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A Microcosm Study of Endosulfan Degradation and its Short-Term Effect on pH and Biological Parameters of Cotton Zones Soils of Burkina Faso

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Abstract: We studied under microcosm conditions the degradation of endosulfan and its effect on soil microbial respiration, soil microbial population and pH evolution in three major soil types of Burkina Faso. Results indicated that the recommended treatment dose of endosulfan (3 μg g^{-1}) did not affect soil pH. But when the dose was 6 μg g^{-1}, a stimulation of the respiratory activity of the soils during the first five days and a disturbance of the pH were observed. There were no significant impact of endosulfan at 3 and 6 μg g^{-1} of soil in the total bacterial number. After 5 days of incubation the degradation rate of endosulfan with initial concentration of 3 μg g^{-1} were 50, 56.5 and 83.5% in the soil from Boni, Farakoba and Kaibo, respectively. But when the initial concentration of endosulfan was 6 μg g^{-1}, the figures were 94.6, 79.6 and 20.4%, respectively. Endosulfan degradation in these three soils led to a production of endosulfan-sulphate.

Key words: Pesticide degradation, cotton, soil microorganisms, soil pH

INTRODUCTION

Endosulfan [6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide] is a chlorinated pesticide, which is a mixture of alpha-endosulfan and beta-endosulfan in an approximate ratio of 70:30 (Tomlin, 2000). In Burkina Faso, this pesticide is largely used to protect cotton plant against devastating insects (Vaissayre *et al*., 2006a, b). The use of this pesticide is justified by its effectiveness against *Helicoverpa armigera* which caused, in 1991, the losses of 27% of the total production of cotton in Burkina Faso (Buès and Boudinhon, 2003). However, endosulfan presents risks for water (Tapsoba and Bonzi-Coulibaly, 2006) and soil (Savadogo *et al*., 2006) pollution. Moreover, this molecule belongs to EDC (Endocrine Disrupting Chemical) pesticides which disturb the hormonal system and lead to an increase of the rate of malformations at birth, man sexual anomaly and reproduction incapacities. Endosulfan is toxic for fish and several other aquatic animals (Tomlin, 2000). In the cotton field in Burkina Faso, farmers misused endosulfan by applying over four treatments while only two are recommended (Savadogo *et al*., 2006).

The fate of endosulfan in the environment is governed by its low hydrosolubility, high volatility and persistence in the soil (Tariq *et al*., 2006). The half-life of the endosulfan in the soil varies according to the type of soil from five months to two years (Tariq *et al*., 2006). Endosulfan may decrease the total number of bacteria as compared to control field (Tu, 1970). Kathpal *et al*. (1997)

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have reported inhibitory effect of insecticides on bacteria. The persistence of endosulfan in agricultural soils has been studied in many laboratories (Awasthi *et al*., 2000; Ismail and Enoma, 2005). However, such studies have been rarely undertaken in Africa and particularly in Burkina Faso (Savadogo *et al*., 2006). Endosulfan transformation in the soil leads to the formation of metabolites such as endosulfan-sulphate, endosulfan-diol, endosulfan-lactone, endosulfan hydroxy-ether, ether-endosulfan (Awasthi *et al*., 2003). Endosulfan-sulphate, the principal metabolite of endosulfan is stable and also toxic (Martens, 1976; Ismail and Enoma, 2005). The aim of the present study was to assess the degradation of endosulfan and its effects on soil pH and the biological activity of the three major types of agricultural soils of Burkina Faso.

MATERIALS AND METHODS

Soils Sampling

Soil samples were taken in October, 2006 at 0-20 cm depth from three long-term trials conducted on experimental station located in the cotton belt of Burkina Faso (Fig. 1). The soil was from Farakoba (11°06' N; 4°20 W), Boni (11°35' N; 3°26 W) and Kaibo (11°49 N; 1°00' W). Selected soil chemical and physical characteristics are given in Table 1. The soils of these experimental stations were receiving endosulfan for about twenty years ago.

Pesticide Used

Endosulfan in granulated form (98% purity) was provided by the "Société Africaine de Produits Phytosanitaires et d'insecticides" (SAPHYTO) specialized in the formulation of insecticides for cotton in Burkina Faso.

