DEGRADATION OF THIOBENCARB AND PROPANIL IN AN HERBICIDE AND THEIR EFFECTS ON BACTERIAL COMMUNITY IN SOIL

Huynh Thi Thanh Thuy[1,2], Nguyen Thanh Hung[1,2], Tran Ngoc Chau[1,2], and Ha Danh Duc[3*]

[1]An Giang University, Vietnam National University Ho Chi Minh City, Vietnam

[2]Vietnam National University Ho Chi Minh City, Vietnam

[3]Faculty of Agriculture and Environment Resources, Dong Thap University, Vietnam

[*]Corresponding author: Ha Danh Duc, Email: hadanhduc@gmail.com

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Abstract

Thiobencarb and propanil are active ingredients in herbicides extensively applied in the agricultural sector to control weeds resulting in serious environmental pollution. This study determined the degradation of thiobencarb (13.5 mg/kg dry soil) and propanil (6.8 mg/kg dry soil) in herbicide Satunil 60EC by soil microorganisms. Moreover, the effects of the herbicides on bacterial community in soil were evaluated. The results showed that 42.2 ± 10.3% of thiobencarb in the herbicide was dissipated, while propanil was mostly transformed after 30 days. Thiobencarb in the herbicide was degraded at a slow rate until the 10th day and then similar to the degradation of the pure substrate. Meanwhile, the degradation of propanil in the herbicide was slower than that of the pure compound. In other experiments, supplementing pure thiobencarb and Satunil 60EC into soil caused a shift of bacterial structure. However, the index values of α-diversity, such as OTUs, ACE, Chao1, Simpson and Shannon, of bacterial community in soil with and without chemical addition were not statistically different. These results indicated that indigenous soil microorganisms could degrade thiobencarb and propanil, and both pure thiobencarb and Satunil 60EC did not negatively affect the richness and diversity of soil bacteria.

Keywords: *Thiobencarb, degradation, soil, bacterial structure.*

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ĐÁNH GIÁ SỰ PHÂN HUỶ THIOBENCARB VÀ PROPANIL TRONG THUỐC DIỆT CỎ VÀ ẢNH HƯỞNG CỦA CHÚNG ĐỐI VỚI HỆ VI KHUẨN TRONG ĐẤT

Huỳnh Thị Thanh Thủy[1,2], Nguyễn Thanh Hùng[1,2], Trần Ngọc Châu[1,2] và Hà Danh Đức[3*]

[1]Trường Đại học An Giang, Đại học Quốc gia thành phố Hồ Chí Minh, Việt Nam

[2]Đại học Quốc gia thành phố Hồ Chí Minh, Việt Nam

[3]Khoa Nông nghiệp và Tài nguyên môi trường, Trường Đại học Đồng Tháp, Việt Nam

[*]Tác giả liên hệ: Hà Danh Đức, Email: hadanhduc@gmail.com

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Tóm tắt

Thiobencarb và propanil là các hoạt chất của thuốc trừ cỏ được ứng dụng rộng rãi trong nông nghiệp, nhưng điều này gây ra sự ô nhiễm môi trường nghiêm trọng. Nghiên cứu này đánh giá sự phân hủy của thiobencarb (13,5 mg/kg đất khô) và propanil (6,8 mg/kg đất khô) trong thuốc diệt cỏ Satunil 60EC bởi vi sinh vật đất và ảnh hưởng của những hoạt chất này đến hệ vi khuẩn đất. Kết quả cho thấy 42,2 ± 10,3% thiobencarb bị phân huỷ, trong khi propanil trong thuốc trừ cỏ hầu hết biến mất trong đất sau 30 ngày. Thiobencarb trong thuốc diệt cỏ bị phân hủy với tốc độ chậm hơn sự phân huỷ thiobencarb nguyên chất cho đến ngày thứ 10, nhưng sau đó tốc độ phân huỷ này là tương đương nhau. Trong khi đó, quá trình phân hủy propanil trong thuốc diệt cỏ chậm hơn so với propanil nguyên chất. Trong các thí nghiệm khác, việc bổ sung thiobencarb nguyên chất và thuốc trừ cỏ Satunil 60EC vào đất làm thay đổi thành phần hệ vi khuẩn đất. Tuy nhiên, các giá trị chỉ số của đa dạng α, chẳng hạn như OTU, ACE, Chao1, Simpson và Shannon của hệ vi khuẩn trong đất có và không bổ sung hóa chất thì không khác biệt về mặt thống kê. Các kết quả trong nghiên cứu này cho thấy hệ vi sinh vật đất có khả năng phân hủy thiobencarb và propanil, và cả thiobencarb tinh khiết và Satunil 60EC đều không ảnh hưởng tiêu cực đến sự phong phú và đa dạng hệ vi khuẩn cho dù chúng làm thay đổi thành phần của hệ vi khuẩn trong đất.

