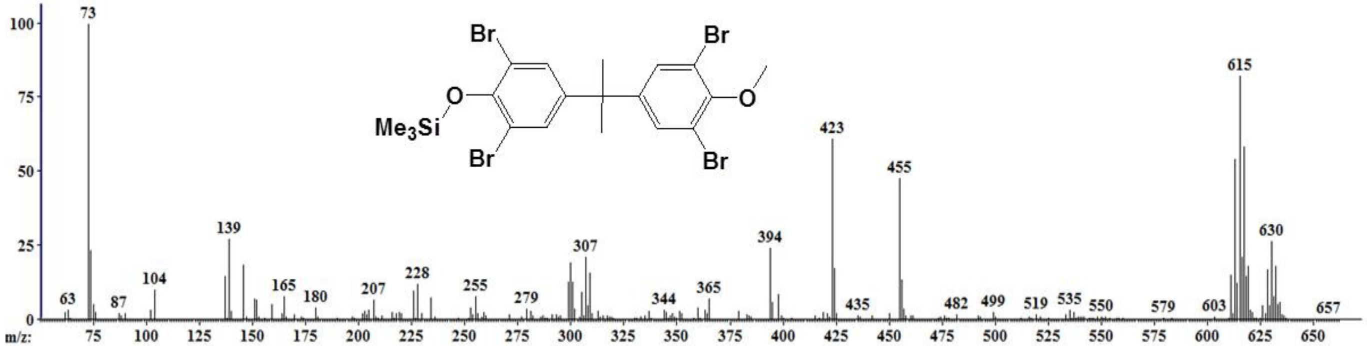


FIGURE S10. Mass spectrum of the TMS derivative of tetrabromobisphenol A monoethyl ether (MeO-TBBPA) (compound **7** in Table S1)

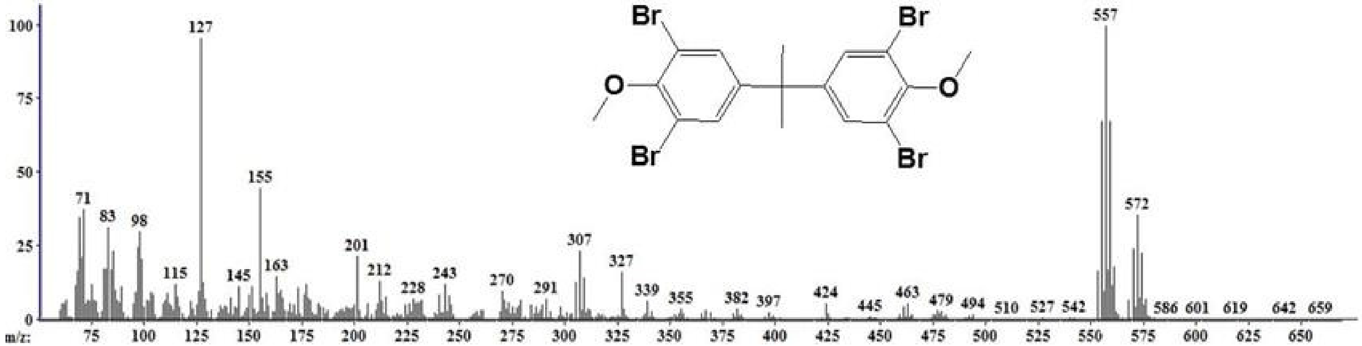


FIGURE S11. Mass spectrum of TBBPA dimethyl ether (diMeO-TBBPA) (compound **8**, Table S1)

