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Modification to degradation of hexazinone in forest soils amended with sewage sludge

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ABSTRACT

Influences of one sewage sludge on degradation of hexazinone and formation of its major metabolites were investigated in four forest soils (A, B, C and D), collected in Zhejiang Province, China. In non-amended forest soils, the degradation half-life of hexazinone was 21.4, 30.4, 19.4 and 32.8 days in forest soil A, B, C and D, respectively. Degradation could start in soil A and C without lag period because the two soils had been contaminated by this herbicide for a long time, possibly leading to completion of acclimation period of hexazinone-degrading bacteria. In forest soils amended with sewage sludge, the degradation rate constant increased by 17.3% in soil A, 48.2% in soil B, 8.1% in soil C and 51.6% in soil D, respectively. The higher degradation rates (soil A and C) in non-amended soils accord with the lower rate increase in sewage sludge-amended soils. Under non-sterile conditions, biological mechanism accounted for 51.8–62.4% of hexazinone degradation in four soils. Under sterile conditions, the four soils had the similar chemical degradation capacity for hexazinone. In non-amended soil B, only one metabolite (B) was detected, while two metabolites (B and C) were found in sewage sludge-amended soil B. Similarly situated in agricultural soils, N-demethylation at 6-position of triazine ring, hydroxylation at the 4-positon of cyclohexyl group, and removal of the dimethylamino group with formation of a carbonyl group at 6-position of triazine ring appear to be the principal mechanism involved in hexazinone degradation in sewage sludge-amended forest soils. These data will improve understanding of the actual pollution risk as a result of forest soil fertilization with sewage sludge.

1. Introduction

More and more wastewater is produced along with the development of industry and agriculture. Sewage sludge is an unwanted and inevitable by-product in sedimentation both before and after wastewater biotreatment process [1]. It is estimated that China is now producing more than two million tons (dry weight) of sewage sludge annually and its disposal represents a major environmental hazard [1]. Organic matter, nitrogen and phosphorus content in sewage sludge are five, three and three times of those in manure (a typical traditional organic waste used for agricultural land in China), respectively, attracting farmers' interest [2]. Being rich in organic matter and plant nutrients, a certain portion of China's sewage sludge has been utilized in a similar way to other traditional organic waste. It is often applied to agricultural and forest land as a soil conditioner and fertilizer, because the physical properties of the soil are improved, and nutrients such as nitrogen and phosphorus are supplied [3].

In recent years, due to the risk of sewage sludge application on agricultural land, the attention on the use of sewage sludge in forest management has been increasing [4]. Sewage sludge may increase nutrients in the soil and thus it has been used in forest plantations. While forest soils may be degraded because of disturbances such as wildfire, erosion, fuel-wood harvesting, etc., improved soil fertility by using sewage sludge may help to restore damaged ecosystems [3]. However, sludge application may potentially lead to the accumulation of hazardous components, e.g. organic chemicals, heavy metals and pathogens [5], and may increase the risk of these components entering the food chain. In China, studies have provided different opinions on whether or not land application of sewage sludge could result in harmful environmental contamination or exposure to people. Many research projects resulted in a positive answer. According to control standard for pollutants in sludge for land use, the maximum permitted content is 20 mg kg^{-1} dry weight

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for Cd, 15 mg kg^{-1} for Hg, 1000 mg kg^{-1} for Pb and Cr, 75 mg kg^{-1} for As, 500 mg kg^{-1} for Cu, 1000 mg kg^{-1} for Zn, 200 mg kg^{-1} for Ni and 3 mg kg^{-1} for Benzo[a]pyrene, respectively. Guo et al. [6] reported that heavy metals in sludge did not accumulate in plants highly, and the concentrations of Hg, As, Pb, Cr and Cd in edible parts of corn, millet and Chinese cabbage did not reach China's national hygienic standards for food when a poor soil was applied with 75 ton/ha of sludge. They also found that heavy metal concentration in poor soil did not reach the background value with the same application, indicating no threat to the soil quality [7]. Zhong et al. reported that concentrations of Ni in tomato growing in the sandy soil with sludge loads of 15 and 30 ton/ha were 1.9 and 1.5 times as those of the control samples, respectively, but still lower than safety limit [8]. They predicted that sludge application in the sandy soil could be carried out safely and continuously for 12 years with a load of 15 ton/ha per year.

Hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione] is an effective herbicide for general weed control in agricultural and forestry area. It is a white solid and water soluble (33 g L^{-1} at 25 °C) with a relatively low vapor pressure [9]. This herbicide is a potent inhibitor of photosynthesis in susceptible species and was often detected in groundwater and soil in the forest areas, which raises great concern about its safety to human health [10]. The previous investigation on environmental fate of hexazinone mainly focused on the development of analytical techniques for the parent and its metabolites at trace levels [11]; dissipation [12]; adsorption–desorption [13]; microbial degradation [14]; leaching potential and mobility in soils [15]. The reported field dissipation half-life of hexazinone was 79 days, and the organic carbon distribution coefficient ranged from 34 to 74 [16]. It is low susceptible to hydrolysis and photolysis [17], and thus residual activity may be expected to last for several months [18]. Metabolites of hexazinone were first identified by Reiser et al. [19] who separated five degradation products in plant seedling. The trione product, i.e. [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6-(1H,3H,5H)-trione], was the most hazardous among five metabolites of hexazinone. Wang et al. [20] observed that the major metabolites formed in a sandy-loam soil were [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] and [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6-(1H,3H,5H)-trione] in bovine manure-amended soil.

As for influences of organic amendments on pesticide behavior, the previous works mainly focused on farm litters. Wang et al. [20] found that bovine manure could significantly increase degradation of hexazinone in a sandy-loam soil and affect forming of its main metabolites. The two amendments affect sorption and leaching of imazaquin in soils [21]. Gupta and Baummer [22] found that farm litter could enhance the biodegradation of atrazine in soil and Gan et al. [23] concluded that the addition of a composted manure to the top 5-cm layer at 5% (w/w) reduced 1,3-dichloropropene emission by 47%. Wang and Yates [24] found that the half-life of oxytetracycline was 8.1 days in manure at 25 °C, 33 days in manure-amended soil and 56 days in non-amended soil. However, the application of organic amendments was proved to slow degradation of triadimefon in soil [25]. Sewage sludge can exert different effects on degradation of different kinds of organic chemicals. On one hand, decreased bioavailability due to increased sorption to the additional organic matter will retard degradation. On the other hand, co-metabolic biotransformation can be enhanced by the general increase in microbial populations and activities. Therefore, the combined effects on pesticide degradation occurred in sludge-amended soil [26].

