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Research Article

Effect of Three Pesticides on Soil Dehydrogenase and Fluorescein Diacetate Activities in Vegetable Garden in Burkina Faso

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Abstract: Many studies have shown that pesticides use have some effect on soil biology. However little information is available concerning the effect of pesticides on soil enzyme activities in semi arid zone of Africa. The aim of this study was to investigate the effect of three pesticides usually used (endosulfan, deltamethrin and profenofos), on soil Fluorescein Diacetate (FDA) and dehydrogenase activities from cultivated and fallow plot. Enzyme activities were followed in 5 days incubated soil, containing 200 mg/kg of each pesticide. The results showed that endosulfan, deltamethrin and profenofos significantly decreased soil dehydrogenase activity ($p<0.005$). In incubated soils no effect of these pesticides on Fluorescein Diacetate (FDA) activity was found ($p>0.005$). These results clearly show the impacts of these pesticides on soils enzyme activites.

Keywords: Burkina faso, dehydrogenase, fluorescein diacetate, pesticide impact, soil biology

INTRODUCTION

Currently, soils are becoming more and more polluted by pesticides molecules because of their wide use in agriculture practices. Pesticides differ in their biodegradability, thus several of them are persistent soil pollutants. Pesticides may influence more or less soil biological activities, which is the result of microbial and enzymatic transformations. Pesticides may be toxic to some important bacterial groups; other microorganisms are able to use some pesticides as energy and nutrient sources (Johnsen *et al*., 2001; Mokiedje and Spiteller, 2002). In addition, some metabolites of pesticides may even be more adverse to microorganisms than the original compound (Topp *et al*., 2004). Different effects of pesticides on soil living components have been reported. Many of pesticides have shown adverse impacts on numbers and functions of diverse range of microorganisms. The reduction of bacterial and fungal biomass, may affect soil respiratory activity, soil enzymes and microbial diversity as well as rates of carbon and nitrogen turnover (Johnsen *et al*., 2001; Lin and Brookes, 1999; Mokiedje and Spiteller, 2002; Naré *et al*., 2010; Pozo *et al*., 1995). However, many factors such as soil structure and texture, pH, organic matter content, temperature and moisture influence pesticides effects on soil microorganisms (Beulke and Malkomes, 2001; Kim *et al*., 2002; Mokiedje and Spiteller, 2002). The persistence, availability and toxicity of pesticides as well as microbial metabolism strongly depend on these factors.

In sub-saharan zone of Africa including Burkina Faso, little studies are available on the effects of pesticides on soil enzymes activities.

The aim of this study was to assess the effects of three pesticides widely used, endosulfan, deltamethrin and profenofos, on soil enzyme activities in two land use systems in Burkina Faso.

MATERIALS AND METHODS

Study site description:

Soil sampling: Soil samples from five spots at 0-20 cm depth were taken from each plot in July 2008 in cultivated fields near the main dam of Ouagadougou (12°27; 1°30 and 300 above the sea level) in Burkina Faso, West Africa. Selected soil chemical and physical characteristics are given in Table 1. Four farmers having similar cultural practices were selected. In the vicinity of each selected field, one fallow plot which had never received any application of pesticides and organic matter was selected as control plot. Samples were sieved through 2 mm mesh for enzymatic test and physical and chemical characterizations.

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Table 1: Selected chemical and physical parameters and Microbial Biomass of soil from cultivated plot and fallow plot (0-20 cm depth)

	Clay[a] %	Silt[a] %	Sand[a] %	Organic Matter[b] (%)	pH[c] (H_2O)	pH[c] (KCl)	N[d] total (%)	K[e] total (mg/kg)	P[f] total (mg/kg)	C[b]/N[d]	MB[g] (mg-C CO_2/g)
Cultivated plot	15.69	31.37	52.94	1.5	8.3	7.8	0.06	900	950	12.97	0.902
Fallow plot	13.73	25.49	60.78	0.9	8.0	8.0	0.04	770	440	12.83	0.508

[a]: Clay, Silt and Sand Were determined according Robinson pipette method; [b]: Total carbon content and organic matter were determined by the Walkley and Black (1934) method; [c]: The pH (H_2O) and pH (KCl) were determined using an electronic pH-meter in 1 g soil in 2.5 mL water and KCl 1M slurries, respectively; [d, e, f]: For total soil nitrogen and total soil phosphorus and potassium analyses, the soil was first mineralized using H_2S0_4-Se-H_2O_2, then P and total N were determined using an automatic colorimeter SKALAR (Walinga *et al*., 1989; Walinga *et al*., 1995); Potassium was determined by photometer method; [g]: Microbial biomass was determined by the fumigation extraction method using a correction factor

Pesticides used: Endosulfan® and Deltamethrin® in granulated form (98% purity), Profenofos® (89% purity) were provided by the company specialized in the formulation of pesticides for cotton in Burkina Faso.