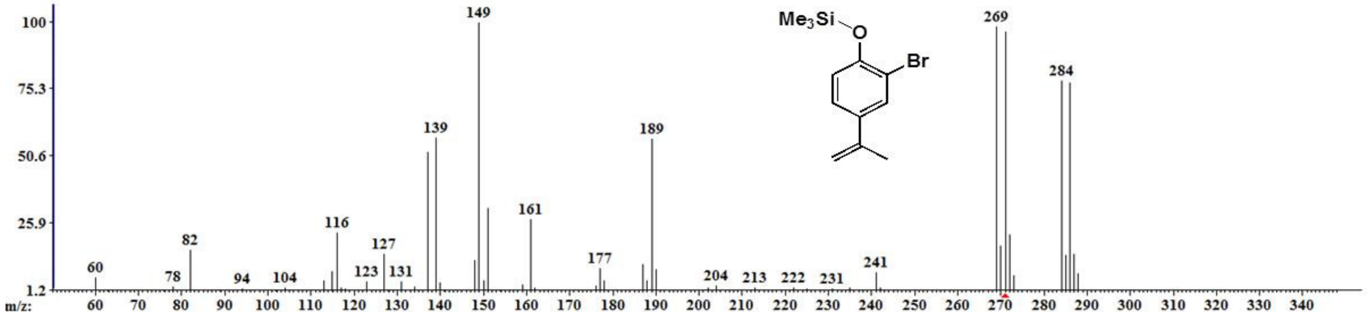


FIGURE S12. Mass spectrum of the TMS derivative of 2-bromo-4(propen-2-yl)-phenol (compound **9**, Table S1)

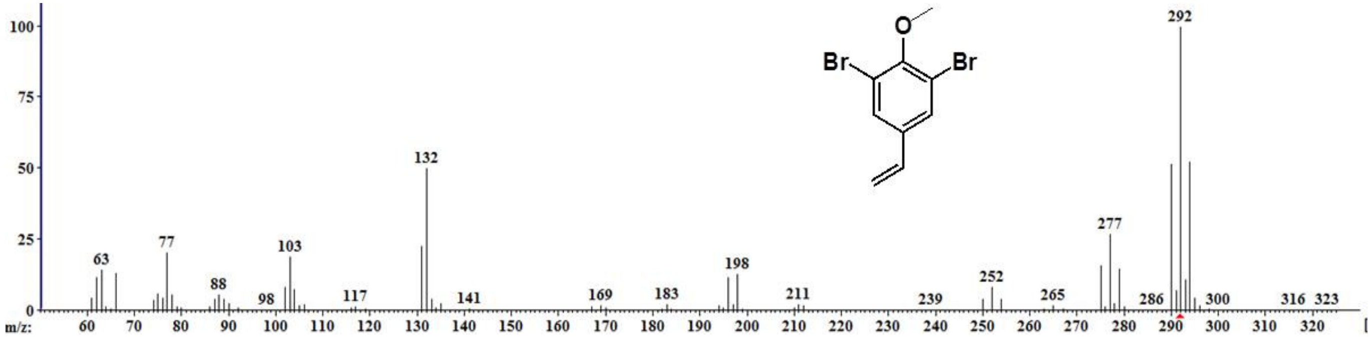


FIGURE S13. Mass spectrum of 1,3-dibromo-2-methoxy-5-vinylbenzene (compound **10**, Table S1)

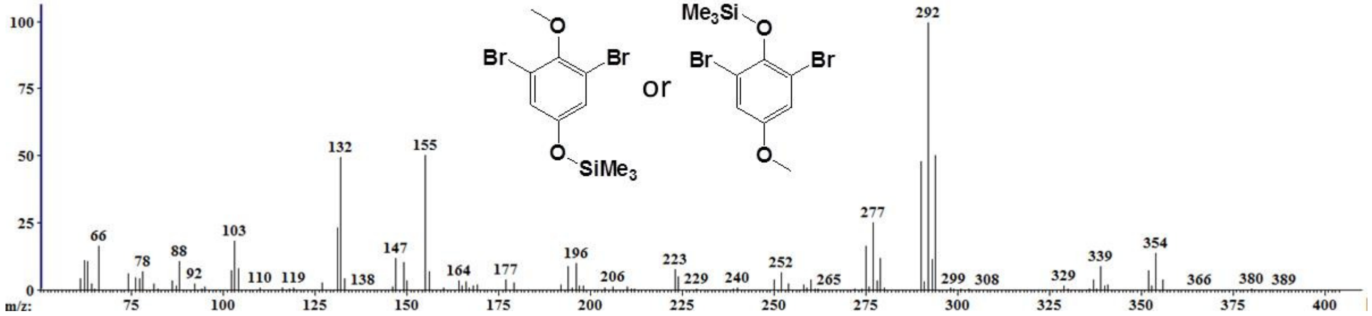


FIGURE S14. Mass spectrum of the TMS derivative of 3,5-dibromo-4-methoxyphenol or 2,6-dibromo-4-methoxyphenol (compound **11**, Table S1)