Từ khóa: *Thiobencarb, phân huỷ, đất, hệ vi khuẩn.*

1. Introduction

Thiobencarb (S-4-chlorobenzyl diethyldithiocarbamate) is commonly used for weed control worldwide, mainly for rice fields (Tanetani et al., 2013). The excessive use of this herbicide causes serious environmental problems. The herbicide has been detected in water taken from rice fields (Sapari and Ismail, 2012) and tap water (Amin et al., 2008). Thiobencarb accumulates in the soil affecting the following crops in rotation (Mahmoudi et al., 2011). The compound is highly toxic to invertebrates and moderately toxic to fish (Cashman et al., 1990; Fernández-Vega et al., 2002). The use of thiobencarb may stimulate or inhibit soil microorganisms (Sato, 1989; Jena et al., 1990; Bhowmick et al., 2014). Moreover, thiobencarb causing a significant shift in a bacterial community in a sediment slurry under anaerobic conditions has been reported (Oanh & Duc, 2022). However, information on the effect of the herbicide on bacteria community in soil under aerobic condition has not been evaluated in detail.

Some active gradients are mixed in an herbicide, such as thiobencarb and propanil, to increase the weed control efficiency (Smith, 1981). Propanil is also extensively applied to kill grasses (Smith, 1965), and it is accumulated at a high-concentration in irrigation water (Primel et al., 2007). Both thiobencarb and propanil show potential contamination in the water system of the rice fields (Sapari and Ismail, 2012); therefore, the presence of a substrate may inhibit the degradation of the other one. Moreover, adjuvants in herbicides may affect the degradation of the main ingredients.

The Mekong Delta is a leading rice producer in Vietnam. Farmers here use herbicides regularly, including herbicides containing thiobencarb and propanil. As a result, many pesticides have been detected in groundwater, surface water, tap water and drinking water (Toan et al., 2013). Moreover, herbicides may accumulate in soil. However, data for monitoring the degradation of thiobencarb and propanil in the soil in Vietnam are still lacking. Therefore, this study determined the degradation of pure thiobencarb compared with the compound in an herbicide in the soil. Also, the effects of pure thiobencarb and an herbicide on the structure of soil bacterial communities were evaluated.

2. Materials and methods

2.1. Degradation of thiobencarb and propanil in an herbicide in soil

The soil was collected from a rice field at a depth of 0-20 cm in Dong Thap Province, Vietnam, as described in a previous report (Huynh et al., 2022). Farmers usually use herbicides to control weeds in this field. Soil samples were transferred into a lab, smashed and sieved to obtain soil less than 2.0 mm in diameter. The granulometric properties were 40.4±3.3% of fine sand, 36.4±3.4 of silk and 22.3±3.2% of clay. Total carbon and nitrogen in the soil were 5.2±0.5% and 0.23±0.0%, respectively. Soil was then transferred to plastic containers with length × width × depth of 15 cm × 15 cm × 10 cm. Each container was added with 500 g soil on dry basis.

A commercial herbicide named Satunil 60EC (Summit Agro Company, Vietnam) with adjuvants, 400 g/L thiobencarb and 200 g/L propanil was also used to determine biodegradation by soil microorganisms. Thiobencarb (>98% purity, Sigma-Aldrich) as a stock was dissolved in absolute ethanol at 0.1 M. The stock solution and Satunil 60EC were diluted in distilled water before being sprayed on the soil at 13.5 mg thiobencarb/kg dry soil. Thiobencarb supplemented in soil using the stock solution was considered to be pure thiobencarb. Distilled water was added into the soil to approximately 40% moisture content. The soil was thoroughly mixed, then capped with a plastic cover and incubated in a dark condition for 30 days.