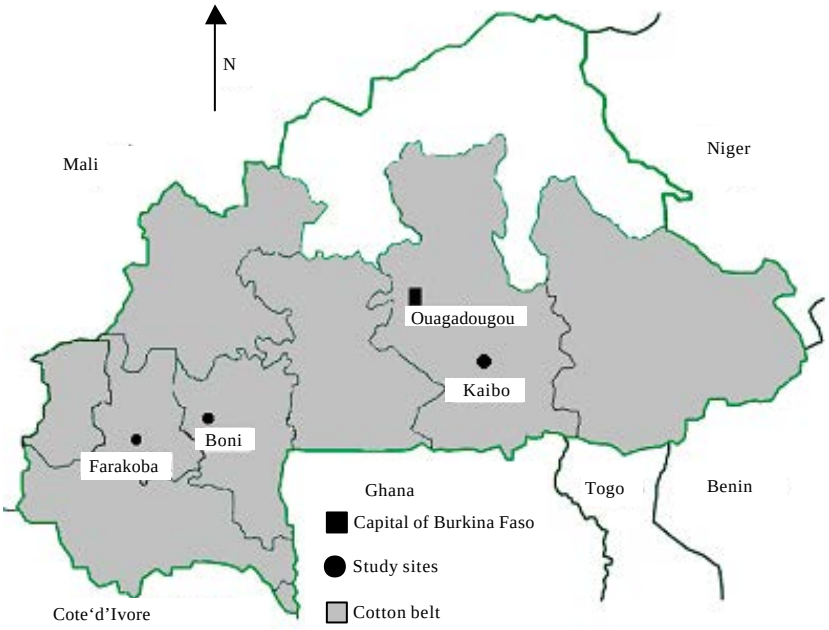

Fig. 1: Map of Burkina Faso, showing the location of the study sites (Adapted from Sofitex, 2004)

Table 1: Selected chemical and physical parameters of soil (0-20 cm depth) from Farakoba, Boni and Kaibo, Burkina Faso

	Soils		
Parameters	Farakoba	Boni	Kaibo
Clay (%)	13.80	19.80	26.10
Silt (%)	34.30	37.80	47.60
Sand (%)	51.70	42.30	26.20
Total organic carbon (%)	0.52	1.13	1.02
Organic matter (%)	0.90	1.90	1.80
Total N (%)	0.04	0.07	0.07
Total P (μg g^{-1})	123.00	179.00	148.00
pH (H_2O)	5.09	5.55	6.13
pH (KCl)	4.50	4.24	5.26
Maximum water holding capacity (g/100 g)	37.40	42.70	43.90

Study of Endosulfan Degradation in the Soil

Thirty five grams of soil humidified to the 2/3 of the water holding capacity and containing 0 (control), 3 and 6 μg g^{-1} endosulfan, were introduced into 40 mL polystyrene flask. The flasks are then placed in random into 1 L glass bottles. In order to trap the evolved carbon dioxide (CO_2), another flask containing 10 mL of sodium hydroxide (NaOH, 2 N) solution was placed in each bottle. To maintain moisture, 10 mL of sterile distilled water was also placed in the 1 L bottle and hermetically closed. The microcosms were incubated at 28°C and soil samples were taken at 0, 5, 10 and 15 days after the start of incubation and stored at -20°C for analysis.

Study of pH (H_2O) and pH (KCl) Evolution

Soil pH (H_2O) was measured using an electronic pH-meter in soil/water slurries (1 g of soil in 2.5 mL of water). The pH (KCl) was measured after addition of a quantity of potassium chloride (KCl) in the preceding slurries to give one molar concentration of KCl.