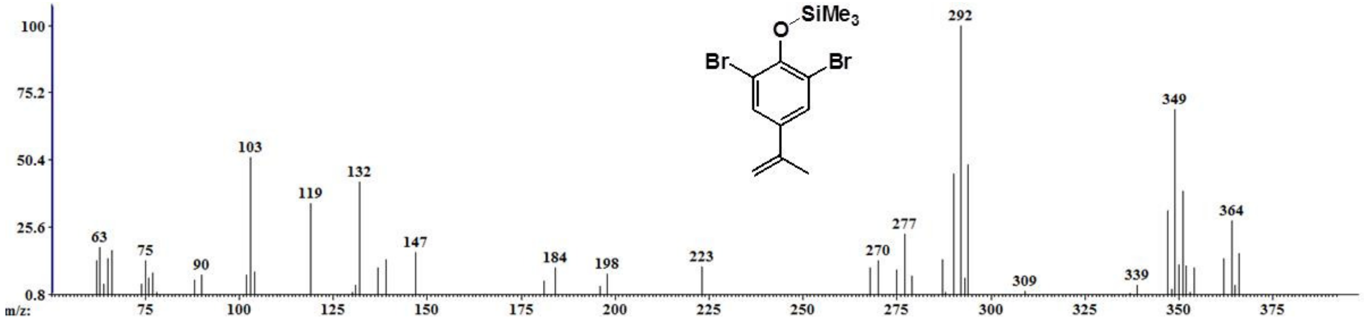


FIGURE S15. Mass spectrum of the TMS derivative of 2,6-dibromo-4(propen-2-yl)-phenol (compound **12**, Table S1)

