Environ Sci Pollut Res (2017) 24:11141–11153
DOI 10.1007/s11356-016-6068-8

Uptake and decomposition of the herbicide propanil in the plant *Bidens pilosa* L. dominating in the Yangtze Three Gorges Reservoir (TGR), China

Zhongli Chen[1] · **Burkhard Schmidt**[1] · **Andreas Schäffer**[1,2,3]

Received: 11 May 2015 / Accepted: 6 January 2016 / Published online: 5 February 2016

Abstract Propanil (3′,4′-dichloropropionanilide) is a selective post emergence herbicide for controlling broad leaf and grass weeds in rice (*Oryza sativa* L.). After being taken up by plants, the fate of propanil in decomposing plant material is of particular importance to the phytoremediation of the environment. Therefore, we investigated the biotransformation of propanil in the plant *Bidens pilosa* under conditions close to those present in the Three Gorges Reservoir (TGR), China. Plants pre-treated with ^{14}C-ring-labeled propanil were either (treatment a) directly submerged in TGR water for 90 days or (treatment b) pre-extracted with organic solvents, and subsequently only insoluble materials and non-extractable residues (NER) of the pesticide fractions were similarly incubated. After incubation in TGR water (treatment a), 30 % of applied radioactivity was released into water and simultaneously, amounts of NER in the plant debris appeared to increase with time finally amounting to 40 % of applied ^{14}C. The radioactivity contained in the extractable fractions were identified as propanil, 3,4-dichloroaniline (DCA), and N-β-D-glucopyranosyl-3,4-dichloroaniline (DCA-Glu). In treatment b, significant ^{14}C amounts were released to the water (6 % of applied ^{14}C) and the solubilized radioactivity fractions were demonstrated to agree with those found in the extractable fractions. Therefore, if residues of the pesticide propanil are taken up by plants, it may enter again the aquatic environment after plant death and submergence. This phenomenon may have a potential impact on aquatic organisms, which to our knowledge has not been reported before. As plant uptake and degradation of xenobiotics are recognized as detoxification, we consider *B. pilosa* with its high uptake potential, at least for propanil, as suitable species for phytoremediation.

Keywords Three Gorges Reservoir (TGR) · *Bidens pilosa* L. · Propanil · Pesticide residues · Non-extractable residues (NER)

Abbreviations

TGR	Three Gorgers Reservoir
DOC	Dissolved organic carbon
NER	Non-extractable residues
AR	Applied radioactivity
CFU	Colony forming units
LSC	Liquid scintillation counter

Introduction

Construction of the Three Gorges Dam created a unique ecosystem in the upper stream of the Yangtze River; the corresponding reservoir, the Three Gorges Reservoir (TGR) area amounts to 58,000 km^2 (water surface area 1080 km^2). For purposes of flood control, energy production, and navigation improvement, the water level of the reservoir fluctuates between 145 m during summer and 175 m above sea level during winter, resulting in a drawdown zone of 350 km^2. The hydrology of TGR is anti-seasonal thus, effects of water

Responsible editor: Philippe Garrigues

✉ Zhongli Chen
zhongli.chen@bio5.rwth-aachen.de

[1] Institute for Environmental Research (Biology V), RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] School of the Environment, Nanjing University, Xianling Av. 163, 210023 Nanjing, China

[3] College of Resource and Environmental Science, Chongqing University, 174 Shazheng Street, 400030 Chongqing, China

fluctuation may strongly influence environmental and ecological processes of the TGR, i.e., sediment deposition, water pollution, self-purification capacity, and increased erosion. In summer of 2009 (after the first 30 m height winter flooding), decline of plant diversity was found in the littoral zone area (Sun et al. 2012; Wang et al. 2012). Surviving plants were either annual fast growing species or perennial species tolerant to submergence, which may absorb nutrients and pollutants present in the water of the TGR and derived from resuspended possibly contaminated sediment. Vegetation in the littoral zone was found with higher plant richness and biomass, in contrast to the vegetation found above the submerged region of TGR (e.g., 175–200 m). This finding implies the potential use of the littoral zone; farmers living around the reservoir are expected to cultivate the land which they have used traditionally. In order to guide sustainable use, an experimental field station has been established in Kaixian County (Chongqing) of the TGR region since 2008, in which several ecological engineering studies have been conducted (Willison et al. 2013). Vegetation at the river bank acts as ecological buffer mainly due to dominant species; these are critical species for investigating ecosystem succession. Plants have been proposed and occasionally used for reducing contamination by organic pollutants in freshwater ecosystems. This technique, designated phytoremediation was examined both, in the laboratory and field (Vymazala and Kröpfelová 2009; Williams 2002). However, the plants of the littoral zone of the TGR are submerged in winter due to inundation and then, natural constituents of the plants and pesticide residues potentially accumulated and metabolized by the plants can be released into TGR water. Thus, phytoremediation techniques applied in the TGR region should include both, pollutant absorption and transformation by the plants and the possible release of residues by plants decaying into the water phase.

In freshwater ecosystems, macrophytes serve key functions by providing primary production, creating habitats for wetland biota, and strongly influencing ecosystem processes, e.g., elemental geochemical cycling (Cronk and Fennessy 2001). In past decades, numerous studies indicated that vascular plants are main source of total organic inputs in wetlands ranging from 10 % to above 75 %. Plant litter decomposition thus, is thought to play an important role in wetlands (Tristan Gingerich and Anderson 2011). Plant litter is decomposed by invertebrate activity (shredders), chemical decay, and microbial decomposition (Webster and Benfield 1986). Freshwater invertebrates fragment and decompose plant litter, and utilize it as a food source. Because of higher surface, litter fragments are prone to more rapid chemical and microbial degradation (Leroy and Marks 2006). Plant litter fragments can release soluble materials shortly after immersion in water; composition and properties (e.g., toxicity, mineral contents, solubility) of these materials change with time. Studies on litter decomposition may first aim at its initial C/N ratio (Battle and Golladay 2001; Taylor et al. 1989); low values point to fast decomposition (Berendse et al. 1987; Coley et al. 1985; Minderman 1968; Nakai et al. 1986). In contrast to rapid loss of soluble, low molecular weight of organic compounds, degradation of polymers, such as cellulose and hemicellulose, is slower and in case of lignin very slow. During degradation, portions of hemicellulose and cellulose thus, decline, whereas relative portions of lignin increase (Godshalk and Wetzel 1978a, b; Paul et al. 1983). Unlike fragmentation by invertebrates and chemical decomposition, microbial action may more or less completely degrade plant materials including polymers, thereby providing essential elements, especially carbon and nitrogen to ecosystems (Austin and Ballare 2010; Belova 1993). In freshwater ecosystems, aquatic hyphomycetes produce polysaccharide hydrolyzing exoenzymes including pectinases, hemicellulases, cellulases, and chitinases (Abdel-Saterar and Ei-Said 2001; Duarte et al. 2006). However, these enzymes do not catalyze the degradation of lignin, which can only be attacked by extracellular oxidative and ligninolytic enzymes produced by certain fungi (Bucher et al. 2004; Fisher et al. 1983; Romaní et al. 2006; Suberkropp and Klug 1980).

Nowadays, large amounts of xenobiotics such as pesticides are present in the environment (Villeneuve et al. 2011). After absorption, pesticides are metabolized by plants in three-phased process (phase I: transformation, phase II: conjugation, phase III: compartmentation) and finally bound by various plant components (Casida and Lykken 1969). Considerable portions of pesticides and their metabolites may be incorporated into plant macromolecules, i.e., proteins, starch, cellulose, hemicellulose, pectin, and lignin (Sandermann 2004). These portions of applied xenobiotics are difficult to extract from plant tissues even using a range of solvents with different polarities and thus, were defined as non-extractable residues (NER) by IUPAC (Mordaunt et al. 2005; Schmidt 1999; Skidmore et al. 1998). NER fractions were thought to be end product of the plant metabolism of xenobiotics; NER formation is regarded as detoxification process. Concerning plants consumed by animals and humans (e.g., crops, herbs), studies on their bioavailability, maximum residue limits (MRLs), and chemical nature of NER are necessary. In a number of cases, NER bioavailability to animals ranged from 3 % to more than 60 % (Khan et al. 1990; Sandermann 2004; Sandermann et al. 1990). Higher amounts of NER in crop plants usually lead to reduction of existing MRLs values (Sandermann 2004). Methods to distinguish among different mechanisms of binding of xenobiotic moieties to polymeric plant constituents have been published (Sandermann 2004). Due to difficulties to isolate NER fractions from plant tissues, structural identification of these residues was only rarely successful (Brunow et al. 1998; Sandermann 2004). To our knowledge, the fate of plant NER of xenobiotics in the

environment has only insufficiently been investigated before, though release of pollutants or their metabolites from plant litter containing extractable and non-extractable residues can be of environmental relevance.

