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Full Length Research Paper

Microcosm study of the long term effect of endosulfan on enzyme and microbial activities on two agricultural soils of Yaounde-Cameroon

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Soil samples with different physical and chemical characteristics were taken from two sites, Minkoa-Meyos and Mendong, in Yaounde. These samples were treated with the insecticide endosulfan, using commercially recommended rates (1.5l ha^{-1}), and incubated in the laboratory at 20°C in glass flask for 75 days in order to evaluate their toxic effects on enzyme activities, organic matter content and the availability of phosphorus in the soils studied. The enzyme activities studied included dehydrogenase, β-D glucosidase, acid and alkaline phosphatases. The results showed that, on one hand, endosulfan inhibits dehydrogenase, acid and alkaline phosphatase activities in both soils. β-D Glucosidase activity was inhibited in Mendong soil, but this activity seems not to be affected by endosulfan in Minkoa-Meyos soil. On the other hand, endosulfan appeared to have no measurable effect on the organic carbon content and on the availability of phosphorus in Minkoa-Meyos soil. However, these two functions were inhibited in the Mendong soil. Moreover, significant correlations in the Mendong soil at the highest concentration were obtained between acid phosphatase activity and the available phosphate (P = 0.024; r = 0.821), and between alkaline phosphatase activity and the available phosphate (P = 0.014; r = 0.858).

Key words: Endosulfan, soil microcosm, enzyme and microbial activities, exposition time.

INTRODUCTION

Soil is a dynamic living system and consists of variety of micro- and macroflora and fauna including a wide range of microorganisms. Microorganisms represent one of the largest reservoirs for essential nutrients. They are considered as the living pool of organic matter, which is vital for the maintenance of soil health. These organisms, through their enzymes activities, have a primary catabolic role in the degradation of plant and animal residues in the environment, which contributes to the cycling of nutrients. Microbes, being in intimate contact with the soil microenvironment, make ideal monitors of soil pollution (Brooks, 1995). Significant increases in agricultural productivity have resulted from control of agricultural pests with man-made chemical pesticides, but on the same hand, there is a wide spread concern about the residues of these man-made chemicals in food and the environment. An appreciable proportion of the insecticides applied often reach the soil either directly from deliberative applications to the soil or indirectly from runoff from leaves and stems of the plants. Persistence and dispersion of pesticides in the soil environment depends not only on the properties of the pesticides but also on the properties of the soil and the prevailing climatic conditions (Khan, 1980). Endosulfan is one of the

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Abbreviations: OCPs, Organochlorine pesticides; **TTC,** triphenyltetrazolium chloride; **TPF,** triphenylformazan; **MUB,** modified universal buffer; **PNP,** p-nitrophenol.

Table 1. Concentration of endosulfan applied on the soils.

Concentration	C_1	C_2	C_3	C_4	C_5	C_6
Dosage µg/g of dry soil	0.5	1	5	10	50	100

organochlorine pesticides (OCPs) and also a candidate to be included in a group of new persistent organic pollutants (UNEP, 2007).

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6,9-metano-2, 4, 3-benzodioxathiepin-3-oxyde) is a mixture of two stereoisomers, α- and ß-endosulfan, in a ratio of 7:3 (Tomlin, 2000). Endosulfan is used extensively throughout the world to control the insect pests of a wide range of crops and most importantly, it has been used for the control of *Helicoverpa sp*. in cotton cropping. However, endosulfan presents risk for water (Tapsoba and Bonzi-Coulibaly, 2006) and soil (Savadogo et al., 2006) pollution. Endosulfan is extremely toxic to fish and aquatic invertebrates and has been implicated increasingly in mammalian gonadal toxicity, genotoxicity, and neurotoxicity (Sutherland et al., 2000). It can bind to soil particles and persist for a relatively long period with half-life of 60-800 days depending on the type of soil (Siddique et al., 2003; Tariq et al., 2006). Accordingly, the manufacturing and use of these chlorinated pesticides has either been banned or severely restricted in most developed and developing countries. In Cameroon, in the group of chlorinated insecticides, only the use of endosulfan is now permitted. In this country, the major use of pesticides is on the coffee, cocoa and cotton crops. Endosulfan and its isomers are designated to be biologically active and their microbial breakdown for carbon or sulphur sources (Siddique et al., 2003) is an important means of biodegradation and bioremediation. However, continuous input of these chemicals into the soil ecosystem could potentially affect the soil microorganisms and their activities. This may lead to the stimulation, decrease, or modification of soil biological processes (Domsch et al., 1983; Tu, 1995) that are essential for soil fertility and crop productivity. Endosulfan may decrease the total number of bacteria as compared to control field (Tu, 1970). However, Savadogo et al. (2009) reported a stimulation of the respiratory activity on the soil during the first five days. Kathpal et al. (1997) have reported inhibitory effect of insecticides on soil bacteria.