Soil pesticides extraction and analysis: Ten (10 g) gram of dry soil were introduced into a 100 mL flask. Then 50 mL of a mixed solution (hexane/isopropanol; 3/1) was added, vigorously shaken for 45 min and decanted for 30 min. Then 10 mL of 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. The pesticide extracts were kept in bottles at 4°C until the analysis.

Pesticides 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 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.

Measurement of soil enzyme activities:

Dehydrogenase activity: For measuring dehydrogenase activity, 1 g of dry soil was incubated with 1 mL of 0.4% (w/p) Triphenyltetrazolium Chloride (TTC) in Tris-HCl (0.1 M) buffer at pH 7.6 for 16 h at 25°C. The triphenylformazan (TPF) formed was extracted with 5 mL of acetone by shaking vigorously for 2 h and filtered. TPF was measured spectrophotometrically at 546 nm (Thalmann, 1968).

Fluorescein diacetate hydrolyzing activity: For measuring Fluorescein Diacetate hydrolyzing Activity (FDA), 1 g of soil was incubated with 15 mL of 60 mM potassium phosphate buffer pH 7.6. Stock solution (1 mg/mL) was added to start the reaction. Blanks were prepared without the addition of the FDA substrate along with a suitable number of sample replicates. Samples were then placed in an orbital incubator at 30°C for 20 min. Once removed from the incubator, 15 mL of acetone was added immediately to end the reaction. The contents were then transferred to centrifuge tubes and centrifuged at 2000 rev/min for 3 min. The supernatant from each sample was then measured at 490 nm on a spectrophotometer.

Statistical analysis: Analysis of Variance (ANOVA) was performed using a General Linear Model (GML) implemented in Minitab (V. 14) statistical software for Window (Minitab Inc.). Differences were significant when $p<0.05$ according to Tukey's test (Tukey, 1977).

RESULTS

Results from cultivated soil showed more pesticides residues than fallow soil (Table 2). Also, o.p DDE (0.008 μg/g of soil), pp-DDE (1.18 μg/g of soil), endrin (0.28 μg/g of soil) et aldrin (0.16 μg/g of soil) were detected in cultivated soil when undetectable in fallow soil. The concentration of heptachlore and endosulfan sulphate were respectively 0.1 μg/g of soil and 0.05 μg/g of soil in cultivated soil but 0.0627 μg/g of soil and 0.035 μg/g of soil in fallow plot. However p.p-DDT was higher in fallow soil (0.068 μg/g of soil) than cultivated soil (<LD).

Effects of pesticides on soil enzyme activities: According to Adam and Duncan (2001) the estimation of FDA is a accepted method for measurement of the total microbial activity in soil. FDA might be considered as a suitable tool for measuring the early detrimental effect of pesticides on soil microbiology (Bjornlund *et al*., 2000). Fluorescein Diacetate Hydrolyzing Activity was significantly higher in cultivated soil (9.04 μg fl/g dry soil/h) than fallow soil (3.96 μg fl/g dry soil/h) before incubation. After 5 days of incubation, there was no significant difference between soil from cultivated plot and soil from fallow plot. The activity of dehydrogenase was almost the same (0.36 μg of TPF/g dry soil/24 h) before incubation and after 5 days of incubation in both soils. Results showed that both activities increased with incubation in both soils (Table 3).

The results showed that endosulfan, deltamethrin and profenofos exhibited an inhibitory effect on dehydrogenase activity after 5 days of incubation in

Table 2: Residual pesticides in soils µg/g of soil

	Heptachlore	o, p-DDE	p, p- DDE	Endrin	Aldrin	Endosulfan sulfate	p, p-DDT
Cultiveted plot	0.1	0.008	1.18	0.28	0.16	0.05	<LD
Fallow plot	0.0627	<LD	<LD	<LD	<LD	0.035	0.068

LD: Limit of Détection; DDT: Dihlorodiphenyl- Trichloroéthane; 25; DDE: Dihlorodiphenyléthane-trichloroéthylène

Table 3: Fluorescein diacetate hydrolyzing and dehydrogenase activities in two plots after 0 and 5 days incubation

	Fluorescein Diacetate Hydrolyzing Activity (ug fl/g dry soil/h)		Dehydrogenase activity µg de TPF/g dry soil /24 h	
Day after incubation	Cultivated plot	Fallow plot	Cultivated plot	Fallow plot
0	9.04±1.25[a]	3.96±0.47[b]	0.37±0.04[a]	0.36±0.10[a]
5	15.83±2.93[a]	10.37±2.58[a]	0.65±0.05[a]	0.65±0.08[a]

In a same line, values affected with the same letter are not significantly different at p<5%

Table 4: Fluorescein diacetate hydrolyzing and dehydrogenase activities in cultivated and fallow plots 5 days after the addition of pesticides