In the present study, ^{14}C-ring-labeled 3′,4′-dichloropropionanilide (common name: propanil), which belongs to the herbicide class of phenylamides, was selected as model substance. Propanil is a selective post emergence herbicide for control of broad leaf and grass weeds in rice (*Oryza sativa* L.). Propanil has a low acute toxicity (Hofstra and Switzer 1968). Its primary metabolite, 3,4-dichloroaniline (DCA), is highly toxic for a number of aquatic organisms (Crossland 1990; Giacomazzi and Cochet 2004). Metabolites, especially 3,3′,4,4′-tetrachloroazobenzene (TCAB) and 3,3′,4,4′-tetrachloroazoxybenzene (TCOAB) are occasionally produced in soils and sediments. Both were considered as embryolethal, teratogenic, and genotoxic, but part of the data is questionable (Birgelen et al. 1999; Poland et al. 1976; Still 1969; Witt et al. 2000). Plant metabolism of propanil leads to considerably less toxic metabolites, e.g., 3,4-dichloroaceteanilide (DCAA) and N-β-D-glucopyranosyl-3,4-dichloroaniline (DCA-Glu), and NER. *Bidens pilosa* (English: e.g., black-jack; Chinese: gui zhen cao) native to the Americas is a dominant annual species in the littoral zone of TGR because of its adaption to fluctuating water levels; it produces seeds before submergence. This plant species was used to evaluate uptake and metabolism of propanil. In addition, we investigated whether extractable and non-extractable residues of propanil present in decaying plant materials are released into the water.

Materials and methods

Chemicals

[Ring-U-^{14}C]-3′,4′-dichloropropananilide (^{14}C-propanil) was purchased from the Institute of Isotopes (Budapest, Hungary); its specific activity was 1013 MBq/mmol, its radiochemical purity 98.42 %. Non-labeled propanil and 3,4-dichloroacetanilide (DCAA) were supplied by Sigma-Aldrich (Taufkirchen, Germany). 3,4-dichloroaniline (DCA) was provided by Riedel-de Haen (Seelze, Germany). N-β-D-glucopyranosyl-3,4-dichloroaniline (DCA-Glu) was synthesized according to a published procedure (Gareis et al. 1992). Water used for the incubation experiment (see later) was obtained from the catchment area of the Yangtze Three Gorges Reservoir, i.e., from Baijia stream (a tributary of the Pengxi River, N 31° 08′ 03.27″ and E 108° 33′ 28.87″) in Kaixian county of Chongqing, China in 2012. The water was assumed to possess the necessary chemical properties for a study with implications on the TGR.

Preparation of plant material and application-pre-incubation phase

Seeds of *Bidens pilosa* L. 1753 (English: e.g., black-jack; Chinese: gui zhen cao) were sampled in the TGR region, i.e., from Daning River (a tributary of the Yangtze River) estuary (N 31° 05′ 11.39″ and E 108° 53′ 12.07″), near Wushan county of Chongqing, China in 2011, transported to the Aachen laboratory (Germany) and were kept at 4 °C prior to use. They were germinated on wet filter-paper for 7 days in darkness at 22 °C. Then, seedlings were transferred onto steel wire gratings (4 mm mesh width) placed upon the surface of 50 ml of Hoagland nutrient solution (Hoagland and Arnon 1950) contained in cultivation flasks (5.0 × 8.5 cm; diameter × height). Seedlings were cultivated until the four leaves stage and were subsequently transferred to 50 ml of fresh Hoagland nutrient solution in identical cultivation flasks.

For the experiment, six plants of *B. pilosa* were separately placed in 150 ml beakers as described before, which contained Hoagland nutrient solution treated with 500 μg of ^{14}C-propanil (47 KBq, dissolved in 60 μl of methanol) prior to introduction of the plants. Plants were cultivated for 7 days pre-incubation phase for absorption and metabolization of the herbicide. Further, plants (six parallels) were treated in a similar way with non-labeled propanil, which were designed for monitoring decay of the plant materials (determined as dissolved organic carbon in water; see below). Control using plants not treated with propanil (six parallels) were utilized to examine microbial concentrations during decay of the plant materials.

Incubation, extraction, and analysis

After pre-incubation, the plants (treated with ^{14}C- or non-labeled propanil and control plants) were each divided into two groups: the first group of plants was introduced into the subsequent incubation experiment without preceding extraction (in the following abbreviated as plant tissue samples: PT), whereas the second group was first extracted and then the extracted plant tissue was incubated (in the following designated as extracted plant tissue: EPT).

The PT plants were cut into 2 cm pieces and were each directly transferred into a flask (500 ml) containing 400 ml of a mixture of filtered water from the TGR filtered 0.45 μm and autoclaved tap water (1:1, *v*/*v* =; designated as TGR water). Flasks were closed with absorption devices containing 15 g soda lime for trapping $^{14}CO_2$. EPT plants were cut into 2 cm pieces and extracted using 30 ml of Bligh-Dyer mixture (chloroform/methanol 1:2, *v*/*v*) (Bligh and Dyer 1959) for 3 h at 22 °C; the procedure was finished by treating the plants tissues with an ultraturrax mixer (IKA, Staufen, Germany) for about 5 min. Extracted plant materials and extract were separated by means of suction filtration. Plant debris were washed with a

modified Bligh-Dyer mixture (chloroform/methanol/H_2O 1:2:0.8, *v*/*v*/*v*) and water consecutively. Assays were then treated as described for PT assays. All assays (a total of 18, three parallels with each treatment modification) were incubated for a total of 90 days on a shaker (50 rpm) in darkness at 22 °C. After 14, 28, and 56 days, the water of all assays was removed and flasks were refilled up with fresh TGR water as described before. After 90 days of incubation, plant debris of PT assays were separated from water phase by suction filtration and extracted as described for plant materials used for EPT assays. Release of solubilization of natural was determined by measuring the DOC content (see below) in all water phases emerging in the course of the experiment. Release of soluble ^{14}C from plant materials were investigated by measurement of the ^{14}C content of the TGR water phase (liquid scintillation counting, LSC; LS 6500, Beckman, Krefeld, Germany). Soda lime contained in $^{14}CO_2$ traps was solubilized in concentrated HCl and $^{14}CO_2$ evolved was absorbed in a suitable scintillation cocktail; resulting samples were examined by LSC. All insoluble plant residues emerging during the experiment were air-dried and subjected to ^{14}C combustion analysis (Biological Oxidizer OX 500, Zinsser/Harvey Instruments, Frankfurt, Germany).

C, H, N analyses of *B. pilosa* plants in their four leaves stage were performed on a vario EL III (Elementar Analysensysteme, Hanau, Germany). DOC concentrations in water phases were determined by Eurofins Umwelt West (Aachen, Germany). Microbial activity (plate count method) (APHA 2005; HC 2006; HPA 2005) of all water samples were analyzed as follows: original samples were first diluted appropriately and spread on solidified media for bacteria or fungi (Standard I nutrient, Merck, Darmstadt, Germany; Difco™ Czapek Dox Agar, Nordwald Hamburg, Germany). Plates were then incubated in the dark for 3 days (room temperature), when colonies were counted as colony forming units (CFU) (Goldman and Green 2008).