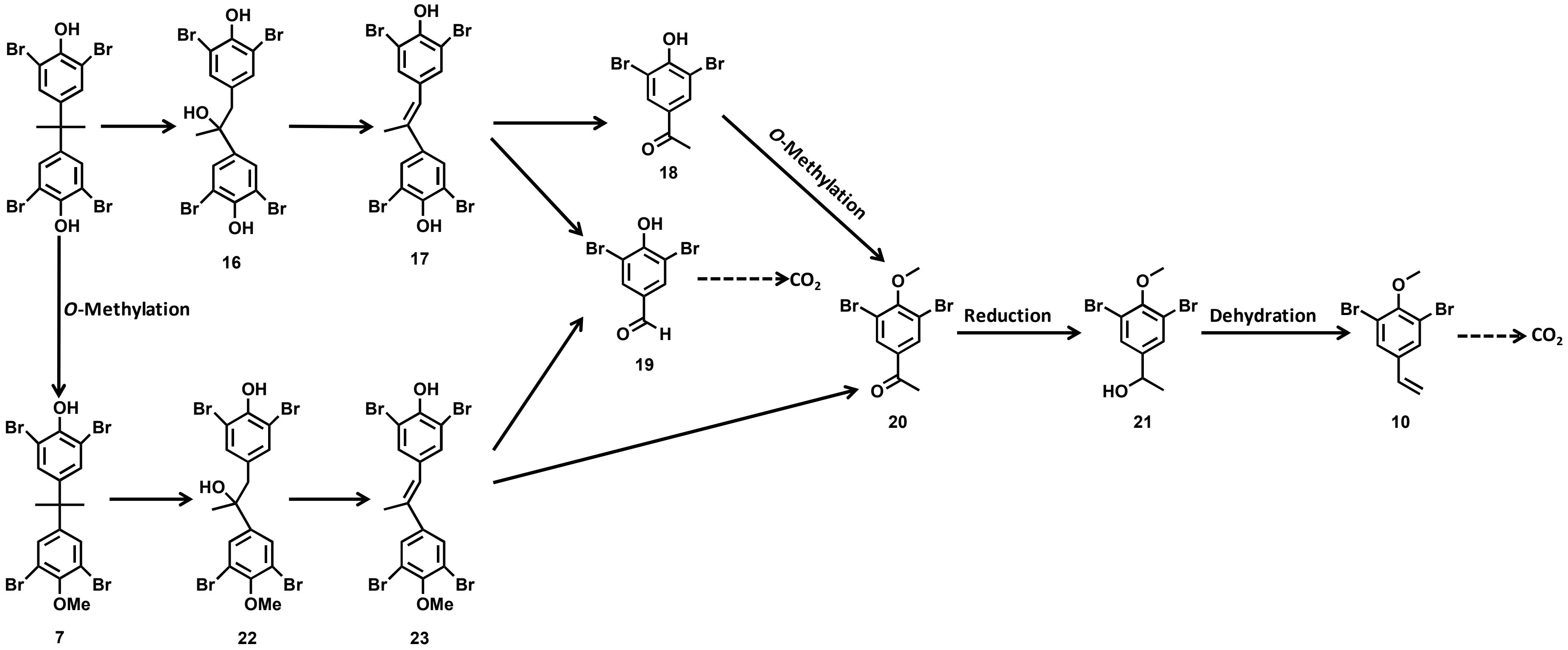


Figure S16. Proposed pathways for the degradation of TBBPA and MeO-TBBPA in submerged soil via oxidative skeletal rearrangements followed by reduction and dehydration.