	Fluorescein Diacetate Hydrolyzing Activity (ug fl/g dry soil/h)		Dehydrogenase activity µg de TPF/g dry sol/24 h	
Pesticides	Cultivated plot	Fallow plot	Cultivated plot	Fallow plot
Endosulfan	27.71±18.67[a]	12.87±4.20[a]	0.12±0.08[b]	0.15±0.10[b]
Deltamethrin	9.00±00.00[a]	12.79±9.50[a]	0.25±0.04[b]	0.17±0.01[b]
Profenophos	23.33±11.49[a]	26.50±15.28[a]	0.19±0.03[b]	0.16±0.03[b]
Control	18.83±5.50[a]	8.25±10.62[a]	0.68±0.08[a]	0.68±0.08[a]

In a same column, values affected with the same letter are not significantly different at p<5%

both soils. Deshydrogenase activity is considered indicators of: biological equilibrium in soil (Frankenberger Jr. and Tabatabai, 1991), quality of cultivated soils (Quilchano and Maran˜o´n, 2002), pollution in soil (Chu *et al*., 2003). Dehydrogenase activity of samples was significantly (p<0.005) lower than control. However, no significant (p>0.005) differences were found in FDA activity, between soils treated with endosulfan, deltamethrin, profenofos and control in both soils. The results showed that deltamethrin had the lowest FDA activity in cultivated soil. The other pesticides increased the FDA activity in both soils (Table 4).

DISCUSSION

The present study showed that before incubation, FDA activity was significantly higher in cultivated soil compared to fallow soil. After 5 days of incubation, no difference was observed with both activities in both soils. The most presence of presence of pesticides residues, organic matter and microbial biomass (Table 2) can explain the significantly higher of FDA in cultivated soil. Also, Araùjo *et al*. (2003), showed that an exposed soil to glyphosate for several years had the positive response in microbial activity. The result was an increase of 10-15% in the CO_2 evolved in the presence of glyphosate compared with the same soil, which never received glyphosate. These results were also in accord with Schnurer and Rosswall (1982) which showed that FDA activity increased with soil organic matter, humidity and microbial biomass. However there was no difference with dehydrogenase activity in both soils. The enzymes of the dehydrogenase complex are associated with respiration (Yegen and Tuzun, 1999).

In the present study, there were no significant effects of endosulfan, deltamethrin and profenofos addition on both soils with FDA activity after 5 days of incubation. Soil incubation permits such properties to stabilize but the effect on microbial activities is not clear (Rose *et al*., 1980). West *et al*. (1986) showed a decrease in biomass C (35%) and an increase in ATP (59-67%) after 7days of incubation. The absence of modification of the FDA activity indicated that the amount of pesticide applied was insufficient to induce an effect. In support to these finding, 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 microbial. Imazethapyr, under field and laboratory studies, applied at field rate application had no adverse effect on FDA but not at the higher rates (Haney *et al*., 2002). Perucci *et al*. (2000) studied the effects of rimsulfuron on FDA of soil under laboratory conditions. The authors did not observe any effect on FDA at the field dose but at the higher doses the effect was prominent. Maarit Niemi *et al*. (2009) showed that stimulation or decreased depended on the enzyme, the herbicide, its concentration and duration of the exposure. Also, Dutta *et al*. (2010) studied the effects of chlorpyrifos on the Fluorescein Diacetate hydrolysing Activity (FDA) and showed that this enzyme activity was not affected by chlorpyrifos at field rate, but at higher dosage significantly decreased was observed. These results were in disaccord with the results we found. Indeed, even with 200 mg/kg didn't find any effect.

Dehydrogenase activity is only present in viable cells and it is useful indicator of overall microbial activity in soil (Kiss *et al*., 1975). The increased activity of dehydrogenase during the periods of incubation in soils treatments with endosulfan, deltamethrin and profenofos in this study, suggests increased microbial activities during this period. Similar observations were also made by Pandey and Singh (2004), Singh and Singh (2005a, b) and Tejada *et al*. (2011) who reported

that dehydrogenase activities were inhibited by insecticides. Inhibition of dehydrogenase activity by chlorpyrifos and quinalphos was also reported by Menon *et al*. (2005). In the other hand some studies showed dehydrogenase activity increasing after pesticides application (Fragoeiro and Magan, 2008; Singh and Kumar, 2008). Yao *et al*. (2006) showed that after 14 days application of acetamiprid at rates from 0.5 to 50 mg/kg soil dehydrogenase activity was stimulated.

CONCLUSION

Repeated application of pesticide in field affected soil FDA activity. In laboratory condition, endosulfan, deltamethrin and profenofos addition decreased soil dehydrogenase activity. This study showed that effect of pesticides on soil enzyme activities depends on the conditions of study. This can be lead to soil fertility decreasing by soil respiration inhibition.

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