Medium from pre-incubation period and water phases from incubation period were extracted with ethyl acetate (2 × 10 and 3 × 100 ml, respectively). The resulting extracts were evaporated to dryness and residues were dissolved in 10 ml of methanol for subsequent radio-thin-layer chromatography (radio-TLC) analysis. Extracts of all plant tissues were treated similarly. Solvents of TLC were A (methylene chloride/toluene 9:1, *v*/*v*) and B (methylene chloride/acetone 10:1, *v*/*v*). A maximum of 500 μl of the extracts containing 85–170 Bq of each sample was placed on pre-coated silica plates (SIL G-25 UV 254, 0.25 mm, Macherey-Nagel, Düren, Germany). Remaining portions of the samples were further concentrated to 1 ml and filtrated (0.45 μm) for radio-HPLC analysis. Eluents were A (H_2O + 0.1 % acetic acid, *v*/*v*) and B (methanol/acetonitrile 4:5, *v*/*v* + 0.1 % acetic acid, *v*/*v*). Flow was 1 ml/min using the following gradient program: 0–5 min, isocratic A:B 80:20 (*v*/*v*); 5–10 min, linear gradient to A:B 55:45; 10–25 min, isocratic A:B 55:45; 25–30 min, linear gradient to A:B 15:85; 30–35 min, isocratic A:B 15:85; 35–45 min, linear gradient to A:B 80:20; 45–50 min isocratic A:B 80:20. The HPLC system, which consisted of HP Agilent 1200 apparatus including degasser, pump, autosampler, and UV/Vis DAD modules (Bad Homburg, Germany) completed with radio detector Ramona Star including a liquid scintillation cell (cocktail: Quick Safe Flow 2; Zinsser Analytic, Frankfurt, Germany) and corresponding LC cocktail pump module (Raytest, Straubenhardt, Germany). Samples and reference compounds were chromatographed on a Nucleosil C18 Column (250 × 4 mm, CS-Chromatographie Service, Langerwehe, Germany) and corresponding pre-column (20 × 4 mm). A volume of 100 μl of the sample containing about 850 Bq was injected into the system; measurement of non-labeled references was executed at 250 and 350 nm. After analytical examination of samples, interesting HPLC fractions were isolated and collected by preparative HPLC (with detached radio detector) for GC-EIMS analysis. GC-EIMS was done using an Agilent 6890 N gas chromatography equipped with an Agilent 5973 mass selective detector (Agilent Technologies, Waldbronn, Germany). Samples were examined by an Optima-35-MS capillary column (Macherey-Nagel, 30 m length, 0.25 mm diameter, 0.25 μm film thickness). Carrier gas was He at a flow of 1.0 ml/min. Temperature program was 50 °C ramped at 10 °C/min to 280 °C, followed by another ramp to 240 °C and finally to 270 °C. Injector temperature 250 °C and MS temperatures were 230 °C (source) and 150 °C (quadrupole). Full scan electron impact mass spectra were recorded with m/z 50–550 and 70 eV ionization energy. Identification of substances in samples was performed by comparison with corresponding data (retention time, mass spectrum) of reference substances (propanil, DCA, DCAA) and with mass spectra reported in NIST library.

Results and discussion

Uptake and distribution of radioactivity

The distribution of radioactivity determined after the defined incubation periods is shown in Table 1. ^{14}C-Propanil was rapidly accumulated by *B. pilosa* during 7 days pre-incubation. Only 2.83 % (PT assays) and 7.88 % of applied ^{14}C (EPT assays) remained in nutrient media. Soluble, extractable radioactivity was the major portion found in the plants after pre-incubation in case of the EPT assays (51.16 %). Extraction was not executed with the PT assays at this stage of the experiment. After 90 days of incubation, only a minor portion of radioactivity was extracted from remaining of the PT assays (3.48 %), while 29.36 % of ^{14}C was released into the TGR water phase. Nevertheless, the plant materials of the EPT

Table 1 Distribution of radioactivity after termination of the experiments; pre-incubation: 7 days, incubation 90 days

Phase	Fraction	PT assays %	EPT assays %
Pre-incubation[a]	Nutrient media	2.17 ± 1.77	7.88 ± 2.29
	Plant extract	Not performed	51.16 ± 2.68
Incubation[b]	Plant tissue extract	3.48 ± 0.54	Not performed
	TGR water[c]	29.36 ± 5.58	6.00 ± 0.37
	Mineralization ($^{14}CO_2$)	0.96 ± 0.09	0.75 ± 0.11
	NER	40.36 ± 7.3	11.19 ± 3.95
Recovery		76.99 ± 11.88	76.98 ± 1.60

Values are in percent of radioactivity applied for pre-incubation and are averages of three parallels ± SD at the 95 % level

[a] Pre-incubation phase: Plants were incubated in Hoagland nutrient solution containing 500 μg of ^{14}C propanil solution for 7 days. Plants from EPT assays were cut into pieces and extracted using Bligh-Dyer solvent mixture ('Plant extract') after the pre-incubation. Plants from PT assays were only cut into pieces

[b] Incubation phase: PT assays: Plant pieces resulting from pre-incubation were transferred to TGR water. After 90 days of incubation, remaining plant materials were extracted using Bligh-Dyer solvent mixture ('Plant tissue extract'). EPT assays: Extracted plant materials were transferred to TGR

[c] TGR water: water from the TGR filtered (0.45 μm) and autoclaved tap water 1:1 (*v*/*v*)

assays, which were extracted after pre-incubation and thus, contained only non-extractable ^{14}C residues (NER), released significant amounts of radioactivity (6 % of applied ^{14}C) into the water phase. With all assays, only traces of ^{14}C (0.96 % for PT and 0.75 % for EPT) were found mineralized by the microorganisms.

Previously published studies indicated that the NER of ^{14}C-propanil or primary metabolite ^{14}C-DCA treated plants increased with time, ranging from 30 % (48 h) to 92 % (at harvest) (Bockers et al. 1994; EFSA 2013; Gareis et al. 1992). In the present study, portions of NER of the PT assays determined after 90 days of incubation (40.36 %) were possibly higher than those present in the EPT assays after pre-incubation and extraction. After 90 days of incubation, only 17.94 % of applied ^{14}C (sum of ^{14}C in TGR water, $^{14}CO_2$ and NER after incubation) were detected in the EPT assays. This would point to an increase of portions of NER in the PT assays during the 90 days of incubation—i.e., after removing plants from pre-cultivation culture media, cutting them into pieces and introducing them into TGR water. Recovery of ^{14}C was 76.99 and 76.98 %, respectively, with the PT and EPT assays. Reasons for losses of radioactivity were not obvious. Besides minor parts of ^{14}C-labeled lost with procedures, e.g., adsorbance to the experimental apparatus, inaccurate determination of applied amounts may have been the main reason for low recovery values. This assumption is at least supported by quite similar recoveries of ^{14}C with both, the PT and EPT assays.

Release of DOC and radioactively during incubation phase

The C, H, N content of maturing plants of *B. pilosa* were 40.93, 5.44, and 4.37 % of the plant dry weight, respectively. The C/N ratio thus, amounted to 9.4; consequently, a rapid decay process was expected. The release of DOC and ^{14}C from the plant materials during the 90 days of incubation are shown in Fig. 1. The results indicated that the main decomposition period of *B. pilosa* was in the first few weeks after introduction of plant materials into TGR water with a massive DOC release. Thus, finding is in line with data on other fast decomposing macrophytes (Facell 1991). Unexpectedly, release of DOC from the pre-extracted plant tissues (EPT assays) was higher (22.50 mg/L after 90 days) than that of non-extracted plant tissues (PT assays; 7.06 mg/L). Explanations for this result are given in the following: (1) due to homogenization and extraction terminating the pre-incubation phase, the contact surface of the plant tissues in case of EPT were enlarged; (2) plant structures were severely damaged with the EPT assays and thus, easier degraded by microbes or via physical and chemical reactions. It is however, questionable, whether these arguments can explain the unexpected higher release of natural organic materials from the EPT as compared to the PT tissues. Questions remained especially because the release of ^{14}C from the same assays was higher in the PT than in the EPT assays. So far, further conclusive assumptions are not at hand—neither from the literature nor from additional data determined during the experiments. DOC concentrations in both groups of assays were in the range of concentrations observed in the environment (Chen et al. 2011). Soluble ^{14}C was released from plant materials of the both groups of assays decayed with highest rate in the first 14 days of incubation (PT assays 18.93 %, EPT assays 3.01 %). After 14 days, release of ^{14}C slowed down, and finally after 90 days, total of 29.36 % (PT) and 6 % (EPT) of applied radioactivity were dissolved into the water phase. It was assumed that in the course of the