Soil Respiration Measurements

Soil respirations were determined in 100 g of soil humidified at the 2/3 of the soil water holding capacity. Endosulfan was added at the rate of 0 (control), 3 and 6 μg g^{-1}. Prior to incubation, the soils were activated during 24 h by humidification. Two flasks of 15 mL, one containing 10 mL of NaOH (0.1 N) to trap released CO_2 and the other containing 10 mL of distilled water to maintain the humidity constant, were put in each bottle. The unit was then incubated at 28°C during 15 days. The amount of CO_2 released was measured daily during the first seven days of incubation, then every two days until the 15th day, according to Isermayer and Eine Eingahge (1952) method. The quantity of released CO_2 per day was calculated using the following formula:

$$Q\ (mg) = [V_{HCl}\ (blank) - V_{HCl}\ (sample)]\ X\ 0.6$$

where, Q is the quantity of CO_2 released and V_{HCl} is the volume of HCl used to neutralize the excess of NaOH.

Soil Microbial Analysis

Plate Count Agar media (PCA, Difco) were prepared according to the directives, sterilized in autoclave at 15 psi at 120°C for 20 min and cooled in petri box at room temperature. Serial soil dilutions were prepared and 0.1 mL of dilution 10^{-4} was spread on an agar-media Petri box in four repetitions to determine population per gram of dry soil. Incubation was carried out at 28°C for 48 h. Soil bacteria were enumerated after 0, 5 and 15 days after the start of incubation. The culture media had the following composition: 5 g L^{-1} of tryptone, 2.5 g L^{-1} of yeast extract, 1 g L^{-1} of dextrose and 9 g L^{-1} of agar. The pH of the media was 7.0±0.2.

Soil Endosulfan Extraction and Analysis

Ten grams of dry soil were introduced into a 100 mL flask. Then 50 mL of a mixed solution (hexane/isopropanol at the ratio of 3 L^{-1}) was added, vigorously agitated for 45 min and decanted for 30 min. Then, 10 mL of the supernatant was taken and introduced into a separating funnel of 500 mL containing 15 mL of distilled water, agitated for 2 min and decanted for 30 min. Then the organic phase was collected, dried using sodium sulphate and filtered through Whatman qualitative filter paper Grade 2V. The pesticide extracts were kept in bottles at -20°C until the analysis.

Endosulfan analysis was carried out using a gas phase chromatograph type HP 5890 A, provided with a HP-5 (25 m×0.2 mm×0.11 μm) column, equipped with an ECD detector, an autosampler and controlled by a computer equipped with Chemstation software for the data processing. The injection was made directly into the column with a pressure of 10 Psi. The chromatograms from the samples were compared with those of the reference samples. The limit of detection was 0.02 μg g^{-1} for the endosulfan.

Statistical Analysis

Prior to run the Analysis of Variance (ANOVA), variables were tested for homogeneity of variance. Means were compared with the test of Student Newman-Keuls using XLSTAT 6.1.9 software.

RESULTS

Effect of Endosulfan on Soil pH

The addition of endosulfan did not affect soil pH (H_2O) and pH (KCl) at day 0 and day 5 as compared with the control (Table 2). However, after 15 days of incubation, addition of endosulfan resulted in significant higher soil pH(KCl) than the control (without endosulfan addition) with the soils from Farakoba and Kaibo, while with the soil from Boni it was lower (Table 2).

Effect of Endosulfan on Soil Respiration

Effect of Endosulfan on the Daily Release of CO_2

There were two phase in the CO_2 evolution curves whatever the treatment and the soil type (Fig. 2):

- A phase of high CO_2 production followed by a decrease from the 1st to the 5th day of incubation. This phase was characterized by a peak at the 3rd day with soils from Farakoba and Kaibo.
- A stationary phase, with a smaller peak at the 7th day. This phase went from the 5th to 15th day of incubation.

The statistical analysis did not reveal a significant difference between the treatments (Table 3).