2.2. Bacterial diversity and relative abundance in soil

Bacterial diversity and relative abundance in soil were analyzed. The relative abundance of the bacterial community in soil was determined through Illumina MiSeq sequencing of 16 S rRNA genes. The total deoxyribonucleic acid (DNA)

was directly extracted from 1.0 g soil using the UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA) under the manufacturer's instructions. All the DNA samples were amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') at the V3-V4 region. The initial denaturation step of 3 min at 95 °C was followed by 27 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C. The final extension was performed for 10 min at 72 °C. The raw sequencing reads were quality-controlled using the Trimmomatic tool (version 0.39). Rarefaction and α-diversity indices, i.e., abundance-based coverage estimators (ACE), Chao1, Simpson, Shannon, were calculated using Mothur software. The Operational Taxonomic Units (OTUs) were classified at 97% similarity.

2.3. Extraction of thiobencarb and propanil from soil

Soil samples were collected during the incubation process, pulverized using a mortar and pestle. Thiobencarb and propanil were extracted twice from soil with *n*-hexane. Five grams soil sample was transferred to a glass tube, added with 15 mL of *n*-hexane and shaken with a speed of 150 rpm for 20 min. The tube was kept stably for 20 min before collecting *n*-hexane above soil. The extract was filtered with a 0.22 µm syringe filter, evaporated under a nitrogen stream before dissolved in methanol. The recovery efficiencies of thiobencarb and propanil from soil were 91.4% and 92.4%, respectively.

2.4. Analytical Methods

The concentrations of the thiobencarb were determined using HPLC (LC-10AD, Shimadzu, Japan), with C18 column (5 µm, 250 mm × 4,6 mm; Hyperclone, Phenomenex, USA). The gradient elution method with the mobile phase composed of acetonitrile and ultrapure water (7 : 3, v/v) with a total velocity of 1.0 mL/min. The injection volume of 10 µL and UV detection of 280 nm were applied.

2.5. Statistical analysis

Data obtained from at least three replicates are shown as the mean ± standard deviation. Significant differences among means were statistically analyzed using one-way Duncan's test ($p < 0.05$) in SPSS program version 22.0.

3. Results and discussion

3.1. Degradation of propanil and thiobencarb in herbicide Satunil 60EC in soil

Because the propanil concentration in the herbicide is a half of thiobencarb, the experiment was conducted at 6.8 mg/kg dry soil. For pure compounds, the concentrations of propanil and thiobencarb were decreased by 10-12% in sterile soil, probably because the substrates were degraded by physical and chemical processes or absorbed into soil components. Pure propanil was not found in non-sterile soil within 20 days (Figure 1A), given a half-life of 3.2 days and a decay constant of 0.21 on average.

For the degradation of active ingredients in the herbicide Satunil 60EC, dissipation rate of thiobencarb was slower than that of pure substrate until 10 days. The low reduction of thiobencarb in the first 10 days was probably due to the toxicity of these compounds to soil microorganisms. However, the degradation at the following time was not statistically different compared to that of pure compound. After 30 days, thiobencarb remaining in the soil was 7.8 ± 0.8 mg/kg dry soil, meaning 42.2 ± 10.3% of the compound in the herbicide was degraded (Figure 1B), given a half-life value of 37.9 days, and decay constant of 0.018 on average. For propanil, the compound was mostly transformed after 30 days. The average values of half-life and decay constant for propanil in soil were 7.9 days and 0.126, respectively.