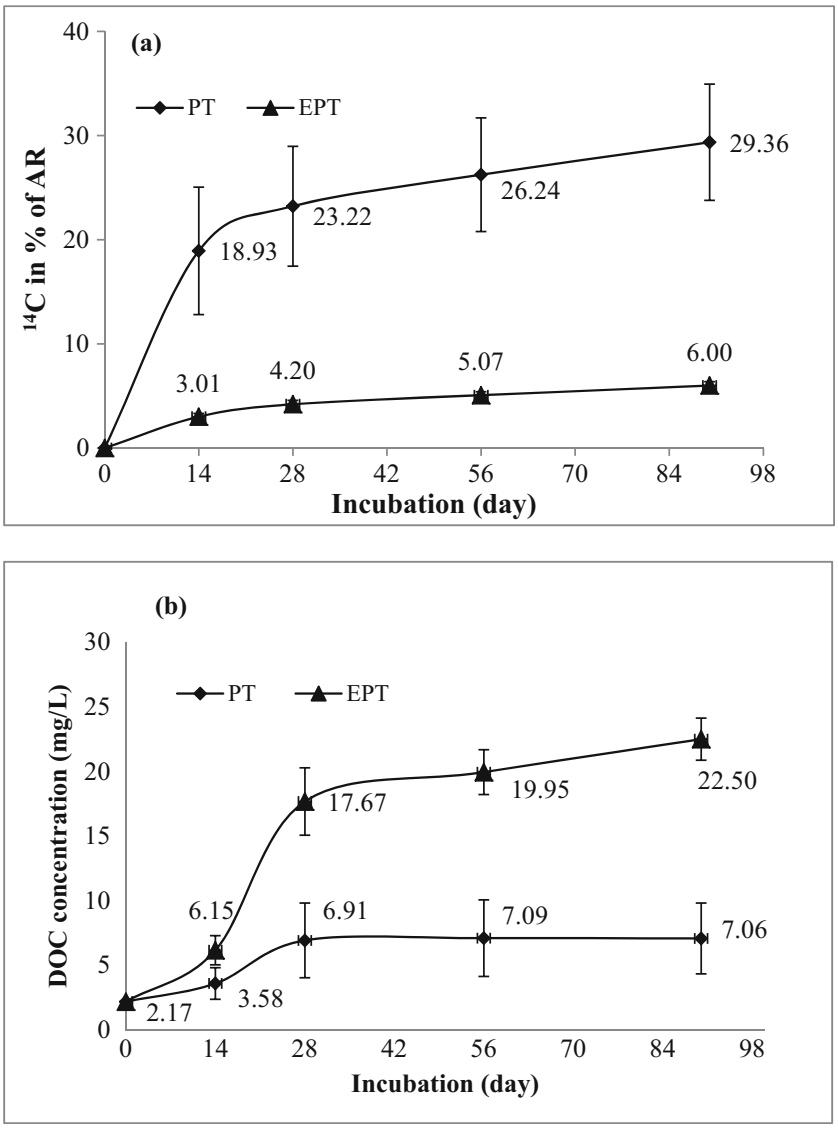

Fig. 1 Release of soluble ^{14}C in % of applied radioactivity (**a**) and total DOC (**b**) from plant debris into TGR water during the incubation phase (90 days). All values are average of three parallels ± SD at the 95 % level

experiment, both soluble and insoluble portions of the ^{14}C residues were released from the plant debris of PT assays, whereas in case of the EPT assays only portions of the NER were released. Consequently, amounts of ^{14}C found in TGR water of PT assays were higher than those of the EPT assays. Non-extractable portions of ^{14}C in insoluble plant materials may be bound to plant macromolecules, which are degraded by microorganism with time. Macromolecules of plant cells such as pectin, cellulose, and hemicellulose are hydrolyzed rapidly. Therefore in the first 14 days of incubation, a high release rate of ^{14}C residues was observed in the present experiment, followed by a slower release of radioactivity possibly because of a slower degradation of the more resistant macromolecules. Non-extractable pesticide residues can be bound either covalently or non-covalently to plant macromolecules, forming NER fractions. In comparison to covalent-bound residues, residues entrapped non-covalently in the macromolecular plant matrix are more easily released (Schmidt 1999). Microbial release of the non-covalent NER fractions is thought to be more effective. Covalent lignin conjugates with chloroanilines have been identified by ^{1}H and ^{13}C NMR spectroscopy (Still et al. 1981; Trenck et al. 1981). Non-extractable residues are regarded the final metabolic products in plants at least in case of xenobiotics containing an aniline function; portions of these residues usually increase with incubation period and become more stable (Casida and Lykken 1969; Sandermann 2004; Schmidt 1999; Skidmore et al. 1998; Still et al. 1981; Trenck et al. 1981). Accordingly, portions of non-extractable residues found in the present experiment with propanil would have increased and become more stable if pre-incubation was extended. Release of ^{14}C from both, PT and EPT assays is thought to decrease with increasing pre-

incubation interval. If the incubation period is extended beyond 90 days, release of ^{14}C in PT and EPT assays may be assumed to slow down possibly even come to an end.

In the present study, microbial abundance associated with the water phase and mineralization of ^{14}C residues was determined in the PT and EPT assays during the incubation phase (90 days) by means of the plate count method and trapping and measure of $^{14}CO_2$ evolved, respectively. Data are shown in Table 2. CFU of bacteria and fungi did not differ significantly between the PT and EPT assays up to 14 days of incubation. Bacterial CFU then increased with time in PT assays, whereas in EPT assays a strong decline (day 28–56) was followed by varying CFU. Fungal CFU exhibited the same trend in both groups of assays. They showed maximum numbers after 14 days, then varied until the end of incubation. Mineralization of ^{14}C residues was rather correlated with the CFU of the fungi than those of the bacteria. This may indicate that fungi rather than bacteria dominated the degradation and mineralization of the plant material including the ^{14}C-propanil residues, which is in line with previous studies (Komínková et al. 2000; Romaní et al. 2006; Sánchez 2009; Sandermann et al. 1998).

During degradation of plant litter, polysaccharides, and other easily degradable constituents enter the water phase and provide additional energy for microbes, which proliferate and further decompose natural and xenobiotic substances. All major fungal phyla are usually present in aquatic habitats, while fungal communities in plant litter are dominated by ascomycetes and hyphomycetes (Gessner et al. 2007). Leachates of *B. pilosa* not only contain nutrition for microbes but also potentially phytotoxins such as pyrocatechin or salicylic acid, which according to Deba et al. (2007) might be responsible for the inhibition of fungal activity in agricultural soil (Deba et al. 2007). Such phytotoxins are water soluble and are rapidly degraded (Sydes and Grime 1981) which in the present study might explain the decrease of fungal CFU in the period 14–28 days.

Analysis of soluble radioactivity

A first characterization of the radioactivity contained in the nutrient media, plant extracts, plant tissue extracts, and water phase was performed using radio-thin layer chromatography (radio-TLC). Radioactivity corresponding with interesting peaks was calculated as percent of applied radioactivity. Parent compound propanil (R_f = 0.49–0.55) and metabolites DCA (R_f = 0.65–0.72), DCAA (R_f = 0.33–0.40) and DCA-Glu (R_f = 0.00–0.04) were used as references. Identification of ^{14}C components in the samples were executed by co-chromatography with these references. The TLC data are presented in Table 3. Parent propanil was found in all fractions ranging from 0.13 to 4.68 %. DCA and DCAA were main metabolic products present in most fractions (0.07–2.17 and 0.13–6.24 %, respectively). An unknown substance with R_f = 0.28 was only present in some samples (0.09 and 1.37 %). Noticeable amount of radioactivity from all samples remained at the start of the plate. In most samples these portions of radioactivity ranged between 0.41 and 5.29 %, with the exception of the plant extract of the EPT assays (34.12 %).