Table 2: Soil pH (H_2O) and pH (KCl) as affected by incubation time and endosulfan application rate

		Soils					
	Endosulfan	Farakoba		Boni		Kaibo	
Days after incubation	concentration (μg g^{-1})	pH (H_2O)	pH (KCl)	pH (H_2O)	pH (KCl)	pH (H_2O)	pH (KCl)
0	0	5.49^a	4.33^a	6.73^a	5.60^a	6.52^a	5.28^a
	3	5.52^a	4.29^a	6.72^a	5.60^a	6.57^a	5.28^a
	6	5.45^a	4.34^a	6.66^a	5.57^a	6.53^a	5.23^a
5	0	5.73^a	4.38^a	6.82^a	5.71^a	6.61^a	5.29^a
	3	5.67^a	4.41^a	6.80^a	5.72^a	6.58^a	5.32^a
	6	5.69^a	4.40^a	6.72^a	5.74^a	6.59^a	5.39^a
15	0	5.81^a	4.50^a	6.91^a	5.81^b	6.77^a	5.50^a
	3	5.97^a	4.66^b	6.87^a	5.74^b	6.91^a	5.56^b
	6	5.99^a	4.63^b	6.90^a	5.69^a	6.77^a	5.65^c

For each incubation day, means with the same letter(s) in the same column are not statistically different at $p<0.05$

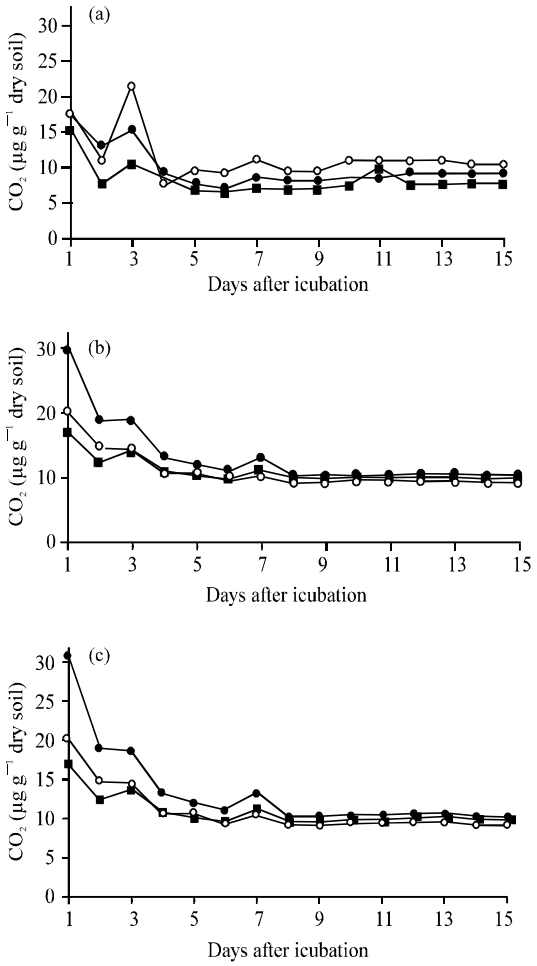

Fig. 2: Daily microbial respiration of soils from (a) Farakoba, (b) Kaibo and (c) Boni after addition of different rate of endosulfan. (■) Control without endosulfan, (○) initial endosulfan concentration of 3 µg g^{-1} and (●) initial endosulfan concentration of 6 µg g^{-1}

Effect of Endosulfan on the Cumulated Release of CO_2

Addition of endosulfan increased the cumulated quantities of CO_2 during 15 days of incubation as compared with the control in the three types of soils. However, these differences were not significant. At each day after incubation, there was no treatment effect on the cumulative CO_2 production with the soil from Farakoba (Table 3). After 5 days of incubation, the addition of 6 µg g^{-1} of endosulfan induced higher significant cumulative CO_2 with soils from Boni and Kaibo. No significant difference was observed between soils with 3 µg g^{-1} and without endosulfan (Table 3). The results showed that the soil from Boni had the higher respiratory activity (280.8 µg CO_2 g^{-1} of dry soil) followed by the soil from Kaibo (150.8 µg $CO_2 g^{-1}$ of dry soil) and the soil from Farakoba (120 µg CO_2 g^{-1} of dry soil).