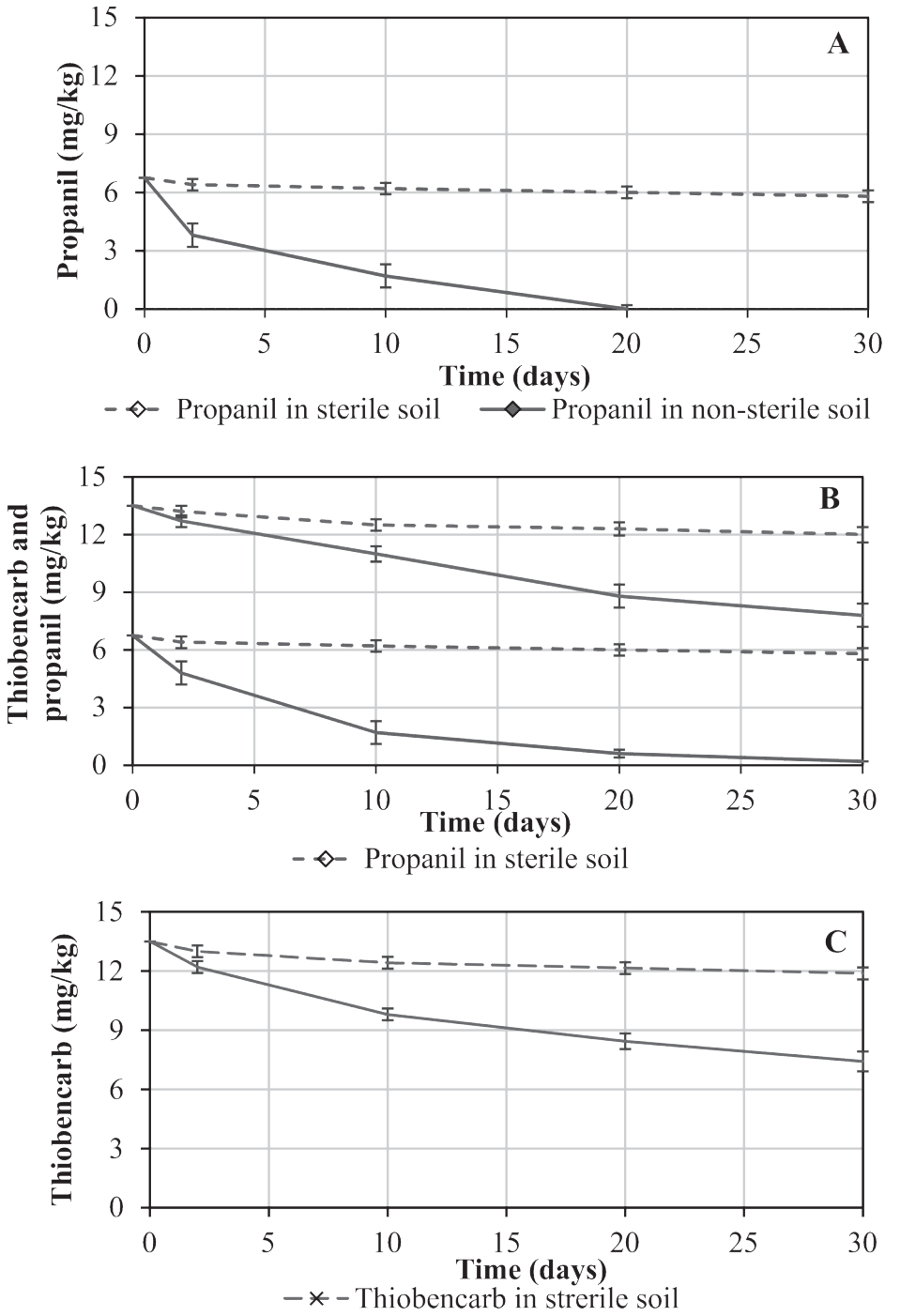

Figure 1. Degradation of (A) pure propanil, and (B) thiobencarb and propanil in the herbicide Satunil 60EC in soil in comparison with (C) pure thiobencarb (the degradation of pure thiobencarb was described by Huynh Thi Thanh Thuy *et al., 2022*).

The degradation of both compounds in non-sterile soil was significantly higher than that in sterile soil due to the activities of soil microorganisms. The degradation rate of pure propanil was significantly higher than that of propanil in the herbicide, while the degradation of thiobencarb in both forms was not statistically different after 30 days. These results indicated that thiobencarb and adjuvants caused a negative effect on propanil degradation, while propanil and adjuvants did not affect thiobencarb degradation.

In a previous report (Huynh et al., 2022), 45.0 ± 5.4% of pure thiobencarb was dissipated after 30 days at the initial concentration of 13.5 mg/kg dry soil in non-sterile soil (Figure 1C). Propanil at higher than 0.5 mM inhibited the degradation of butachlor by *Pseudomonas* sp. But2 in liquid media (Duc et al., 2020). Adjuvants in herbicides are considered to range from likely harmless to serious toxicity (Cox & Surgan, 2006; Haller & Stocker, 2003). Pérez-Bárcena et al. (2014) showed that adjuvants inhibited the degradation of prometryn.