Results from high performance liquid chromatography (HPLC) coupled to a radioactivity detector are shown in Table 4. Roughly, the data derived from HPLC analysis resembled those of TLC analysis discussed before. Differences, however, were observed especially with DCA-Glu and DCA. In case of the HPLC data, portions of DCA-Glu were lower as those detected with TLC analysis (vice versa regarding portions of DCA). These differences were thought to result from the chemically labile N-glucoside DCA-Glu, which was assumed to be hydrolyzed to DCA (and glucose) during sample preparation for HPLC analysis. Propanil (R_t = 28.75 min) and its metabolites

Table 2 Development of bacterial and fungal populations associated with the decay of plant materials of *B. pilosa* (pre-incubated for 7 days with 500 μg of ^{14}C-propanil; PT: non-extracted plant tissues, EPT: extracted plant tissues) in TGR water, as well as mineralization of ^{14}C residues contained in the plant materials

		0 day	14 days	14–28 days	28–56 days	56–90 days
PT	Bacteria[a]	40 ± 11	698 ± 107	1134 ± 417	1668 ± 155	984 ± 216
	Fungi[a]	2 ± 0	882 ± 76	141 ± 19	611 ± 241	278 ± 69
	$^{14}CO_2$[b]	0.00	0.07 ± 0.01	0.03 ± 0.01	0.16 ± 0.07	0.69 ± 0.16
EPT	Bacteria[a]	40 ± 11	650 ± 242	141 ± 97	25 ± 6	427 ± 60
	Fungi[a]	2 ± 0	534 ± 250	57 ± 63	343 ± 164	151 ± 48
	$^{14}CO_2$[b]	0.00	0.09 ± 0.03	0.06 ± 0.01	0.41 ± 0.1	0.19 ± 0.07

All values are averages of three parallels ± SD at the 95 % level

[a] Bacteria and fungi were counted by colony forming units (CFU) per microliter sample

[b] The $^{14}CO_2$ is presented in percent of applied ^{14}C

Table 3 Radio-TLC analysis (solvent system: A dichloromethane/toluene 9:1 *v/v*; B dichloromethane/acetone 10:1 *v/v*) of nutrient media, extracts, and water phases emerging during pre-incubation of *B. pilosa* plants with ^{14}C-propanil and subsequent incubation of resulting *B. pilosa* plant tissues in TGR water

	DCA-Glu R_f = 0.00–0.04 %	Unknown R_f = 0.28 %	DCAA R_f = 0.33–0.40 %	Propanil R_f = 0.49–0.55 %	DCA R_f = 0.65–0.72 %
PT assays:					
Nutrient media[a]	1.05	n.d.	0.37	0.57	n.d.
Plant tissue extract[b]	2.59	n.d.	n.d.	0.24	0.24
14 days water[b]	8.29	1.37	2.53	2.97	2.17
14–28 days water[b]	1.94	0.34	0.54	0.88	n.d.
28–56 days water[b]	2.09	n.d.	n.d.	0.29	n.d.
56–90 days water[b]	2.11	n.d.	0.27	0.23	n.d.
EPT assays:					
Nutrient media[a]	4.02	n.d.	1.25	1.00	0.43
Plant extract[a]	34.12	n.d.	6.24	4.68	n.d.
14 days water[b]	1.50	n.d.	0.50	0.32	0.19
14–28 days water[b]	0.59	n.d.	0.23	0.13	0.07
28–56 days water[b]	0.41	0.09	0.13	0.13	n.d.

Parent and metabolites were identified by co-chromatography with authentic reference compounds. Data are given as the percent of applied ^{14}C

n.d. not detected, *DCA-Glu* N-β-D-glucopyranosyl-3,4-dichloroaniline, *DCAA* 3,4-dichloroacetanilide, *DCA* 3,4-dichloroaniline

[a] Pre-incubation phase (7 days)

[b] Incubation phase (up to 90 days)

DCA (R_t = 21.83 min), DCA-Glu (R_t = 14.15 min), and a fraction with R_t = 34.87 min were detected in all fractions; DCAA (R_t = 24.38 min) was only observed in the media from pre-incubation phase. Portions of propanil only amounted to 0.14–1.14 and 0.11–3.81 % in all soluble fractions of PT and EPT assays, respectively, which demonstrated the ability of *B. pilosa* to absorb and metabolize propanil. In the course of the incubation phase, release of

Table 4 Radio-HPLC analysis of nutrient media, extracts, and water phases emerging during pre-incubation of *B. Pilosa* plants with ^{14}C-propanil and subsequent incubation of resulting *B. pilosa* plant tissues in TGR water

	DCA-Glu R_t = 14.15 min %	DCA R_t = 21.83 min %	DCAA R_t = 24.38 min %	Propanil R_t = 28.75 min %	Unknown R_t = 34.87 min %
PT assays:					
Nutrient media[a]	0.45	0.24	0.63	0.20	0.66
Plant tissue extract[b]	0.27	0.29	n.d.	0.14	2.79
14 days water[b]	0.10	14.64	n.d.	0.99	3.20
14–28 days water[b]	0.04	2.62	n.d.	0.37	0.60
28–56 days water[b]	0.12	1.58	n.d.	0.80	0.32
56–90 days water[b]	0.24	0.79	n.d.	1.14	0.71
EPT assays:					
Nutrient media[a]	0.52	2.48	0.53	0.65	3.71
Plant extract[a]	8.81	30.84	n.d.	3.81	7.69
14 days water[b]	0.18	1.87	n.d.	0.31	0.47
14–28 days water[b]	0.11	0.47	n.d.	0.11	0.50
28–56 days water[b]	0.07	0.48	n.d.	0.06	0.26
56–90 days water[b]	0.19	0.33	n.d.	0.11	0.30

Parent and metabolites were identified by co-chromatography with authentic reference compounds. Data are given as the percent of applied ^{14}C

n.d. not detected, *DCA-Glu* N-β-D-glucopyranosyl-3,4-dichloroaniline, *DCAA* 3,4-dichloroacetanilide, *DCA* 3,4-dichloroaniline

[a] Pre-incubation phase (7 days)

[b] Incubation phase (up to 90 days)

DCA from the plant materials decreased from 14.62 % (PT) and 1.87 % (EPT) at day 14 to 0.79 % (PT) and 0.33 % (EPT) at days 56–90. After incubation of 90 days of the PT assays, 4.64 and 26.86 % of the ^{14}C residues released into TGR water and those in the plant extracts were confirmed as propanil and DCA, respectively; 40.36 % remained non-extractable. In EPT assays, portions of propanil and DCA, respectively amounted to 3.29 and 17.56 %, while a total of 62.35 % of applied ^{14}C comprised NER fractions (non-extractable residues after pre-incubation + non-extractable residues after incubation).

The radioactivity contained in the plant tissue extract of EPT assays (pre-incubation 7 days) and the TGR water extract of PT assays emerging after 14 days of incubation was fractionated and collected according to the HPLC signals. Resulting fractions were prepared for analysis by gas chromatography mass-spectrometer (GC-EIMS) in order to confirm identities of propanil and DCA (Fig. 2). Both compounds were identified in the samples examined by means of their retention times and EI mass spectra as well as corresponding data obtained by references of DCA and propanil and the NIST data base. Molecular ion of DCA was found at