Effect of the Endosulfan on Soil Microflora

With the soil from Farakoba, counting of Colony Forming Units (CFU) indicated that at day 5 after incubation, addition of 6 µg g^{-1} endosulfan increased significantly the microbial population as

Table 3: Cumulative soil respiration (μg CO_2 g^{-1} dry soil) as affected by incubation time and endosulfan application rate

Days after incubation	Endosulfan concentration (μg g^{-1})	Farakoba	Boni	Kaibo
0	0	10.50^{a}	30.40^{a}	10.56^{a}
	3	10.74^{a}	30.42^{a}	20.02^{ab}
	6	10.53^{a}	40.28^{a}	30.06^{b}
5	0	40.72^{a}	120.36^{a}	60.33^{a}
	3	60.62^{a}	120.81^{a}	70.06^{a}
	6	60.06^{a}	140.38^{b}	90.34^{b}
15	0	120.00^{a}	280.80^{a}	150.81^{a}
	3	160.88^{a}	290.76^{a}	160.90^{a}
	6	160.98^{a}	290.72^{a}	170.10^{a}

For each incubation day, means with the same letters in the same column are not statistically different at $p<0.05$

Table 4: Total number of bacteria (cfu×10^{7} g^{-1} dry soil) as affected by incubation time and endosulfan application rate

Days after incubation	Endosulfan concentration (μg g^{-1})	Farakoba	Boni	Kaibo
0	0	9.67^{a}	16.7^{a}	35.1^{a}
	3	7.95^{a}	20.1^{a}	37.6^{a}
	6	9.76^{a}	14.8^{a}	25.1^{a}
5	0	4.89^{a}	22.5^{a}	18.7^{a}
	3	8.33^{ab}	23.3^{a}	15.9^{a}
	6	9.93^{b}	14.2^{a}	14.8^{a}
15	0	5.77^{a}	17.3^{a}	22.1^{a}
	3	8.12^{a}	12.5^{a}	17.4^{a}
	6	6.18^{a}	22.1^{a}	16.4^{a}

For each incubation day, means with the same letters in the same column are not statistically different at $p<0.05$

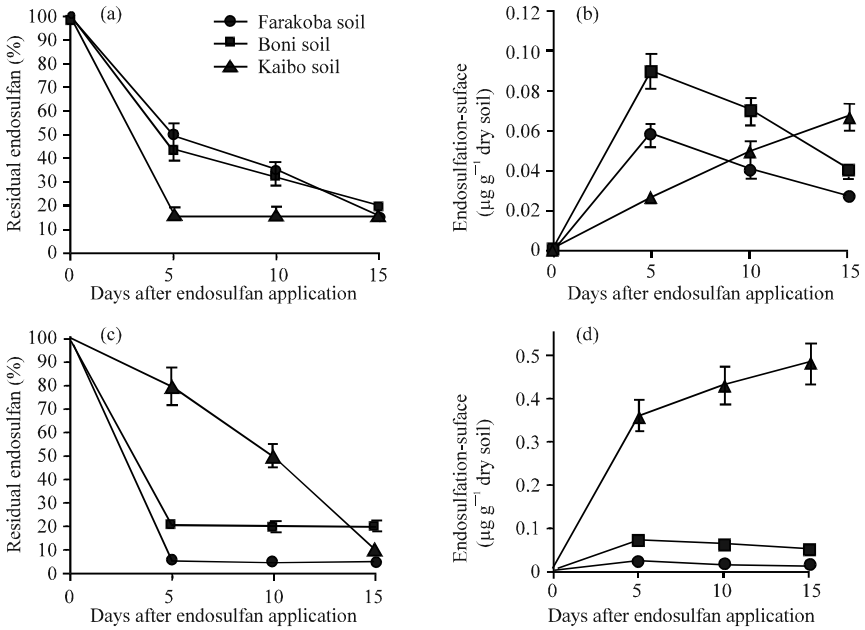

Fig. 3: Residual endosulfan and endosulfan-sulphate production in soils from Farakoba, Boni and Kaibo during 15 days of incubation at 28°C. (a) and (b) were respectively the rate of residual endosulfan and the production of endosulfan-sulphate when endosulfan was applied at 3 μg g^{-1}. (c) and (d) were respectively the rate of residual endosulfan and the production of endosulfan-sulphate when endosulfan was applied at 6 μg g^{-1}. Bars indicated standard deviation. Note the difference in scale between Fig. 3b and d

compared with the control. However, at day 0, 5 and 15 there were no significant endosulfan addition effect on microbial population for soil from Boni and Kaibo (Table 4).