The degradation rate of thiobencarb in soil mainly depends on soil components and organic materials (Moon and Kuwatsuka, 1984). A previous study showed that the half-life of thiobencarb in aerobic soil was 77 days (Doran et al., 2006), while the degradation in flooded soil occurred with a significantly slower rate (Nakamura et al., 1977). For propanil, the half-lives of dissipation in the upper soil layer were between 0.17 and 1.8 days (Kanawi et al., 2016).

3.2. Effects of pure thiobencarb and herbicide Satunil 60EC on phyla and genera of bacterial community in soil

At the phylum level, 9 phyla with abundances ≥ 1% found in original soil were shown in table 1. Proteobacteria was dominant and quite stable in all treatments after incubation for 30 days. Actinobacteria was the second abundance increased overtime in all treatments with and without chemical addition. The abundance of Firmicutes was also increased in all treatments. On the other hand, the percentages of other phyla which were less than 5.5% in the original soil were decreased (Table 1).

At the genus level, 25 genera with abundances ≥ 1% in the original soil were shown in table 2. *Aeromonas* was the most abundant and quite stable in the control without any chemical addition, but it was decreased in soil supplemented with pure thiobencarb and the Satunil 60EC. (Table 2). Similarly, *Acinetobacter, Acrobacter* and *Olivibacter* were quite stable in the control, but the abundances of the genera reduced in soil with pure thiobencarb and the Satunil 60EC. Other genera, i.e., *Enterobacter*, *Arthrobacter* and *Acidovorax* were quite stable in all treatments. Meanwhile, data for *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Cupriavidus* and *Dechloromonas* in the control were not statistically different compared the original time; however, the relative abundances of these genera increased in other treatments. *Streptomyces*, *Thiobacillus* and *Variovorax* increased in all treatments. On the other hand, the abundances of *Sphingomonas*, *Sphingobacterium*, *Methylopila* and *Cyanothece* decreased in all treatments during the experiment.

Table 1. Relative bacterial abundance at phylum level in original soil and soil after 30 days.

Phyla	Relative abundance (%)(*)			
	Original soil	Soil without chemical addition	Soil treated with pure thiobencarb	Soil treated with Satunil 60EC
Proteobacteria	56.6 ± 3.5^{a}	56.3 ± 5.8^{a}	58.8 ± 6.2^{a}	58.1 ± 6.8^{a}
Actinobacteria	12.2 ± 1.1^{a}	16.2 ± 2.2^{b}	15.2 ± 2.5^{b}	16.6 ± 2.7^{b}
Firmicutes	5.6 ± 0.3^{a}	7.0 ± 1.1^{b}	7.7 ± 1.4^{b}	7.8 ± 1.2^{b}
Bacteroidetes	5.4 ± 0.3^{a}	3.7 ± 0.6^{b}	3.7 ± 0.5^{b}	2.5 ± 0.4^{b}
Chloroflexi	3.4 ± 0.2^{b}	2.5 ± 0.4^{a}	2.0 ± 0.4^{a}	2.0 ± 0.3^{a}
Bacteroidota	2.3 ± 0.1^{b}	1.7 ± 0.3^{a}	1.0 ± 0.2^{a}	0.8 ± 0.2^{a}
Cyanobacteria	1.8 ± 0.1^{b}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}
Nitrospirota	1.1 ± 0.1^{b}	1.0 ± 0.2^{b}	0.7 ± 0.1^{b}	0^{a}
Acidobacteria	1.0 ± 0.0^{ab}	1.3 ± 0.2^{b}	0.8 ± 0.1^{a}	2.0 ± 0.3c
Others	5.4	4.0	4.3	4.5
Unclassified	5.2	6.0	5.6	6.6

(*)Different small superscript letters indicate statistically significant differences ($p < 0.05$) in the same treatment groups (within a line).

Table 2. Relative bacterial abundance at genus level in original soil and soil after 30 days.