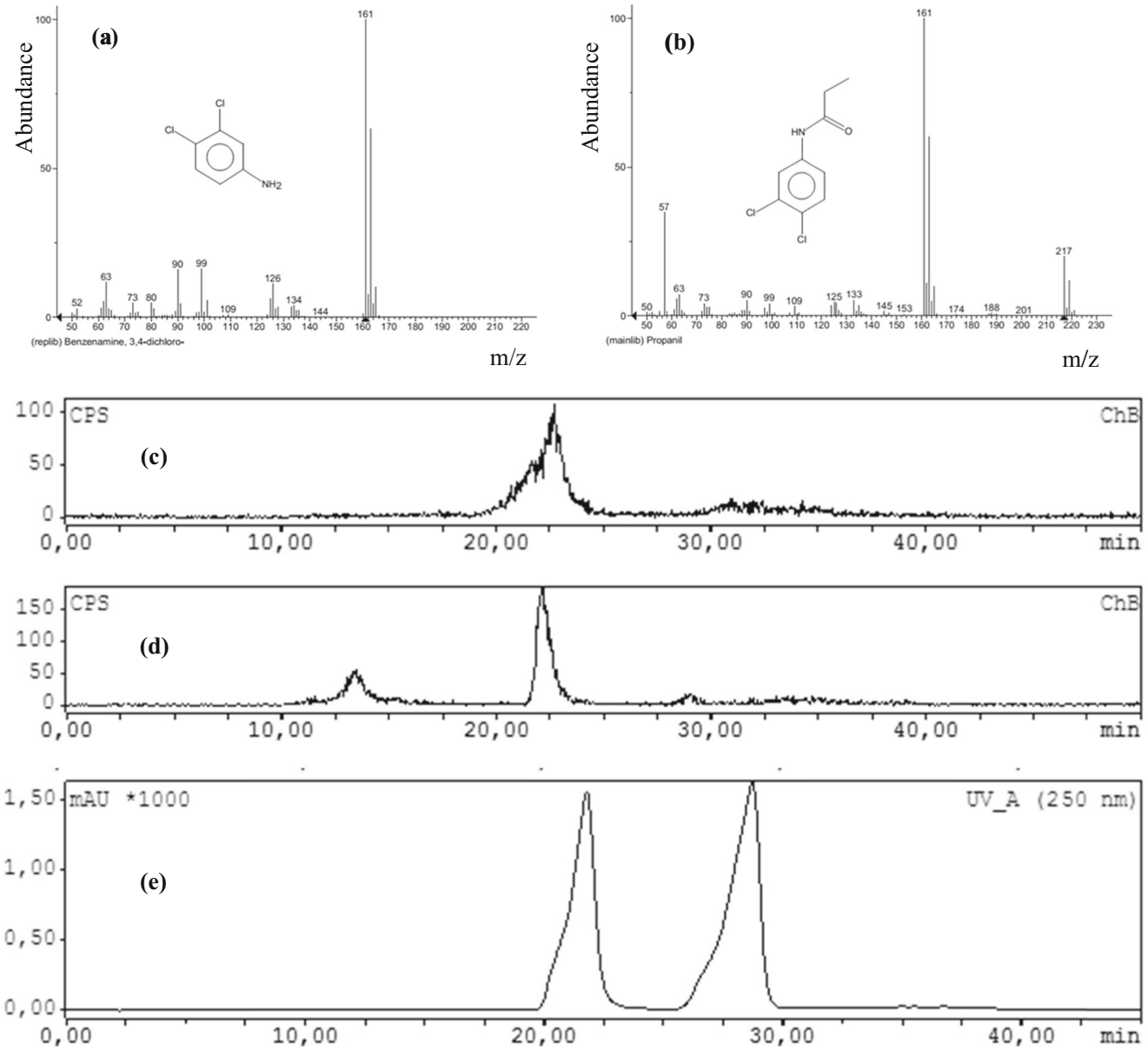

Fig. 2 HPLC analysis and fractionation and GC-EIMS analysis of propanil and DCA contained in samples resulting from the incubation study (plant tissue extract of EPT assays, 7 days of pre-incubation; TGR water extract emerging after 14 days of incubation from PT assays); mass spectra were similar with both samples. **a** EI mass spectrum of 3,4-dichloroaniline (DCA). **b** EI mass spectrum of propanil. **c** Radiochromatogram of 14 days water sample of PT assay. **d** Radiochromatogram of plant sample from EPT assay. **e** UV/Vis chromatograms (250 nm) of references propanil (R_t = 28.75 min) and DCA (R_t = 21.83 min)

m/z = 161/163/165, which clearly shows the presence of three chlorine substituents. Similarly, propanil showed a molecular ion at m/z = 217/219/211, and a prominent ion fragment at 161/163/165 due to mass spectral cleavage of the propionic acid moiety. DCA-Glu could not be identified by GC-EIMS, because it is volatile under such conditions. The HPLC fraction R_t = 12–16 min was collected and re-analyzed by TLC (methylene chloride and chloroform/methanol/H_2O 65:25:4, *v*/*v*/*v*) (Gareis et al. 1992). The radiochromatogram showed a single peak with R_f = 0.35, which co-chromatographed with that of reference DCA-Glu. The HPLC fraction eluting between R_t = 32–36 min was collected and re-injected into the HPLC system. The radiochromatogram exhibited three peaks (peak 1, R_t = 21.83 min; peak 2, R_t = 28.75 min; peak 3, R_t = 34.87 min). It was concluded that the radioactivity appearing in the first radiochromatogram at R_t = 32–36 min represented a HPLC artifact possibly due to interfering effects by plant matrix compounds. In summary, the only metabolites of propanil in plant *B. pilosa* were DCA and DCA-Glu.

It is known that the first metabolic step of propanil is aryl acylamidase-catalyzed hydrolysis, to yield propionic acid and DCA; this step is observed in soil, plants, and animals (McMillan et al. 1990a, b; Pothuluri et al. 1991). Propionic acid is rapidly mineralized to CO_2, whereas the fate of DCA is complex. Still (1968) studied the metabolism of propanil in rice and found that the DCA formed could exist either freely or in form of DCA-carbohydrate conjugates, for instance DCA-Glu (Bockers et al. 1994; EFSA 2013; Gareis et al. 1992), DCA-Fructose and DCA-Xylose (Yih et al. 1968). These rather labile conjugates can be hydrolyzed enzymatically or chemically again releasing DCA. Beside its strong affinity to soils and sediments, DCA may dimerize to form 3,3′,4,4′-tetrachloroazoxybenzene (TCAB) and also azo-products catalyzed by microbial peroxidases (Singh and Bingley 1991; Zhang 2013). However, no TCAB was isolated from plants (such as *Oryza sativa* L.) treated with propanil or DCA, but TCAB was absorbed and translocated by rice plants (Lieb and Still 1969; Still 1969). DCAA has been found as metabolite in water-sediment (Oda and Yukimoto 1975; Zhang 2013), soils (Oda and Yukimoto 1975), fish (Zok et al. 1991), and plants (EFSA 2011; Mitsou et al. 2006) and is formed by acetyltransferase catalysis from DCA (Martins et al. 2009; Volnova et al. 1980).

The herbicide propanil is a phytoxic chemical, but with regard to animals and humans, acute toxicity, and potential for genotoxicity, carcinogenicity, and mutagenicity is low (EFSA 2011; EPA 2003). The acute toxicity of its main metabolite DCA is higher, and several short term and long term toxicity data of DCA are available on various species of fish, invertebrates, and algae (Crossland 1990; Schmitz and Nagel 1995). DCA-Glu is rapidly cleaved by enzymes (or even water) in organisms; thus, it is considered low or even non-toxic. Date on metabolites TCAB and TCOAB are limited (Poland et al. 1976). All before mentioned metabolites are however, not phytotoxic. Propanil is widely used around the world except in Europe (EFSA 2011), and it exhibited very low persistence both in lab and field studies under anaerobic and aerobic conditions. DCA is formed also from other herbicides (e.g., linuron and diuron) and because of its persistence, environmental concentrations are higher than that of propanil (Gooddy et al. 2002; Still and Mansager 1969). Plants absorb and metabolize propanil and DCA, which was considered as detoxification process in the environment (Schmidt 1999).

Substances with aromatic or aniline structures are often found incorporated in high amounts into insoluble plant constituents (Schmidt 1999). For example, rice plants with foliar application may generate up to 92 % of non-extractable residues (EFSA 2013), which were also formed in plant cell cultures (Bockers et al. 1994; Gareis et al. 1992; Schmidt 1999; Schmidt and Schuphan 2002). The release of such residues from plant debris after death should be further investigated. In our study, the most interesting finding is that pesticide residues were released from plant NER fractions after plant death and that these residues resembled those of the extractable fractions. Plants contribute mainly to organic input into wetland and are main source for forming organic matter both in soils and sediments (Aumen et al. 1983; Zemek et al. 1985). According to our finding, xenobiotics metabolized and released from plants will also be used as carbon source by microbes also a common process in soil (Kästner et al. 2014).