Commonly studied microbial reactions that are impacted by inorganic or organic pollutants include carbon mineralization, nitrogen mineralization, CO_2 production, and enzyme activities (Domsch et al., 1983). The potential reduction of these parameters can be used as an indicator of stress. Moreover, soil enzymatic activities are good soil quality indicators, as they are related to processes of transformation of soil organic matter and with the biogeochemical cycles. The importance of soil enzymes resides in their relationship to the soil microbiology, ease of measurement and rapid response to changes in soil management. It is conceptually wrong to rate a single enzyme activity as criteria of soil quality or soil microbial activity (Nannipieri, 1994). Thus, many enzyme activities should be considered. Phosphatase activities are considered as useful indicators of both positive and negative effects of soil management practices on soil quality (Dick, 1994; Jordan et al., 1995). Glucosidase activity is often included in studies searching for sensitive biomarkers of soil quality (Bucket and Dick, 1998). Its activity is considered to be a useful index for measuring side effects of pesticides on microbial activity in soil (Schäffer, 1993). Dehydrogenase activity in soil provides an index of the overall microbial activity (Nannipieri et al., 1990).

The effect of endosulfan on microbial functions in agricultural soils has been studied in many laboratories. However, such studies have been rarely undertaken in Central Africa particularly in Cameroon to the best of knowledge. The aim of this study is to assess the effect of endosulfan on enzymes (dehydrogenase, β-D-glucosidase, acid and alkaline phosphatases) activities and to assess the effect of this pesticide on the organic carbon content and phosphorus availability in two types of agricultural soils in Yaounde, Cameroon.

MATERIALS AND METHODS

Insecticide used

Thiosulfan 350 g/L, the trade name of endosulfan in the emulsifible concentrated form (EC) was provided by ADER (Action pour le Dévelopement et l'Economie Rurale), a company specialized in the distribution of crop protection products in Cameroon. The pesticide was applied at the recommended dosage for the coffee and cocoa crops (0,466 µg/g of dry soil) and according to the rate in the literature (Table 1).

Soil sampling

The potential reduction of these parameters can be used Soil samples (0 - 20 cm depth) were taken at random from 5 places in each field using a plastic soil auger and mixed thoroughly to prepare one composite sample. The soil was from Mendong and Minkoa-Meyos. Both sites are located in Yaounde (3° 52′ 12″N; 11°31′ 12″E) and have not been treated with pesticides for at least

Table 2. Chemical and physical parameters of soil (0-20 cm depth) from Minkoa-Meyos and Mendong.

Parameter	Soil from Minkoa-Meyos	Soil from Mendong
Facies	Ferrallitic red soil	Ferrallitic black soil
Clays (%)	31	26
Silt (%)	24	18
Sand (%)	45	57
Organic Matter (%) = C x 1,724	5.19	5.52
Total N (%)	0.17	0.26
Total P (mg/kg)	0.11	17.28
C/N	17.70	12.40
pH (H2O)	4.8	6.4
Maximum Water Holding Capacity (%)	54.4	39.7
Ca^{2+} (meq/100g)	0.84	9.8
Mg^{2+} (meq/100g)	0.50	1.37
K^{+} (meq/100g)	0.08	0.36
Na^{+} (meq/100g)	0.01	0.05

the past five years. The crops grown irrespective of the season and they include maize, peanut, sweet potato, cassava and beans. The chemical and physical parameters of soil are summarized in Table 2.