Most studies on pesticide degradation have been preformed with agricultural soils and information on their decomposition dynamics in forest soils is scarce. Especially, so far no data are available on the effect of sewage sludge on the degradation and metabolism of hexazinone in forest soils. Therefore, the main objective of this study is to assess the effect of a sewage sludge on environmental behavior of hexazinone in four forest soils. This information will improve the understanding of the actual pollution risk as a result of forest soil fertilization with sewage sludge.

Table 1
Main physico-chemical features of the four forest soils.

Kind of sediment	A	B	C	D
pH	7.33	5.97	8.74	8.03
OC (g kg^{-1})	27.82	12.43	21.41	8.47
CEC (cmol kg^{-1})	14.57	8.92	8.26	10.65
TN (g kg^{-1})	3.34	2.56	1.84	2.13
Sand (%)	23.78	34.22	27.76	28.92
Silt (%)	25.15	39.56	25.80	42.42
Clay (%)	51.07	26.22	46.44	28.66
Texture class	Clay	Loam	Clay	Loam
Hexazinone (ng g^{-1} d.w.)	4.68	ND	3.73	ND

OC, TN and CEC indicate the abbreviations of organic carbon, total nitrogen and cation exchanging capacity, respectively.

2. Materials and methods

2.1. Chemicals

Hexazinone (99.7% purity) was purchased from Shenyang Chemical Engineering Institute, Shenyang, China. Metabolite A [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione]; metabolite B [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione] and metabolite C [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione] were gifts from DuPont de Nemours (Experimental station, Wilmington, DE, USA). The structures of hexazinone and its three metabolites are shown in Fig. 1 and their purities were all 99.0%. Working standards of hexazinone and its metabolites in methanol were prepared freshly every 2 weeks and stored in dark bottles at −20 °C until use. Analytical grade reagents and solvents were locally procured, purified and redistilled before use. The water used in this experiment was purified with a Mill-Q-Plus system (Millipore, Molsheim, France).

2.2. Soil and sewage sludge

The four forest soils were collected from the 0–20 cm soil profile of four forest farms. Soil A, B and C were collected from Fenkou, Yeqi and Fuxi forest farms, respectively, located in Chunan County, Zhejiang Province, China. Soil D was collected from Ruian Forest farm, located in Ruian City, Zhejiang Province, China. All the forest soil samples were collected in November 2008, and their main physico-chemical features and contamination status are shown in Table 1. The soil A and C have a high content of clay as compared with soil B and D. The soil D shows a very low percentage of organic carbon, and soil C and D have a basic pH. According to our investigation on the four forest farms, hexazinone (25% water-soluble formulation) was applied twice yearly in the middle of May and September in Fenkou and Fuxi forest farms during the past 4 years (2005–2008). However, no application history of hexazinone was recorded from 2005 to 2008 in Yeqi and Ruian forest farms. In the four forest soils, hexazinone residue was not detected in soil B and D, while it was found at 4.68 and 3.73 ng g^{-1} d.w. in soil A and C samples, respectively. The collected soil samples were air-dried, ground, and passed through a 2-mm sieve before use. The experimental temperature was kept constantly at 25 °C with the operation conducted in a dark thermostatic incubation room.

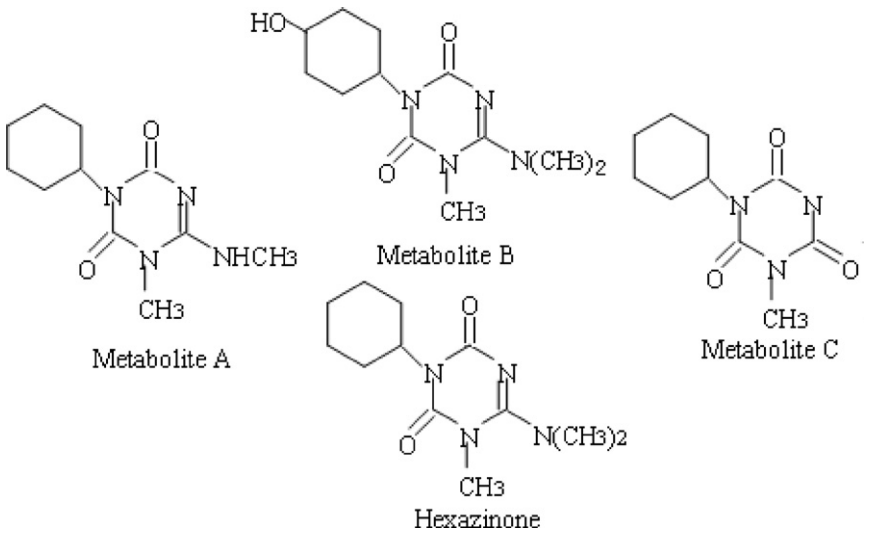

Fig. 1. Structures of hexazinone and its main metabolites.

The sewage sludge used was produced by a domestic water treatment plant, located in Wenzhou Central Wastewater Treatment Plant, Wenzhou, China, and was subjected to an aerobic stabilization process. The sludge was treated by gravity thickening, dewatered by air-drying (a common practice before forest application) and enriched in heavy metals. The sources of heavy metals in sludge were industrial waste, domestic sewage, road surface runoff, precipitation airborne products and tire abrasion products. The concentrations of these heavy metals in final enriched sludge were 2358.6 mg kg^{-1} dry weight for Zn, 257.6 for Cu, 88.6 for Ni, 113.7 for Cr, 81.7 for Pb, 2.4 for Hg, and 2.2 for Cd, respectively. The contents of water and organic matter were 81.7% before air-drying treatment and 43.8%, respectively. Sludge pH was 7.2 and the concentration of hexazinone in sludge was below detectable level (<0.05 mg kg^{-1}).

Microorganisms in four forest soils and sewage sludge were enumerated by plate counting on nutrient agar for bacteria [27]. At the three sampling times (5, 20 and 40 days), the bacterial numbers were about in the range of 2.1–45.3 × 10^6 CFU g^{-1} (colony-forming units) in four soils, while it was from 79.6 to 98.2 × 10^6 CFU g^{-1} in sewage sludge. No bacterial inhibition was found in the spiked soils when comparing the bacterial numbers in the spiked to non-spiked soils.