Before the Three Gorges Dam started, vegetation (especially woods and shrubs) and solid waste in this area were removed and thus, total carbon source was reduced (Chen et al. 2011). Since 2008, plants growing at the river banks are strongly impacted by water fluctuation (Sun et al. 2012). *B. pilosa* originated from South America and spread widely around the world; in many areas, it has traditionally been used as food and medicine (Yang 2014). Due to its fast growth, propagation, and adaption to TGR hydrology, it became a dominant species in the area of the water level fluctuation zone. After water falls in the reservoir (spring), seeds of *B. pilosa* besides other annual and perennial plants germinate and rapidly grow. Pollutants in the TGR area, mainly from re-suspended contaminated sediment may consequently also be absorbed into plants at the river banks. To avoid this pollution transfer, some eco-friendly wetland constructions based on the principles of non-fertilizer and non-pesticide usages have already been implemented in this area (Yuan et al. 2013). Other actions, such as the installation of dike-pond system (Li et al. 2011) on one hand help to resolve regional environmental problems (uptake of pollutants), and on the other hand provide new habitats for biodiversity conservation and benefit to local residents (Li et al. 2013). The natural and artificial vegetation in the littoral zone of TGR thus, acts as ecological barrier between terrestrial and aquatic ecosystems

and according to our findings plays a significant role in the fate and bioavailability of environmental pollutants.

Conclusion

B. pilosa has a high ability to absorb pesticides such as the rice herbicide propanil. After plant's death, considerable amounts of the pesticide residues taken up by plants including non-extractable residues are released into TGR water (30 % of the applied radioactivity after 90 days). The extractable fractions of ^{14}C-compounds were confirmed as propanil, DCA and sugar conjugate DCA-Glu. Most of the remaining portions in the plant tissue were still non-extractable residues (40 % NER after 90 days). Residues released from NER amounted to 6 % of applied radioactivity and the compounds were identified as the same as those in the extractable fractions. Therefore, *B. pilosa* is considered as potential species for phytoremediation of xenobiotics, such as the herbicide propanil in the TGR area.

Acknowledgments This research has been supported by the Yangtze project of German Federal Ministry of Education and Research (BMBF) (No. FKZ 02WT1141) and the China Scholarship Council (CSC). We thank Dr. Joachim Jahnke for help with the analysis of C, H, N contents of *B. pilosa* and great advices regarding the microbial plate count method.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Abdel-Saterar MA, Ei-Said AHM (2001) Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. Int Biodeter Biodegr 47:15–21

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association, Washington

Aumen NG, Bottomley PJ, Ward GM, Gregory SV (1983) Microbial decomposition of wood in streams: distribution of microflora and factors affecting [14 C] lignocellulose mineralization. Appl Environ Microbiol 46:1409–1416

Austin AT, Ballare CL (2010) Dual role of lignin in plant litter decomposition in terrestrial ecosystems. Proc Natl Acad Sci U S A 107:4618–4622

Battle JM, Golladay SW (2001) Hydroperiod influence on breakdown of leaf litter in cypress-gum wetlands. Am Midl Nat 146:128–145

Belova M (1993) Microbial decomposition of freshwater macrophytes in the littoral zone of lakes. Hydrobiologia 251:59–64

Berendse F, Berg B, Bosatta E (1987) The effect of lignin and nitrogen on the decomposition of litter in nutrient poor ecosystems: a theoretical approach. Can J Bot 65:1116–1121

Birgelen APJM, Hebert CD, Wenk ML et al (1999) Toxicity of 3,3′,4,4′-Tetrachloroazobenzene in rats and mice. Toxicol Appl Pharmacol 156:147–159

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bockers M, Rivero C, Thiede B, Jankowski T, Schmidt B (1994) Uptake, translocation, and metabolism of 3,4-dichloroaniline in soybean and wheat plants. Z Naturforsch 49c:719–726

Brunow G, Raiskila S, Sipilä J (1998) The incorporation of 3,4-dichloroaniline, a pesticide metabolite, into dehydrogenation polymers of coniferyl alcohol (DHPs). Acta Chem Scand 52: 1338–1342

Bucher VVC, Hyde KD, Pointing SB, Reddy CA (2004) Production of wood decay enzymes, mass loss and lignin solubilization in wood by marine ascomycetes and their anamorphs. Fungal Divers 15:1–14

Casida JE, Lykken L (1969) Metabolism of organic pesticide chemicals in higher plants. Annu Rev Plant Physiol 20:607–636

Chen H, Yuan X, Chen Z, Wu Y et al (2011) Methane emissions from the surface of the Three Gorges Reservoir. J Geophys Res 116: 1984–2012

Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant antiherbivore defense. Science 230:895–899

Cronk JK, Fennessy MS (2001) Wetland plants: biology and ecology. CRC Press, USA

Crossland NO (1990) A review of the fate and toxicity of 3,4-dichloroaniline in aquatic environments. Chemosphere 21: 1489–1497

Deba F, Xuan TD, Yasuda M, Tawata S (2007) Herbicidal and fungicidal activities and identification of potential phytotoxins from Bidens pilosa L. var. radiata Scherff. Weed Biol Manag 7:77–83

Duarte S, Pascoal C, Cassio F, Barlocher F (2006) Aquatic hyphomycete diversity and identity affect leaf litter decomposition in microcosms. Oecologia 147:658–666

EFSA (2011) Conclusion on the peer review of the pesticide risk assessment of the active substance propanil. EFSA J 9:2085–2147

EFSA (2013) Review of the existing MRLs for propanil. EFSA J 11: 3280–3301

EPA (2003) The propanil RED and fact sheet. United States Environmental Protection Agency, Washington, DC

Facell JM (1991) Plant litter: its dynamics and effects on plant community structure. Bot Rev 57:1–32

Fisher PJ, Davey RA, Webster JR (1983) Degradation of lignin by aquatic and aero-aquatic hyphomycetes. Trans Br Mycol Soc 80:166–168

Gareis C, Rivero C, Schuphan I, Schmidt B (1992) Plant metabolism of xenobiotics. Comparison of the metabolism of 3,4-dichloroaniline in soybean excised leaves and soybean cell suspension cultures. Z Naturforsch 47c:823–829

Gessner MO, Gulis V, Kuehn KA, Chauvet E, Suberkropp K (2007) Fungal decomposers of plant litter in aquatic ecosystems. Environmental and microbial relationships. Mycota 4:301–324

Giacomazzi S, Cochet N (2004) Environmental impact of diuron transformation: a review. Chemosphere 56:1021–1032

Godshalk GL, Wetzel RG (1978a) Decomposition of aquatic angiosperms.1. Dissolved components. Aquat Bot 5:281–300

Godshalk GL, Wetzel RG (1978b) Decomposition of aquatic angiosperms. 2. Particulate components. Aquat Bot 5:301–327

Goldman E, Green LH (2008) Practical handbook of microbiology, second edition. CRC Press

Gooddy DC, Chilton PJ, Harrison I (2002) A field study to assess the degradation and transport of diuron and its metabolites in a calcareous soil. Sci Total Environ 297:67–83

HC (2006) Heterotrophic plate count in guidelines for Canadian drinking water quality—guideline technical document. Health Canada, Ottawa

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil, 347. Berkeley, Calif.: University of California, College of Agriculture, Agriculture Experiment Station

Hofstra G, Switzer CM (1968) The phytotoxicity of propanil. Weed Sci 16:23–28

HPA (2005) Colony count by the pour plate method, national standard method w4. Health Protection Agency, UK

Kästner M, Nowak KM, Miltner A, Trapp S, Schäffer A (2014) Classification and modelling of nonextractable residue (NER) formation of xenobiotics in soil—a synthesis. Crit Rev Environ Sci Technol 44:2107–2171

Khan SU, Kacew S, Akhtar MH (1990) Bound 14C residues in stored wheat treated with [14C] deltamethrin and their bioavailability in rats. J Agric Food Chem 38:1077–1082

Komínková D, Kuehn KA, Büsing N, Steiner D, Gessner MO (2000) Microbial biomass, growth, and respiration associated with submerged litter of Phragmites australis decomposing in a littoral reed stand of a large lake. Aquat Microb Ecol 22:271–282

Leroy CJ, Marks JC (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshw Biol 51:605–617

Li B, Yuan X, Xiao H, Chen Z (2011) Design of the dike-pond system in the littoral zone of a tributary in the Three Gorges Reservoir, China. Ecol Eng 37:1718–1725