Endosulfan Degradation Rate

After the 5 days of incubation with soil from Boni, we got a slower degradation of endosulfan applied at 3 μg g^{-1} (50.6%, Fig. 3a) compared to 6 μg g^{-1} (79.6%, Fig. 3c). With soil from Kaibo, addition of endosulfan at the rate of 3 μg g^{-1} had higher degradation rate (83.5%) than endosulfan added at the rate of 6 μg g^{-1} (20.3 %) (Fig. 2). With soil from Farakoba, the degradation of endosulfan was higher in soil containing 6 μg g^{-1} (94.5%) than that containing 3 μg g^{-1} (50%). So, the degradation of the endosulfan in the soils from Kaibo and Boni is slower when the amount of the pesticide is higher. Endosulfan-sulfate, a metabolite of endosulfan was produced after 5 days of incubation. From day 5 to day 15 after incubation the amount of endosulfan-sulphate decreased in the soils from Farakoba and Boni but increased in the soil from Kaibo (Fig. 3b, d).

DISCUSSION

There were no significant effect of endosulfan addition on soil pH (H_2O) and pH (KCl) after 5 days of incubation. After 15 days of incubation the pH (H_2O) was not affected but the pH (KCl) was significantly affected by the presence of endosulfan. Endosulfan seemed to modify the cation exchange of the soils and reduce its buffer capacity. Soils have normally a buffer capacity which enables them to avoid acidity or excess of alkalinity due to incorporation of acidic or basic products. Endosulfan at high concentration could disturb soil buffer capacity (Awasthi *et al*., 2000). Soils respiration studies showed that the soil from Boni had the higher respiration ability, followed by the soil from Kaibo and finally the soil from Farakoba. The increase in respiration rate may be explained partially by organic carbon mineralization (Martens, 1976; Khan and Anjaneyulu, 2005). The importance of the respiratory activity was related to soil carbon content. Indeed soil from Boni which had the higher organic carbon content had the highest respiratory activity (11.25 μg g^{-1}) and followed by soil from Kaibo (9.69 μg g^{-1}) and soil from Farakoba (5.24 μg g^{-1}). Generally, the endosulfan with amount of 6 μg g^{-1} induced a stimulation of the respiratory activity of the soils during the first 5 days of incubation. But with 3 μg g^{-1} the endosulfan does not significantly modify soil respiration. In support to our findings, Barriuso *et al*. (1996) reported that the presence of low quantities of pesticides can result in lack of significant effect on the enzymatic activity of soil microflora. Endosulfan at 6 μg g^{-1} could stimulate the production of enzymes able to degrade organic matter. In fact, several studies indicated that endosulfan degradation in soil is carried out by mushrooms, bacteria and actinomycetes (Martens, 1976; Awasthi *et al*., 2000).

The effect of pesticides on microbial population is delicate and sometimes difficult to explain since soil remains a black box in the biological functioning point of view. Nevertheless, the absence of modification of the microbial population indicated that the amount of pesticide applied was insufficient to induce an effect. In liquid medium, Drobnikova and Bacilek (1982) reported that Fenitrothion at the concentration of 200 μg L^{-1} inhibits microbial growth which is characterized by a longer lag-time of microorganisms growth phase.

The effect of pesticides on the number of soil microorganisms were in the same direction as for respiratory activity. The lowest number of soil bacteria was obtained with the soil from Farakoba while the soils from Boni and Kaibo had a highest soil bacterial population. Repeated use of a pesticide in the soil can induce the increase of specific microbial population. In this case, the pesticide becomes a source of carbon for those groups of microorganisms (Iqbal *et al*., 2001; Ikeda *et al*., 2005; Savadogo *et al*., 2006, 2007).

The residual endosulfan evolution in the soils from Farakoba and Boni showed a more significant degradation when the concentration is 6 μg g^{-1}, compared to 3 μg g^{-1}. This means that the

concentration of 6 μg g^{-1} of endosulfan induced a microbial activity suitable to endosulfan degradation. Present results were in lines with the findings of Fournier and Soulas (1984) who reported that after 20 days, the degradation rate of herbicide 2,4-Dichlorophenol were 20 and 40% when the concentration is 0.1 μg g^{-1} of soil and 10 μg g^{-1}, respectively. This was explained by the fact that the very low concentrations of pesticides are unfavourable for the development of the degrading activity and specially the emergence of the metabolic populations.