In order to investigate the contribution of chemical and biological degradation, the degradation of hexazinone was compared under sterile and non-sterile soils amended by sewage sludge. As for sterile treatments, the soil and sludge samples were autoclaved for 1 h at 121 °C [28] and then collected, air-dried, ground, and passed through a 2-mm sieve before use. The previous operation procedures were conducted under the conditions of aseptic manipulation room.

2.3. Hexazinone degradation in forest soils

The soil sample was spiked with stock solution of hexazinone to obtain an appropriate concentration on a dry weight basis, adjusted to 60% of maximum water-holding capacity, and introduced into a 100-mL flask for each set. Each flask containing soil was weighed and incubated at 25 ± 1 °C in the dark. The weight loss due to evaporation of soil moisture was minimized by periodical addition of sterile deionized water at intervals of 2 days over the whole incubation period. Each set was in triplicate and processed as described below for residual analysis of hexazinone at intervals of 0 (2 h after spiking), 5, 10, 20, 40, and 60 days after incubation.

2.4. Effect of sewage sludge on degradation of hexazinone and metabolite formation

To determine if sewage sludge had a significant impact on hexazinone degradation, sewage sludge was added into the soil at a concentration of 10% (w/w). The amended soil was mixed thoroughly, and then spiked with stock solution of hexazinone to obtain a final concentration of 10 mg kg^{-1} on a dry weight basis. The soil was then prepared as described in Section 2.3 to compare the difference of hexazinone degradation under non-amended and sewage sludge-amended conditions. At regular intervals, triplicate samples were removed for each set and processed for analyses of hexazinone residues. If the samples were not analyzed immediately, they were frozen at −20 °C until analyzed.

In non-amended and sewage sludge-amended (10% by w/w) soil, the major metabolite formation was studied in concomitant with residual analyses of hexazinone. The major metabolites of hexazinone were identified and quantified by co-chromatography using authentic standards. A few minor metabolites were not taken into account due to lack of authentic standards in this experiment.

2.5. Extraction and clean-up of soil samples

Each soil sample (10 g) was extracted with 20 ml of methanol, shaken vigorously for 2 h on a mechanical shaker and filtered through a Buchner funnel. The former extraction process was performed in triplicate. The filtered solutions was mixed into a flask, evaporated to near dryness (ca. 1–2 mL) on a rotary evaporator, and the final residue was accurately dissolved to 5 ml using methanol in a test tube. Aliquots (2 mL) of the methanol portion were further purified by passing through a 0.2-μm filter to remove any soil particles, and then determined for hexazinone and its metabolite residues by HPLC.

2.6. Analyses of hexazinone and its metabolites by HPLC

The residues of hexazinone and its major metabolites (A, B and C) were determined using an Agilent 1100 model HPLC equipped with diodearray detector. The operation was run under the following conditions: cartridge column, Nova-Pak C_{18} (4.6 mm × 150 mm i.d., 5 μm particle size); flow rate, 1 mL min^{-1}; detection wavelength, 247 nm and injection volume, 20 μL. The mobile phase consisted of methanol and water acidified to pH 5 with formic acid, and

the chromatograph started with 30/70 methanol–water (v/v) for 7 min and then 70/30 for 25 min. An external standard method was used for calibration. The retention time of hexazinone, metabolite A, B, and C was about 15.8, 12.8, 4.3 and 18.7 min, respectively. The detection limits for hexazinone and its three metabolites were 0.05 mg kg^{-1}.

The analytical performance of the proposed method was evaluated by fortifying non-amended or amended soil (10% by w/w) at a series of concentrations (0.1–10 mg kg^{-1}) of hexazinone, metabolite A, B, C and D. The spiked soil was extracted and analyzed as described above. The average recoveries ranged from 83.2 to 105.7% for hexazinone, and from 76.3 to 95.8% for three metabolites in non-amended or amended soil, respectively. The coefficients of variance ranged from 4.3% to 11.2% for hexazinone, and from 3.6% to 12.8% for three metabolites, respectively. As a result, the method adopted for residual analyses of hexazinone and its three metabolites was satisfactory.

2.7. Statistical analysis

The half-life value ($t_{1/2}$) reported in this study was calculated using the pseudo-first-order model. Microsoft Excel 2003 and Origin 6.0 graphing software were used to fit the data to the model. Analysis of variance (ANOVA) was conducted, and Duncan's multiple range test was used to determine significant difference at $p = 0.05$ among treatments with statistical analysis software (SAS Version 8).

3. Results and discussion

3.1. Degradation of hexazinone in four forest soils

Influences of forest soil types on hexazinone degradation were examined in four soils with an initial nominal concentration of 10 mg kg^{-1}, and the results are shown in Fig. 2. During the 60-day incubation period, the hexazinone concentration dropped from 9.28 ± 1.24 to 1.57 ± 0.26 mg kg^{-1} in soil A, from 9.45 ± 1.53 to 2.61 ± 0.29 mg kg^{-1} in soil B, from 9.37 ± 1.68 to 1.12 ± 0.38 mg kg^{-1} in soil C and from 9.61 ± 1.42 to 2.68 ± 0.64 mg kg^{-1} in soil D, respectively. Accordingly, the percentages of hexazinone degraded in forest soils were 80.9% in soil A, 72.4% in soil B, 88.1% in soil C and 72.1% in soil D, respectively. The data fitting results using the first-order kinetic equation showed that coefficients of linear regression (R^2) ranged from 0.8629 to 0.8906. As a result, hexazinone degradation can be well described by the first-order kinetic equation:

$$C_t = C_0 \times e^{-kt}$$

where C_0 is the initial concentration of hexazinone (mg kg^{-1}), C_t is the concentration (mg kg^{-1}) at time t, t is the incubation time (days) and k is the degradation rate constant. The half-life is expressed by $\ln(2/k)$ and the degradation rate constant (k) is determined using

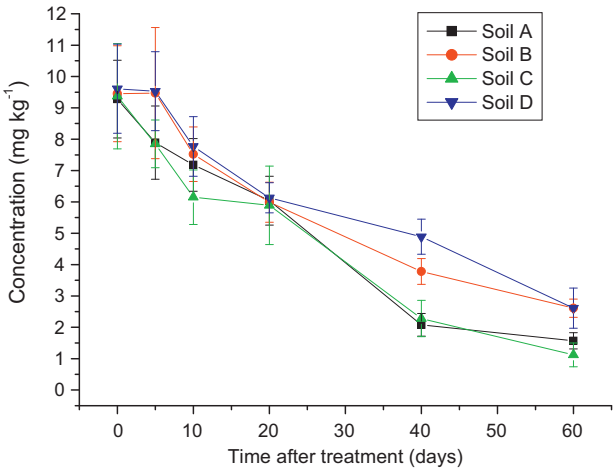

Fig. 2. Hexazinone degradation in four forest soils at the spiked concentration of 10 mg kg^{-1}.