Li B, Xiao H, Yuan X, Willison JHM, Liu H, Chen Z, Zhang Y, Deng W, Yue J (2013) Analysis of ecological and commercial benefits of a dike-pond project in the drawdown zone of the Three Gorges Reservoir. Ecol Eng 61:1–11

Lieb HB, Still GG (1969) Herbicide metabolism in plants: specificity of peroxidases for aniline substrates. Plant Physiol 44:1672–1673

Martins M, Rodrigues-Lima F, Dairou J, Lamouri A, Malagnac F, Silar P, Dupret JM (2009) An acetyltransferase conferring tolerance to toxic aromatic amine chemicals: molecular and functional studies. J Biol Chem 284:18726–18733

McMillan DC, Freeman JP, Hinson JA (1990a) Metabolism of the arylamide herbicide propanil. I. Microsomal metabolism and in vitro methemoglobinemia. Toxicol Appl Pharmacol 103:90–101

McMillan DC, Leakey JE, Arlotto MP, McMillan JM, Hinson JA (1990b) Metabolism of the arylamide herbicide propanil. II. Effects of propanil and its derivatives on hepatic microsomal drug-metabolizing enzymes in the rat. Toxicol Appl Pharmacol 103: 102–112

Minderman G (1968) Addition, decomposition and accumulation of organic matter in forests. J Ecol 56:355–362

Mitsou K, Koulianou A, Lambropoulou D, Papps P, Albanis T, Lekka M (2006) Growth rate effects, responses of antioxidant enzymes and metabolic fate of the herbicide propanil in the aquatic plant Lemna minor. Chemosphere 62:275–284

Mordaunt CJ, Gevao B, Jones KC, Semple KT (2005) Formation of non-extractable pesticide residues: observations on compound differences, measurement and regulatory issues. Environ Pollut 133:25–34

Nakai A, Shiwaku T, Hasegawa H, Hashimoto T (1986) Spinodal decomposition of polymer mixtures with a thermotropic liquid-crystalline polymer as one-component. Macromolecules 19: 3008–3010

Oda M, Yukimoto M (1975) Fate of propanil in soils 1. The formation of 3,4-dichloroacetanilide (DCAA). J Weed Sci Technol 20:12–17

Paul RWJ, Benfield EF, Cairns JJ (1983) Dynamics of leaf processing in a medium-sized river. See Ref 97:403–423

Poland A, Clover E, Kende AS, DeCamp M, Giandomenico CM (1976) 3,4,3′,4′-Tetrachloro azoxybenzene and azobenzene: potent inducers of aryl hydrocarbon hydroxylase. Science 194:627–630

Pothuluri JV, Hinson JA, Cerniglia CE (1991) Propanil: toxicological characteristics, metabolism, and biodegradation potential in soil. J Environ Qual 20:330–347

Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacterial and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194

Sandermann H Jr (2004) Bound and unextractable pesticidal plant residues: chemical characterization and consumer exposure. Pest Manag Sci 60:613–623

Sandermann H Jr, Arjmand M, Gennity I, Winkler R (1990) Animal bioavailability of defined xenobiotic lignin metabolites. J Agric Food Chem 38:1877–1880

Sandermann JH, Heller W, Hertkorn N et al (1998) A new intermediate in the mineralization of 3,4-dichloroaniline by the white rot fungus Phanerochaete chrysosporium. Appl Environ Microbiol 64:3305–3312

Schmidt B (1999) Non-extractable residues of pesticide and xenobiotics in plants—a review. Recent Res Dev Agric Food Chem 3:329–354

Schmidt B, Schuphan I (2002) Metabolism of the environmental estrogen bisphenol A by plant cell suspension cultures. Chemosphere 49:51–59

Schmitz A, Nagel R (1995) Influence of 3,4-dichloroaniline (3,4-DCA) on benthic invertebrates in indoor experimental streams. Ecotoxicol Environ Saf 30:63–71

Singh J, Bingley R (1991) Levels of 3,3′,4,4′-tetrachloroazobenzene in propanil herbicide. Bull Environ Contam Toxicol 47:822–826

Skidmore MW, Paulson GD, Kuiper HA, Ohlin B, Reynolds S (1998) Bound xenobiotic residues in food commodities of plant and animal origin. IUPAC, Great Britain

Still GG (1968) Metabolism of 3,4-dichloropropionanilide in plants: the metabolic fate of the 3,4-dichloroanilide moiety. Science. 159:992–993

Still GG (1969) 3,4,3′,4′-Tetrachloroazobenzene: its translocation and metabolism in rice plants. Weed Res 9:211–217

Still GG, Mansager ER (1969) The presence of 3,4-dichloroaniline in rice grain hydrolysates. Weed Res 9:218–223

Still GG, Balba HM, Mansager ER (1981) Studies on the nature and identity of bound chloroaniline residues in plants. J Agric Food Chem 29:739–746

Suberkropp K, Klug MJ (1980) The maceration of deciduous leaf litter by aquatic hyphomycetes. Can J Bot 58:1025–1031

Sun R, Yuan X, Chen Z, Zhang Y, Liu H (2012) Effect of Three Gorges Reservoir (Yangzi River) on the plant species richness in drawdown zone of downstream the tributary river (Pengxi River). Russ J Ecol 43:307–314

Sydes C, Grime JP (1981) Effects of tree leaf litter on herbaceous vegetation in the deciduous woodlands. II An experimental investigation. J Ecol 69:249–262

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70:97–104

Trenck KT, Hunkler D, Sandermann JH (1981) Incorporation of chlorinated anilines into lignin. Z Naturforsch 36c:714–720

Tristan Gingerich R, Anderson JT (2011) Litter decomposition in created and reference wetlands in west Virginia, USA. Wetl Ecol Manag 19: 449–458

Villeneuve A, Larroudé S, Humbert JF (2011): Pesticides - formulations, effects, fate. Herbicide contamination of freshwater ecosystems: impact on microbial communities. In Tech, 285–313 pp

Volnova AI, Surovtseva EG, Vasieva GK (1980) Acetylation of 3,4-dichloroaniline by representatives of the genus Pseudomonas. Microbiologiia 49:167–170

Vymazala J, Kröpfelová L (2009) Removal of organics in constructed wetlands with horizontal sub-surface flow: a review of the field experience. Sci Total Environ 407:3911–3922

Wang Q, Yuan X, Liu H et al (2012) Effect of long-term winter flooding on the vascular flora in the drawdown area of the Three Gorges Reservoir, China. Pol J Ecol 60:95–106

Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17:567–594

Williams JB (2002) Phytoremediation in wetland ecosystems: progress, problems, and potential. Crit Rev Plant Sci 21:607–635

Willison JHM, Li R, Yuan X (2013) Conservation and ecofriendly utilization of wetlands associated with the Three Gorges Reservoir. Environ Sci Pollut Res 20:6907–6916

Witt KL, Zeiger E, Tice RR, Birgelen APJM (2000) The genetic toxicity of 3,3′,4,4′-tetrachloroazobenzene and 3,3′,4,4′-tetrachloroazoxybenzene: discordance between acute mouse bone marrow and peripheral blood subchronic mouse micronucleus test results. Mutat Res 472:147–154

Yang WC (2014) Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant Bidens pilosa L. Evid Based Complement Alternat Med 2014:698617

Yih RY, McRae DH, Wilson HF (1968) Mechanism of selective action of 3′,4′-dichloropropionanilide. Plant Physiol 43:1291–1296

Yuan X, Zhang Y, Liu H, Xiong S, Li B, Deng W (2013) The littoral zone in the Three Gorges Reservoir, China: challenges and opportunities. Environ Sci Pollut Res 20:7092–7102

Zemek J, Linek K, Kadlecikova B, Kucar S, Somogyi L (1985) Antimicrobial effect of osazones of sugars and anhydro sugars. Folia Microbiol 30:396

Zhang P (2013) The fate of 14C-labeled 3,4-dichloroaniline in a water-sediment system. RWTH Aachen University, Aachen, Germany

Zok S, Görge K, Kalsch W, Nagel R (1991) Bioconcentration, metabolism and toxicity of substituted anilines in the zebrafish (Brachydanio rerio). Sci Total Environ 109–110:411–421