Genera	Relative abundance (%)(*)			
	Original soil	Soil without chemical addition	Soil treated with pure thiobencarb	Soil treated with Satunil 60EC
Aeromonas	6.0 ± 0.2^{b}	6.3 ± 0.6^{b}	4.3 ± 0.5^{a}	4.0 ± 0.5^{a}
Pseudomonas	5.6 ± 0.3^{a}	5.2 ± 0.5^{a}	6.8 ± 0.7^{b}	7.2 ± 0.7^{b}
Enterobacter	5.6 ± 0.3^{a}	5.6 ± 0.7^{a}	5.0 ± 0.7^{a}	5.0 ± 0.6^{a}
Acrobacter	5.4 ± 0.2^{b}	6.3 ± 0.6^{b}	3.7 ± 0.4^{a}	3.0 ± 0.4^{a}
Bacillus	5.3 ± 0.2^{a}	6.0 ± 0.5^{ab}	7.0 ± 0.8^{b}	6.8 ± 0.7^{b}
Comamonas	5.1 ± 0.2^{bc}	5.5 ± 0.5^{c}	4.3 ± 0.4^{ab}	3.6 ± 0.4^{a}
Acinetobacter	5.0 ± 0.4^{a}	5.0 ± 0.5^{a}	6.0 ± 0.4^{b}	6.8 ± 0.5^{bc}
Arthrobacter	4.8 ± 0.2^{a}	5.0 ± 0.6^{a}	4.8 ± 0.5^{a}	4.7 ± 0.5^{a}
Cupriavidus	4.7 ± 0.2^{a}	4.0 ± 0.5^{a}	6.8 ± 0.7^{b}	6.6 ± 0.7^{b}
Acidovorax	4.3 ± 0.3^{a}	3.7 ± 0.4^{a}	4.0 ± 0.5^{a}	4.2 ± 0.5^{a}
Sphingomonas	4.1 ± 0.2^{b}	2.4 ± 0.2^{a}	2.2 ± 0.2^{a}	2.7 ± 0.3^{a}
Sphingobacterium	4.0 ± 0.2^{b}	2.7 ± 0.3^{a}	2.7 ± 0.3^{a}	2.5 ± 0.2^{a}
Mycobacterium	3.8 ± 0.2^{bc}	4.4 ± 0.5^{c}	3.3 ± 0.4^{ab}	3.0 ± 0.3^{a}
Dechloromonas	3.5 ± 0.2^{a}	3.1 ± 0.4^{a}	4.8 ± 0.5^{b}	5.0 ± 0.6^{b}
Methylopila	3.3 ± 0.2^{b}	2.3 ± 0.2^{a}	2.3 ± 0.3^{a}	2.2 ± 0.3^{a}
Anaerolinea	3.0 ± 0.2^{bc}	2.5 ± 0.3^{ab}	2.0 ± 0.2^{a}	2.0 ± 0.2^{a}
Streptomyces	2.8 ± 0.2^{a}	3.8 ± 0.4^{b}	4.4 ± 0.5^{bc}	4.2 ± 0.5^{bc}
Olivibacter	2.1 ± 0.1^{bc}	1.7 ± 0.2^{b}	1.0 ± 0.1^{a}	0.8 ± 0.1^{a}
Cyanothece	1.6 ± 0.1^{c}	0.3 ± 0.0^{b}	0.2 ± 0.0^{ab}	0.1 ± 0.0^{a}
Rhodococcus	1.4 ± 0.1^{a}	2.0 ± 0.2^{b}	1.7 ± 0.2^{ab}	2.7 ± 0.3^{c}
Nitrospira	1.1 ± 0.0^{c}	1.0 ± 0.2^{bc}	0.7 ± 0.1^{b}	0^{a}
Hydrotalea	1.1 ± 0.0^{b}	1.0 ± 0.2^{cb}	1.0 ± 0.2^{b}	0^{a}
Acidobacterium	1.0 ± 0.0^{c}	1.3 ± 0.2^{c}	0.8 ± 0.1^{c}	2.0 ± 0.3^{c}
Thiobacillus	1.0 ± 0.0^{a}	2.3 ± 0.3^{b}	2.5 ± 0.3^{bc}	2.0 ± 0.3^{b}
Variovorax	1.0 ± 0.0^{a}	2.6 ± 0.3^{b}	3.1 ± 0.4^{bc}	3.5 ± 0.5^{c}
Others	8.4	8.0	9.0	8.8
Unclassified	5.0	6.0	5.6	6.6

(*)Different small superscript letters indicate statistically significant differences ($p < 0.05$) in the same treatment groups (within a line).