regression of $\ln(C_t/C_0)$ [29]. The computed half-life of hexazinone was 21.4, 30.4, 19.4 and 32.8 days in soil A, B, C and D, respectively (Table 2). The previous results were consistent with Bottni et al.'s finding [15] of a half-life of 30 days in a soil from Medena Province, Italy. Zhu and Li [17] also observed the field half-life of hexazinone varied from 24 to 74 days. This long half-life of hexazinone in aerobic soils may reflect its structural feature, which is recalcitrant to microbial and chemical degradation.

As can be seen from Table 2, soil A and C showed the higher degradation rate than soil B and D. The statistical results demonstrated that higher degradation in soil A and C was dependent on higher organic carbon (OC) content in them. In most cases, higher OC was always concomitant with higher microbial numbers and activity. Microbial activity in forest soils was estimated by counting soil bacterial numbers at three sampling times (5, 20 and 40 days). As listed in Table 3, soil C possessed the most culturable bacteria, ranged between 35.8 and 45.3 $\times$ 10^6 CFU g^{-1}, among the four forest soils, and followed by soil A (between 32.6 and 38.2 $\times$ 10^6 CFU g^{-1}) at three sampling times (5, 20 and 40 days). The bacterial population in soil A and C was about 10–20 times as many as in soil B and D, which was possibly one of the most important reason for the higher biodegradation capacity of soil A and C. Moreover, no bacterial inhibition was found in the spiked soils when comparing numbers in the spiked and non-spiked soils.

In addition, it is interesting to note that hexazinone degradation in soil A and C started when the experiment began without an obvious lag period (Fig. 2). Soil A and C were collected from Fenkou and Fuxi forest farms, respectively, located in Chunan County, Zhejiang Province, China, and they were applied with 25% water-soluble hexazinone formulation twice annually from 2005 to 2008. Hexazinone residue was detected to be 4.68 and 3.73 ng g^{-1} d.w.

Table 2
The degradation kinetic parameters of hexazinone in forest soils.

Soil types	Soil non-amended with sewage sludge		Soil amended with sewage sludge	
	Degradation constant (k, day^{-1}) (mean ± SD) × 10^{-3}	Half-life ($t_{1/2}$, days) (mean ± SD)	Degradation constant (k, day^{-1}) (mean ± SD) × 10^{-3}	Half-life ($t_{1/2}$, days) (mean ± SD)
A	32.3 ± 2.6	21.4 ± 1.5	37.8 ± 4.8	18.3 ± 1.7
B	22.8 ± 2.9	30.4 ± 3.0	33.8 ± 2.4	20.5 ± 1.9
C	35.7 ± 3.1	19.4 ± 1.7	38.6 ± 3.6	17.9 ± 1.6
D	21.1 ± 1.7	32.8 ± 3.6	32.0 ± 4.2	21.6 ± 2.0

SD indicates standard deviation of triplicate samples in 60 days incubation time.

Table 3
Plate counts of bacteria in sewage sludge and forest soils.

Time (days)	CFU g^{-1} ($\times 10^6$)									
	Soil A		Soil B		Soil C		Soil D		Sewage sludge	
	Non-spiked	Spiked	Non-spiked	Spiked	Non-spiked	Spiked	Non-spiked	Spiked	Non-spiked	Spiked
5	32.6 ± 10.2	23.8 ± 16.2	2.9 ± 1.2	3.4 ± 0.7	35.8 ± 11.3	22.7 ± 6.6	2.8 ± 1.1	2.5 ± 1.3	98.2 ± 9.6	79.6 ± 14.5
20	38.2 ± 8.9	25.4 ± 15.4	3.8 ± 1.6	3.1 ± 1.5	45.3 ± 13.8	40.7 ± 9.2	2.2 ± 1.6	2.1 ± 1.5	92.3 ± 11.2	86.7 ± 8.4
40	34.6 ± 11.9	36.8 ± 7.6	3.6 ± 2.2	3.1 ± 2.7	36.7 ± 16.2	29.1 ± '5.3	2.6 ± 2.2	3.1 ± 2.7	96.7 ± 13.3	92.4 ± 10.8

in soil A and C samples, respectively, suggesting that two soils had been contaminated by this herbicide for a long time. As a result, the hexazinone-degrading bacteria completed their acclimation period, during which little or no biodegradation occurs and often appears as a lag period in the course of biodegradation, and acquired the ability to biodegrade this chemical. However, no degradation was observed in soil B and D during the 5-day incubation period, suggesting the requirement of an acclimation period. As shown in Fig. 2, the degradation of hexazinone started at 10-day incubation period, which implied the completion of acclimation period. The former observation was in accordance with Hirano's findings [30], which investigated biodegradation of chlordane and hexachlorobenzenes in river sediment, and found that high carbon content and contamination by the target chemicals can enrich microorganisms such as sulfate-reducing bacteria and eubacteria which are responsible for degrading organic pollutants. Therefore, high OC and parent contamination in soil A and C led to the enhanced biodegradation of hexazinone in this investigation. In addition, the previous results also indicated that at the onset of experiment, the biodegradation was the main drivers for the degradation of hexazinone.