The collected samples were passed through 2 mm sieve and transported in polyethylene bags to the laboratory. In the laboratory, 200 g of soil humidified to 60% of the maximum water holding capacity and containing 0 (control), 0.5, 1, 5, 10, 50 and 100 µg/g endosulfan, were conveniently and aseptically mixed in laminar airflow, and placed in 500 ml glass bottles. The microcosms were incubated at 22 ± 2°C. Soil samples were taken at 5, 10, 15, 20, 30, 60 and 75 days for investigating the potential effects of this toxic to the parameters studied and the moisture was monitored weekly.

Soil characteristics

Soil characteristics were determined using standard methods.

pH

Soil pH (H_2O) was measured using saturated soil solution (1/2.5 that means 1 g of soil in 2.5 ml of water).

Organic matter content

The organic matter content was estimated by the method of Walkley and Black (1934), using potassium dichromate. Organic carbon was evaluated by dividing the value of the organic matter content by 1,724 according to Hamaker and Thompson (1972).

Phosphorus availability

The phosphorus available was estimated following the method of Olsen et al. (1954). Soil samples for the available phosphorus were extracted with NaHCO3, pH 8.5 and were analyzed spectrophotometrically at 882 nm. The results were reported on an oven dry - weight basis, determined by drying the soils for 24 h at 105°C.

Analysis of soil enzyme activities

Dehydrogenase activity

Dehydrogenase activity was estimated using the method of Casida et al. (1964). This method implies the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC), 3% to triphenylformazan (TPF) with methanol. Each soil sample (20 g) was thoroughly mixed with $CaCO_3$ (0.2 g) to ease methanol extraction and 6 g of this mixture was treated with 3% (w/v) TTC incubated for 24 h at 37 ± 1°C. The TPF formed was extracted quantitatively from the reaction mixture with methanol and assayed at 485 nm in a Hach DR 2000 spectrophotometer.

β-D-Glucosidase activity

β-D-Glucosidase activity was estimated using the method of Eivazi and Tabatabai (1988), which consists of measuring the P-nitrophenol released from soil when treated with P-nitrophenyl-β-D-glucopyranoside. 4 ml of modified universal buffer (MUB) at pH 6.0 and 1 ml of P-nitrophenyl-β-D-glucopyranoside were added to the soil (1 g) and the reaction mixture was incubated at 37°C for 1 h. Then 0.5 M $CaCl_2$ (1 ml) and 0.5 M NaOH (4 ml) were added and the mixture was centrifuged at about 1500 x g for 10 min. The P-nitrophenol (PNP) in the supernatant was determined colorimetrically at 400 nm.

Acid and alkaline phosphatase activities

Acid and alkaline phosphatase activities were determined using the colorimetric methods of Tabatabai and Bremmer (1969) and Eivazi and Tabatabai (1977), with slight modifications. These methods consist of quantifying the P-nitrophenyl phosphate at pH 6.5 and 11 for acid and alkaline phosphatase activities, respectively. Soil