The relative abundances of bacteria in soil treated pure thiobencarb and with Satunil 60EC were not statistically different except for *Rhodococcus*, *Nitrospira*, *Hydrotalea* and *Acidobacterium*. Some species were not present in treated soil. In addition, a number of species could not be classified based on obtained sequences. All obtained results showed that thiobencarb and the herbicide Satunil 60EC caused shifts in the relative abundances of bacterial community in the collected soil.

3.3. Effects of pure thiobencarb and herbicide Satunil 60EC on relative indices of bacterial diversity in soil

The relative indices of bacterial diversity after 30 days were also determined. The sequence numbers significantly increased in all treatments. Soil samples incubated in favorable conditions, such as suitable moisture and light ray, were deemed to be the reason to enhance the sequence richness. Other indices of α-diversity, including OTUs, ACE, Chao1, Simpson and Shannon, were not statistically altered after one month in all treatments (Table 3). The sequences, OTU number, Chao1 and ACE denote the richness of microbial community; meanwhile, Shannon and Simpson indices show the species diversity. The increase in sequence numbers and OTUs indicated the enhancement of richness. The results in this study showed that thiobencarb and the Satunil 60EC did not reduce richness and species diversity of the bacterial community in this soil.

The effects of thiobencarb on soil microorganisms have been reported in some previous studies. Thiobencarb applied at a standard concentration did not apparently affect the density of bacteria and fungi in soils under aerobic condition (Huynh et al., 2022). The presence of thiobencarb in soil at a low concentration enhanced microbial biomass and mineralization of oxidizable organic C and N (Bhowmick et al., 2014). Jena et al. (1990) showed that adding 2 and 4 ppm to an alluvial soil reduced the population of N_2-fixing *Azospirillum*, anaerobic N_2 fixers and *Azotobacter*.

Thiobencarb also caused the shifts of bacterial structure of both genera and phyla under anaerobic conditions (Oanh & Duc, 2021). For example, Proteobacteria were dominant in a sediment slurry, which increased in treatment with thiobencarb (Oanh & Duc, 2021). The application of the compound resulted in the disappearance of some bacterial genera, but the dominance of genera *Dechloromonas*, *Azoarcus*, and *Thauera* during enrichment (Oanh & Duc, 2021). The abundances of some phyla and genera were also changed due to the supplementation of the substrate under anaerobic condition (Oanh & Duc, 2021). This study provides information on the effects of thiobencarb and the herbicide Satunil 60EC on the community of soil bacteria under aerobic conditions.

Table 3. Diversity and richness of bacterial community in original soil, soil without chemical addition (control), amended with pure thiobencarb and Satunil 60EC.

	Original soil	After one 30 days(*)		
		No chemical amendment	Pure thiobencarb	Satunil 60EC
Sequences	40941±332[a]	50115±6132[a]	48207.2±5154[b]	47254.7±5022[b]
OTUs	1145.1±67[a]	1225.1±135[a]	1244.7±133[a]	1233.4±134[a]
ACE	1635.8±77[a]	1732.7±191[a]	1777.2±182[a]	1541.8±171[a]
Chao1	1418.0±70[a]	1552.0±171[a]	1471.6±161[a]	1542.3±165[a]
Simpson	0.034±0.001[a]	0.033±0.003[a]	0.035±0.004[a]	0.036±0.004[a]
Shannon	4.1±0.05[a]	4.2±0.04[a]	4.4±0.5[a]	4.3±0.5[a]

(*) Different small superscript letters indicate statistically significant differences ($p < 0.05$) in the same treatment groups (within a line).

4. Conclusion

The degradation of thiobencarb and propanil in the herbicide Satunil 60EC in soil occurred at different rates. The half-life values of thiobencarb and propanil in soil were 37.9 and 7.9 days on average, respectively. The amendment of pure thiobencarb and the Satunil 60EC in the soil resulted in changes of the bacterial community, both in genera and phyla. However, pure thiobencarb and the Satunil 60EC did not reduce the richness and diversity of soil bacteria, even though they caused the shifts of bacterial community structure./.

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