3.2. Degradation of hexazinone in soils amended with sewage sludge

Sewage sludge is a kind of amendment of organic carbon, which is often recommended as a forest management practice to increase forest soil fertility and to restore damaged ecosystems. The different sewage sludges have different physicochemical and biological features, which will lead to different effect on pesticide degradation and metabolism in forest soils [31]. Therefore, hexazinone degradation was compared in forest soils non-amended and amended with sewage sludge (10%, w/w) and the results are shown in Fig. 3. With the elapse of time (0–60 days), the hexazinone residue

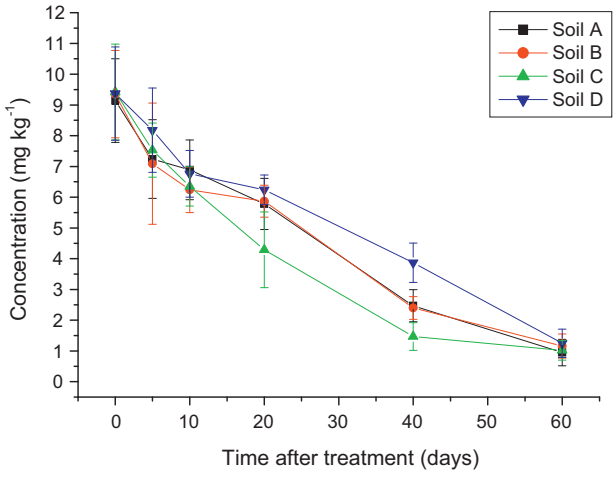

Fig. 3. Hexazinone degradation in four forest soils amended with sewage sludge at the spiked concentration of 10 mg kg^{-1}.

decreased to 0.95 ± 0.26 mg kg^{-1} in sewage sludge-amended soil A, to 1.16 ± 0.39 mg kg^{-1} in soil B, to 1.02 ± 0.39 mg kg^{-1} in soil C and to 1.25 ± 0.46 mg kg^{-1} in soil D, respectively. Accordingly, the estimated half-life of hexazinone in four forest soils was 18.3, 20.5, 17.9 and 21.6 days, respectively, based on first-order reaction kinetics (Table 2). As compared with non-amended soils, the degradation rate was approximately enhanced by 17.3% in soil A, 48.2% in soil B, 8.1% in soil C and 51.6% in soil D, respectively, in the mixture soil-sludge. The previous results demonstrated that the higher degradation rates (soil A and C) in non-amended forest soils were in concomitant with the lower increase of rate in sewage sludge-amended soils, but that the lower degradation rates correspond with the higher increase (soil B and D). The bacterial population of sewage sludge was about 3–20 times more than those of the forest soils (Table 3). Higher microbial densities possibly introduced more hexazinone-degraders, which improved the degradation rate of hexazinone, especially in the forest soils with less microbial population and activity. Gomez-Rico et al. [3] found that the average decay rates of nonylphenolic compounds in forest soils amended with sewage sludge were similar to those observed in agricultural soils. The application of sewage sludge appears to have a complex effect on the degradation of pesticides in soil. Additional adsorption by sewage sludge would make microbes hard to access and retard the degradation, but co-metabolic biotransformation can increase the degradation. The lower percentage of residue for those easily degraded pesticides indicates that the addition of sludge increases the microbial population and degradation capacity in the medium. For the pesticide with more complex chemical structure, its main degradation route is chemical rather than biological, in which case, the addition of sludge means a reduction in its availability and therefore a greater persistence of the residue in comparison with the non-amended soils. In this investigation, fast degradation of hexazinone in forest soils amended with sewage sludge may result in a fast decrease in toxicity, and suggests that it may represent a relatively low risk for the environment when applied in the conditions of our experiment.

3.3. Effect of sewage sludge rates on degradation

As shown in Table 4, the mean degradation half-life ($t_{1/2}$) was 30.4 days for 0%, 26.5 days for 5%, 20.5 days for 10% and 20.8 days for 15% rate treatment in soil B, respectively, suggesting that it decreased with the rate increase of sewage sludge except for 15% rate treatment. The $t_{1/2}$ values at 5%, 10% and 15% rates decreased by 14.9%, 48.2% and 46.1% (Fig. 4), respectively, compared with that of control (0% treatment), and the significant difference (at $p < 0.05$ level) of the half-life was observed among control, 5% and 10% rate treatments of sewage sludge (Table 5). However, the difference was not significant between 10% and 15% rate treatments, which indicated that the higher mix ratio did not necessarily increase hexazinone degradation. In the rate range of sewage sludge (5–15%), it appears that the optimal active degradation lies at the 10% amendment rate. The conclusion was in general agreement with the observations of Namkoong et al. [32] that addition of organic amendment could increase degradation rate of target

Table 4
The kinetic parameter of hexazinone in sterile soils and influence of amendment rate on degradation kinetics.

Soil types	Sterile soils amended with sewage sludge		Influence of amendment rate in soil B		
	Degradation constant (k, day^{-1}) (mean ± SD) × 10^{-3}	Half-life ($t_{1/2}$, days) (mean ± SD)	Amendment rate (%)	Degradation constant (k, day^{-1}) (mean ± SD) × 10^{-3}	Half-life ($t_{1/2}$, days) (mean ± SD)
A	15.2 ± 1.9	45.6 ± 4.7	0	22.8 ± 2.9	30.4 ± 3.0
B	16.3 ± 1.3	42.5 ± 3.2	5	26.2 ± 2.1	26.5 ± 1.6
C	14.5 ± 2.1	47.8 ± 3.1	10	33.8 ± 2.4	20.5 ± 1.9
D	13.6 ± 1.2	50.9 ± 6.3	15	33.3 ± 2.8	20.8 ± 1.2

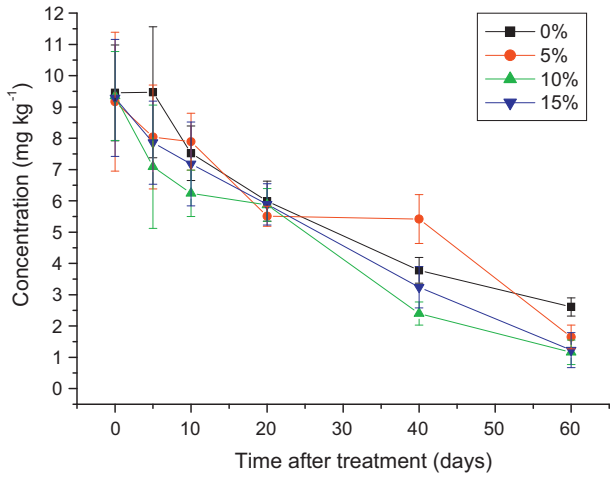

Fig. 4. Influences of the different sewage sludge rates on hexazinone degradation in forest soil B.