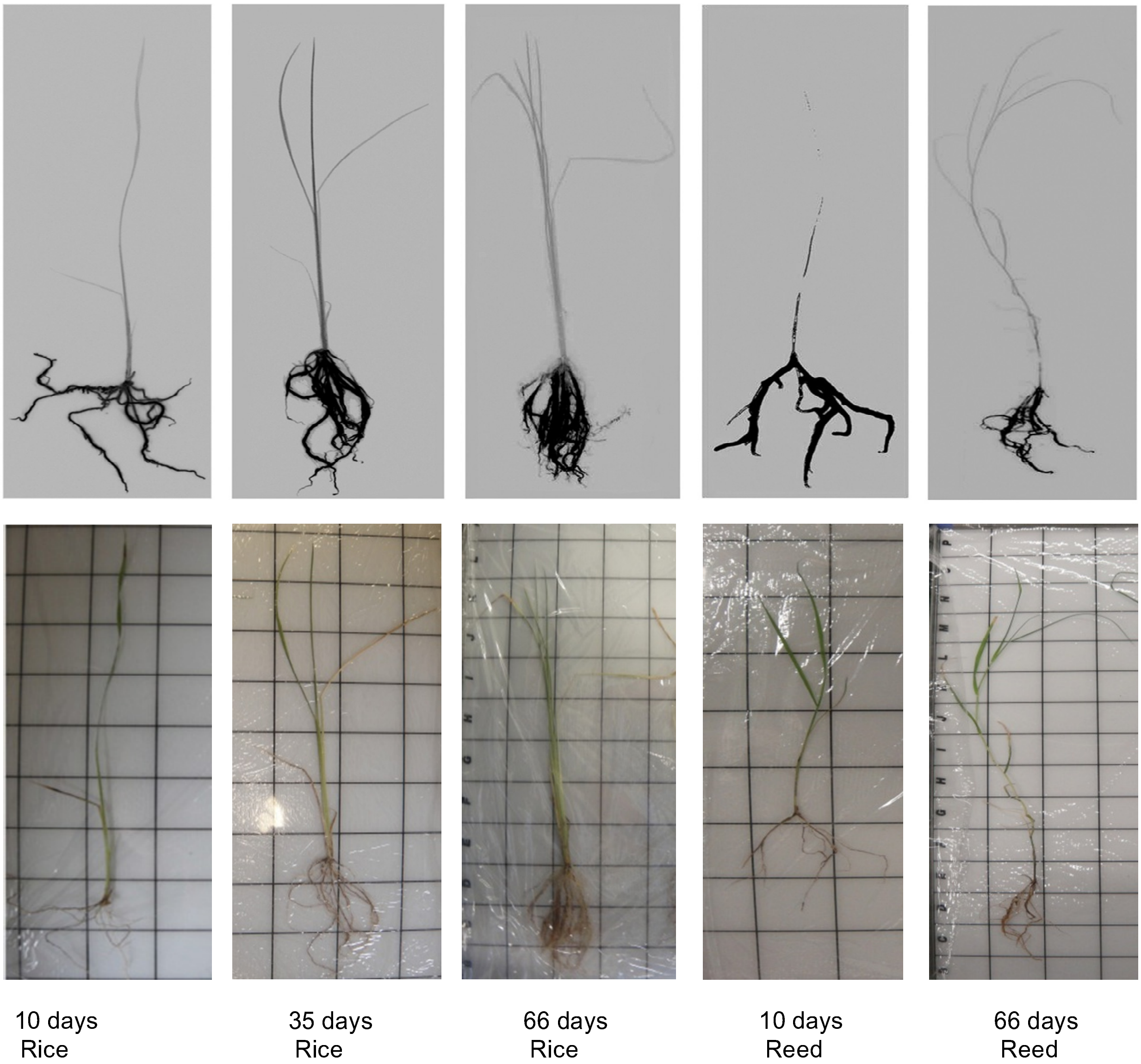


FIGURE S17**.** Autoradiograms (top) and photograms (bottom) of rice and reed plants grown for 10, 35, and 66 days in submerged soil containing ^{14}C-TBBPA.

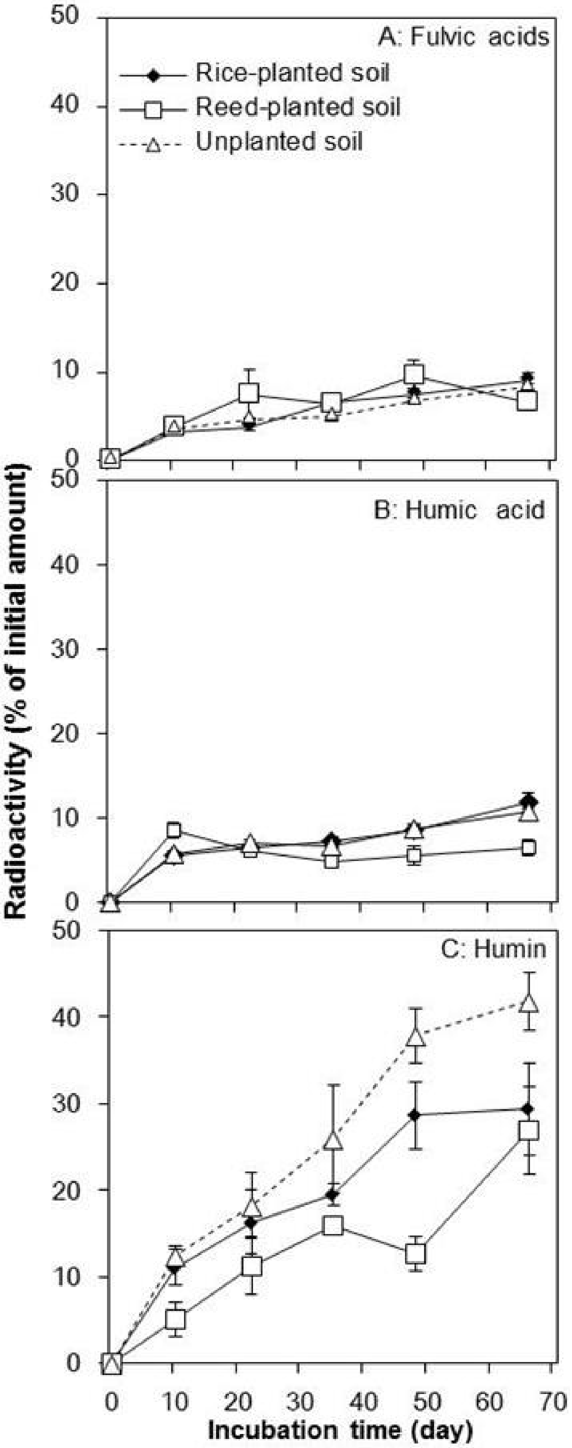


FIGURE S18. Fractionation of bound residues of ^{14}C-TBBPA in submerged soil planted or not with rice or reed seedlings during 66 days of incubation. The bound residues were fractionated into fulvic acids (A), humic acids (B), and humin (C) fractions. The values are the means of three individual experiments ± one standard deviation.