After five days of incubation, the degradation rate was higher with the soil from Kaibo than that from Boni which in turn was higher than that from Farakoba when the endosulfan concentration was 3 μg g^{-1}. However, the degradation rate was inversed when the endosulfan was added at 6 μg g^{-1}, as the degradation was higher with the soils from Farakoba and Boni. Higher degradation rate in the presence of 3 μg g^{-1} of endosulfan in the soils from Boni and Kaibo can be explained by the fact that these soils contain more clays (respectively 19.80 and 26.10%) and organic matter (respectively 1.9 and 1.8%) compared with the soil from Farakoba which contains 13.80% of clay and 0.9% of organic matter (Boivin *et al*., 2005; Ikeda *et al*., 2005). Thus, if this amount of endosulfan was not suitable to induce biodegradation, then its degradation remained primarily abiotic. Abiotic degradation would be more intense in clay and organic matter rich soils (Boivin *et al*., 2005). Kumar and Philip (2006) showed that the content of organic matter is correlated with the coefficients of adsorption. Clays can cause chemical conversion of adsorbed organic molecules. Khan and Anjaneyulu (2005) carried out a study with three types of soil (Isnapur, Muttangi and Bolarum) and four types of phenolic pesticides (Phenol, p-Nitrophenol, 4-Chloro-2-nitrophenol and 2,4-Dichlorophenol) applied at the rate of 5 and 25 μg g^{-1}. They found that with Muttangi soil samples (7% of clay and 0.93% of organic carbon), the amount of 2,4-Dichlorophenol adsorbed were 33.3 μg g^{-1} of soil and 175 μg g^{-1} of soil when the application rate is 5 and 25 μg g^{-1}, respectively. With the Isnapur soil samples (28.3% of clays and 1.5% of organic carbon), the amount of 2,4-Dichlorophenol adsorbed were 58.3 and 216.6 μg g^{-1} of soil when the application rate was 5 and the 25 μg g^{-1}, respectively. For the Bolarum soil samples (10% of clay and 2.4% of organic carbon), the figures were 66.6 and 333.3 μg g^{-1} of soil, respectively. These findings support the role played by clays, silts and organic matter in the adsorption of the pesticides.

Generally, the endosulfan degradation rate in the soil is lower compared to our results. This can be explained by the fact that endosulfan had been used for more than ten years in the long-term experimental stations. Consequently the soils would contain a specific microflora for endosulfan degradation. However, contrasting findings have been reported on pesticide degradation rate as result of repetitively application (Ukai *et al*., 2003; Ikeda *et al*., 2005; Savadogo *et al*., 2007).

The degradation of endosulfan in the soils from Farakoba, Boni and Kaibo produced endosulfan-sulphate which was accumulated in the soil from Farakoba while it was an intermediate metabolic in the soils from Boni and Farakoba. In the latter soils, the endosulfan-sulphate produced after 5 days of incubation tended to be degraded as incubation proceeded. Similar studies showed that endosulfan-sulphate is the major metabolite formed during the mineralization of the endosulfan (Martens, 1976). According to these authors, this metabolite may undergo to degradation to give endosulfan-diol, endosulfan-ether or endosulfan-hydroxyether. These metabolites may be accumulated in the soil for several months to several years according to soil type.

CONCLUSION

We found that endosulfan employed at the rate of 3 μg g^{-1} did not affect the respiratory activity and the cultivable aerobic microorganisms of three soils from cotton belt of Burkina Faso. At higher endosulfan application rate, 6 μg g^{-1} of soil, carbon mineralization rate was activated during the first 5 days of incubation. The study also showed that the endosulfan at 6 μg g^{-1} increased microbial population in soil containing lower amount of clay and organic matter. Consequently, endosulfan at

high application rate may induce a mineralization rate of the soil organic matter and disturbance of biological balance in the early stage after the pesticide application.

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