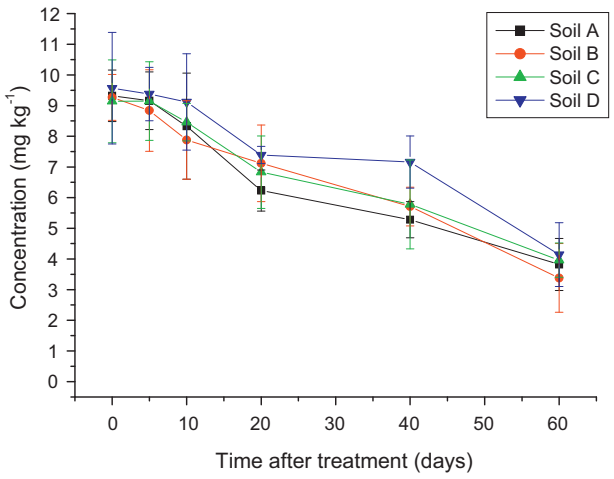

Fig. 5. Hexazinone degradation in four sterile forest soils amended with sewage sludge at the spiked concentration of 10 mg kg^{-1}.

contaminant, but might inhibit the degradation rate when an excessive amount of organic amendment was added. The carbon source in the manure must not represent a preferential carbon source that preempts degradation of the target contaminant. When the added carbon source is preferentially degraded over the target compounds, microbial activity for degrading the target contaminant may be inhibited. In this investigation, sewage sludge added as carbon source did not act as competing energy source, and thus it led to an increase of hexazinone degradation rate.

3.4. Degradation mechanism of hexazinone in soils amended with sewage sludge

The degradation of hexazinone in non-sterile soil is a result of biological and chemical transformation, but only chemical process occurs in sterile soil. Because this experiment was carried out in the dark, photolysis was negligible, and thus hydrolysis was the only chemical process involved. Differences between k values in non-sterile and sterile sludge-amended soils were considered to be attributable to biological degradation. The rate constants (k) of hexazinone in non-sterile and sterile soils with different amendment rates of sewage sludge are listed in Tables 2 and 4, respectively. k values dropped by 59.8% in sterile soil A, 51.7% in sterile soil B, 62.4% in sterile soil C and 57.5% in sterile soil D (Fig. 5) when compared with those in non-sterile soils. It was obvious that the former decreasing in k values was due to the loss of biological degradation. Moreover, an interesting finding was that no significant difference was observed among the four half-lives (42.5, 45.6, 47.8 and 50.9 days) in four sterile forest soils. The previous results suggested that the investigated four soils had the similarly chemical degradation capacity for hexazinone under the conditions of lacking biological degradation. As described above, the differences of k values in sterile and non-sterile soils were deemed to be due to biological mechanism. As a result, up to 51.8–62.4% of hexazinone

Table 5
The statistical result for the half-life of hexazinone in sewage sludge-amended soil B.

Amendment rate (%)	Half-life ($t_{1/2}$, days) (mean ± SD)	Subset for alpha = 0.05 (N = 3)			
		1	2	3	4
0	30.4 ± 3.0	30.4 a			
5	26.5 ± 1.6		26.5 b		
10	20.5 ± 1.9			20.5 c	
15	20.8 ± 1.2			20.8 c	

		Sum of squares	df	Mean square	F
ANOVA	Between groups	205.470	3	68.490	16.995
	Within groups	32.240	8	4.030	
	Total	237.710	11		

The different lower cases (a, b and c) indicate that it is significant at $p < 0.05$ level.

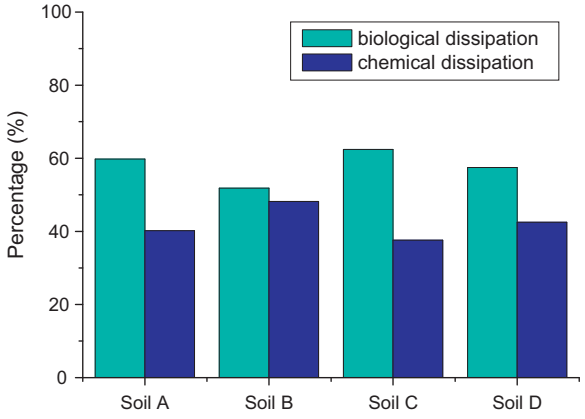

Fig. 6. Contribution rate of biological and chemical mechanisms to hexazinone degradation.

degradation was concerned with biological mechanism in four non-sterile soils. In contrast, 37.6–48.2% of the degradation was chemically associated (Fig. 6). The greatest percentage (62.4%), involved in biological mechanism, of hexazinone degradation occurred in non-sterile soil C, while the least (51.8%) was observed in non-sterile soil B amended with sewage sludge. As occurred under non-sterile conditions, microbial mechanism was the primary pathway for hexazinone degradation in forest soils, while chemical processes also played a role in the degradation. However, this must be determined on a case-by-case basis, since different kinds of pesticides will have the different structural features and degradation behavior. For example, Dungan et al. [33] reported that biological mechanism only accounted for 14–44% and 20–42% of the (Z)-1,3-dichloropropene and (E)-1,3-dichloropropene degradation, which demonstrated that chemical transformation was the primary pathway for 1,3-dichloropropene degradation.

3.5. *Hexazinone metabolism in non-amended and sewage sludge-amended soil*

The formation and changing tendency of three major metabolites (A, B and C) were analyzed in soil B at an initial concentration of 10 mg kg^{-1}. The metabolites formed were identified by co-chromatography technique, i.e. comparing the retention time of metabolite with that of the authentic standard. As shown in Figs. 7 and 8, only one metabolite formed in non-amended soil

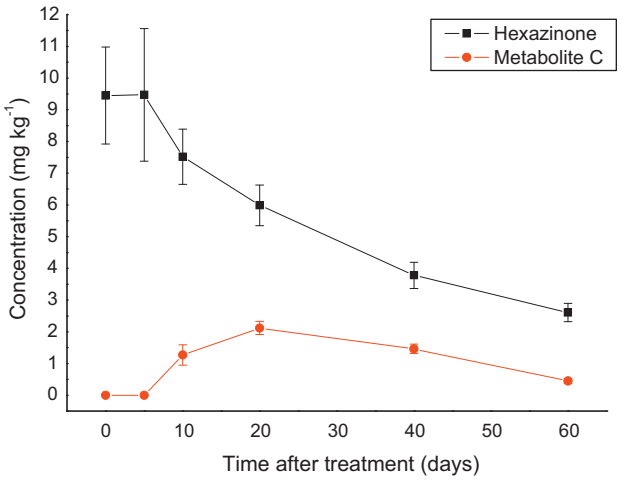

Fig. 7. Degradation and metabolism of hexazinone in non-amended soil B.

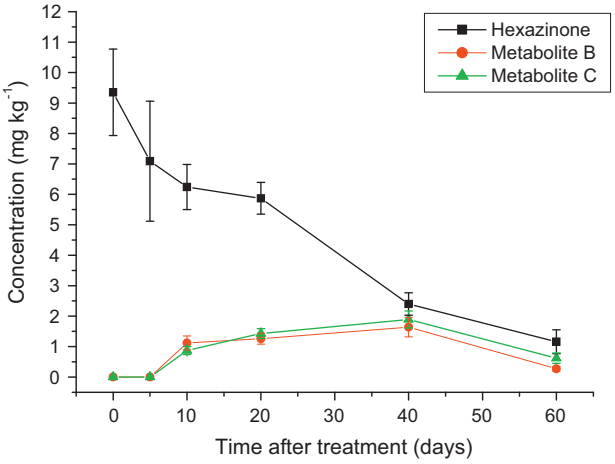

Fig. 8. Degradation and metabolism of hexazinone in soil B amended with sewage sludge.

B was metabolite C, resulting from detachment of dimethylamino group and forming of carbonyl group at 6-position of triazine ring. Metabolite C started forming from the 10th day of incubation at a concentration of 1.27 ± 0.32 mg kg^{-1}, reached its maximum level (2.12 ± 0.21 mg kg^{-1}) at the 20-day incubation period, and then declined gradually to 0.45 ± 0.08 mg kg^{-1} on the 60th day (Fig. 7), which proved the further degradation of this metabolite. However, metabolite A and B was not detected over the whole experimental period in non-amended soil B.

As shown in Fig. 8, the maximum monitoring concentration (1.89 ± 0.28 mg kg^{-1}) for metabolite C was detected at the 40-day incubation period in sewage sludge-amended soil B. Additionally, metabolite B was found to start forming at 10-day incubation period, reached its maximum level (1.64 ± 0.32 mg kg^{-1}) at 40 days and then declined to 0.27 ± 0.08 mg kg^{-1} at the end of experiment. However, metabolite B was not detected in non-amended soil B during the whole incubation period, suggesting that addition of sewage sludge promoted formation of this metabolite. Metabolite B, bearing a hydroxyl group at 4-position of cyclohexyl group (Fig. 1), is a reducing form of hexazinone and therefore reducing conditions contribute to formation of this metabolite. Singh [25] reported that reduction potential in soil amended by composted manure increased faster than that in non-amended soil, which hastened the onset and attainment of soil reducing conditions. Therefore, the application of sewage sludge to soil promoted formation of the reducing metabolite B. Similarly as in non-amended soils, metabolite A was not detected through the whole incubation period (60 days) in sewage sludge-amended soil B.

Overall, the parent molecule of hexazinone was detected to be the highest peak, followed by those containing its degradation products in non-amended and sewage sludge-amended soil B. Therefore, by combining the findings of this experiment under different conditions, a possible degradation pathway of hexazinone was deduced and is presented in Fig. 9. The above metabolite data were similar to our previous research on hexazinone degradation modified by bovine manure [20]. However, the metabolism difference of hexazinone lies in that another metabolite A was also detected in bovine manure-amended soil, while not in sewage sludge-amended soil. Based on these findings, it can be inferred that N-demethylation at 6-position of triazine ring, hydroxylation at the 4-positon of cyclohexyl group, and removal of the dimethylamino group with formation of a carbonyl group at 6-position of triazine ring appear to be the principal mechanism involved in hexazinone degradation in sewage sludge-amended forest soils.

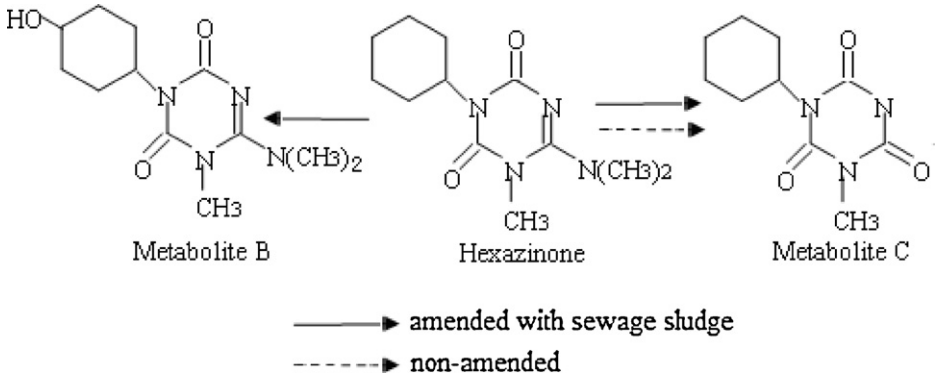

Fig. 9. The difference of hexazinone metabolites in non-amended and amended forest soil B with sewage sludge.

4. Conclusions

In non-amended forest soils, the high-to-low order of hexazinone degradation was related to order of soil organic carbon content, and microbial population and activity. In soil A and C, hexazinone degradation could start without lag period because the two soils had been contaminated by this herbicide for a long time, which resulted in completion of acclimation period of hexazinone-degrading bacteria. As compared with non-amended soils, the incorporation of sewage sludge into soils enhanced degradation rate by 17.3% in soil A, 48.2% in soil B, 8.1% in soil C and 51.6% in soil D, respectively. The higher degradation rates (soil A and C) in non-amended soils were in concomitant with the rate increase in sewage sludge-amended soils. The higher mix ratio of sewage sludge did not necessarily increase degradation of hexazinone, and the optimal active degradation lies in the 10% amendment rate. Biological mechanism accounted for 51.8–62.4% of hexazinone degradation in four non-sterile soils. No significant difference was observed among the four half-lives (42.5, 45.6, 47.8 and 50.9 days) in four sterile soils, indicating that the investigated soils had the similar chemical degradation capacity for hexazinone under the condition of lacking biological degradation. Only one metabolite formed in non-amended soil B was metabolite C. However, in sewage sludge-amended soil B, metabolite B was detected to be up to $1.64 \pm 0.32\,mg\,kg^{-1}$ at 40-day incubation period. N-demethylation at 6-position of triazine ring, hydroxylation at the 4-positon of cyclohexyl group, and removal of the dimethylamino group with formation of a carbonyl group at 6-position of triazine ring appear to be the principal mechanism involved in hexazinone degradation in forest soils amended with sewage sludge. This study, along with further research on heavy metal dynamics and plant growth in sludge-amended soils, can help to optimize the management of biosolids and minimize the environmental risks associated to their use.

Acknowledgements

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References

[1] J. Yang, G.H. Guo, T.B. Chen, G.D. Zheng, Concentrations and variation of heavy metals in municipal sludge of China, China Water Wastewater 25 (2009) 122 (in Chinese).
[2] M.J. Wang, Land application of sewage sludge in China, Sci. Total Environ. 197 (1997) 149.
[3] F.M. Gomez-Rico, R. Font, J. Vera, D. Fuentes, K. Disante, J. Cortina, Degradation of organic pollutants in Mediterranean forest soils amended with sewage sludge, Chemosphere 71 (2008) 2129.
[4] J. Horsewll, V. Ambrose, L. Clucas, A. Leckie, Survival of Escherichia coli and salmonella spp. after application of sewage sludge to a Pinus radiate forest, J. Appl. Microbiol. 103 (2007) 1321.
[5] D.A. Bright, N. Healey, Contaminated risks from biosolids land application: contemporary organic contaminant levels in digested sewage sludge from five treatment plants in Greater Vancouver, British Columbia, Environ. Pollut. 126 (2003) 39.
[6] M.L. Guo, R.T. Tian, Y.Q. Wang, Q.X. Zhang, Crop accumulation of heavy metals from soil amended with municipal refuse and sewage sludge, Agro-environ. Protect. 14 (1995) 67.
[7] M.L. Guo, R.F. Mi, R.T. Tian, M.Q. Xi, Effects of city sewage sludge and sludge waste compost on the soil as a fertilizer resource, Agro-environ. Protect. 13 (1994) 204.
[8] X.G. Zhong, Y. Lin, C.R. Zhang, Q.T. Wu, Preliminary study of ecological effects of sewage sludge on agricultural soil, Trop. Subtrop. Soil Sci. 1 (1992) 91.
[9] S. Dousset, C. Chauvin, P. Durvet, M. Thevenot, Transfer of hexazinone and glyphosate through undisturbed soil columns under Christmas tree cultivation, Chemosphere 57 (2004) 265.
[10] D. Kubilius, R. Bushway, Determination of hexazinone and its metabolites in groundwater by capillary electrophoresis, J. Chromatogr. A 793 (1998) 349.
[11] J. Fischer, J. Michael, Thermospray ionization liquid chromatography–mass spectrometry and chemical ionization gas chromatography–mass spectrometry of hexazinone metabolites in soil and vegetation extracts, J. Chromatogr. A 704 (1995) 131.
[12] M.J. Calderson, M. Ortega, M.C. Hermosin, J. Cornejo, Hexazinone and simazine dissipation in forest field, Chemosphere 54 (2004) 1.
[13] A.I. Garcia, J.L. Tadeo, Influence of soil moisture on sorption and degradation of hexazinone and simazine in soil, J. Agric. Food Chem. 47 (1999) 3895.
[14] X.D. Wang, H.L. Wang, C.X. Tan, Degradation and metabolism of hexazinone by two isolated bacterial strains from soil, Chemosphere 61 (2005) 1468.
[15] P. Bottni, J. Keizer, E. Funari, Leaching indices of some major triazine metabolites, Chemosphere 32 (1996) 1401.
[16] I. Toiber-Yasur, M. Rosner, A. Hadas, D. Russo, B. Yaron, Leaching of terbuthylazine and bromacil through field soils, Water Air Soil Pollut. 113 (1999) 319.
[17] Y. Zhu, Q. Li, Movement of bromacil and hexazinone in soils of Hawaiian pineapple fields, Chemosphere 49 (2002) 669.
[18] M.D. Fernandez, R.A. Perez, J.L. Tadeo, Persistence of simazine and hexazinone in soil, Fresenius Environ. Bull. 10 (2001) 490.
[19] R. Reiser, I. Belasco, R. Rhodes, Identification of metabolites of hexazinone by mass spectrometry, Biomed. Mass Spectr. 10 (1983) 581.
[20] H.L. Wang, Y.Y. Li, Y.J. Lu, X.D. Wang, Influence of bovine manure on dissipation of hexazinone in soil, Ecotoxicol. Environ. Saf. 72 (2009) 93.
[21] T. Undabeytia, E. Morillo, C. Maqueda, Effect of organic amendments on the retention and mobility of imazaquin in soils, J. Agric. Food Chem. 52 (2004) 4493.
[22] G. Gupta, J. Baummer, Biodegradation of atrazine in soil using poultry litter, J. Hazard. Mater. 45 (1996) 185.
[23] J. Gan, S. Yates, D. Crowley, J. Becker, Acceleration of 1,3-dichloropropene degradation by organic amendments and potential application for emissions reduction, J. Environ. Qual. 27 (1998) 408.
[24] Q. Wang, S.R. Yates, Laboratory study of oxytetracycline degradation kinetics in animal manure and soil, J. Agric. Food Chem. 56 (2008) 1683.
[25] N. Singh, Factor affecting triadimefon degradation in soil, J. Agric. Food Chem. 53 (2005) 70.
[26] E. Barriuso, S. Houot, C. Serra, Influence of compost addition to soil on the behaviour of herbicides, Pestic. Sci. 49 (1997) 65.
[27] S. Houot, E. Barriuso, V. Bergheaud, Modification to atrazine degradation pathways in a loamy soil after addition of organic amendments, Soil Biol. Biochem. 30 (1998) 2147.

[28] G. Gian, B. John, Biodegradation of atrazine in soil using manure, J. Hazard. Mater. 45 (1996) 185–192.
[29] M.E. Sanchez, I.B. Estrada, O. Martinez, A. Aller, Influence of the application of sewage sludge on the degradation of pesticides in the soil, Chemosphere 57 (2004) 673.
[30] T. Hirano, T. Ishida, O. Kokyo, R. Sudo, Biodegradation of chlordane and hexachlorobenzenes in river sediment, Chemosphere 67 (2007) 428.
[31] M.B. McBride, Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? Adv. Environ. Res. 8 (2003) 5.
[32] W. Namkoong, E. Hwang, J. Park, Bioremediation of diesel-contaminated soil with composting, Environ. Pollut. 119 (2002) 23.
[33] R.S. Dungan, J. Gan, S.R. Yates, Effect of temperature, organic amendment rate and moisture content on the degradation of 1,3-dichloropropene in soil, Pest Manag. Sci. 57 (2001) 1107.