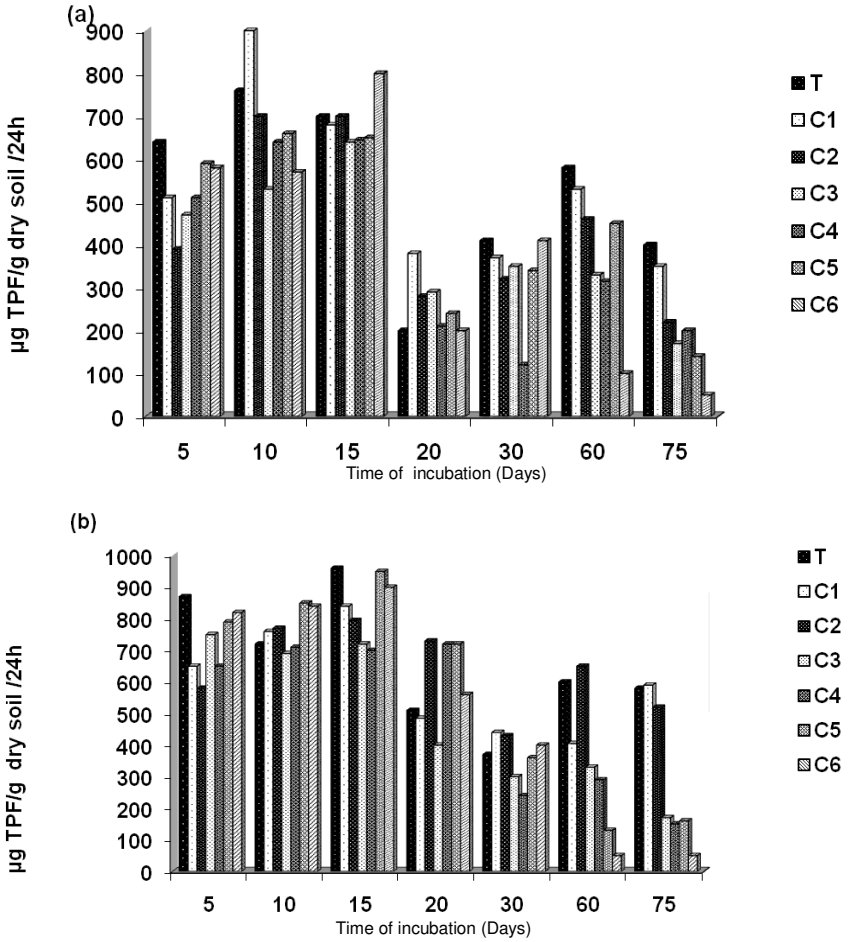

Figure 1. Effect of endosulfan on dehydrogenase activity of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

samples (1 g) were mixed with 4 ml of modified universal buffer (MUB) at pH 6.5 and 11 for acid and alkaline phosphatase assays, respectively; and 0.05 M P-nitrophenyl-phosphate (1 ml) was incubated at 37°C for 1 h. Then 0.5 M $CaCl_2$ (1 ml) and 0.5 M NaOH (4 ml) were added and the mixture was centrifuged at about 1500 x g for 10 min. The PNP in the supernatant was determined colorimetrically at 400 nm. To evaluate the activities of these hydrolases (glucosidase and phosphatase), toluene was not used since it increases the enzyme activities (Tabatabai, 1982) and can be used as carbon source for microorganisms of the soil (Kaplan and Hartenstein, 1979).

Analysis of correlation and determination of IC_{50}

Pearson correlation coefficients were calculated with the Statistical Package for Social Sciences (SPSS) 80 software, and were tested at 95% of security threshold. This correlation was studied on one hand between organic matter and β-D-Glucosidase activity, and between phosphorus available and phosphatase activities on the other hand. The IC_{50} (median inhibitory concentration at which a 50% reduction in response relative to controls was predicted) values were determined for enzyme activity responses that are adversely affected by endosulfan, using least squares linear regression of percentage control of enzyme activity responses against the logarithm of test chemical concentrations.

RESULTS

The soils studied were typical ferrallitic soils. According to the triangle soil, the soil of Mendong was medium loam while the soil of Minkoa-Meyos belonged to the clay loam. Minkoa-Meyos soil seemed to be more acid and holds more water. Mendong soil contained more kations, phosphorus, organic carbon, and total nitrogen than Minkoa-Meyos soil (Table 2).

Effect of endosulfan on dehydrogenase activities

The effects of endosulfan on dehydrogenase activities on soils are illustrated in Figure 1. There were two phases in the evolution of dehydrogenase activity histograms regardless of the soil type. One phase was from the 1st

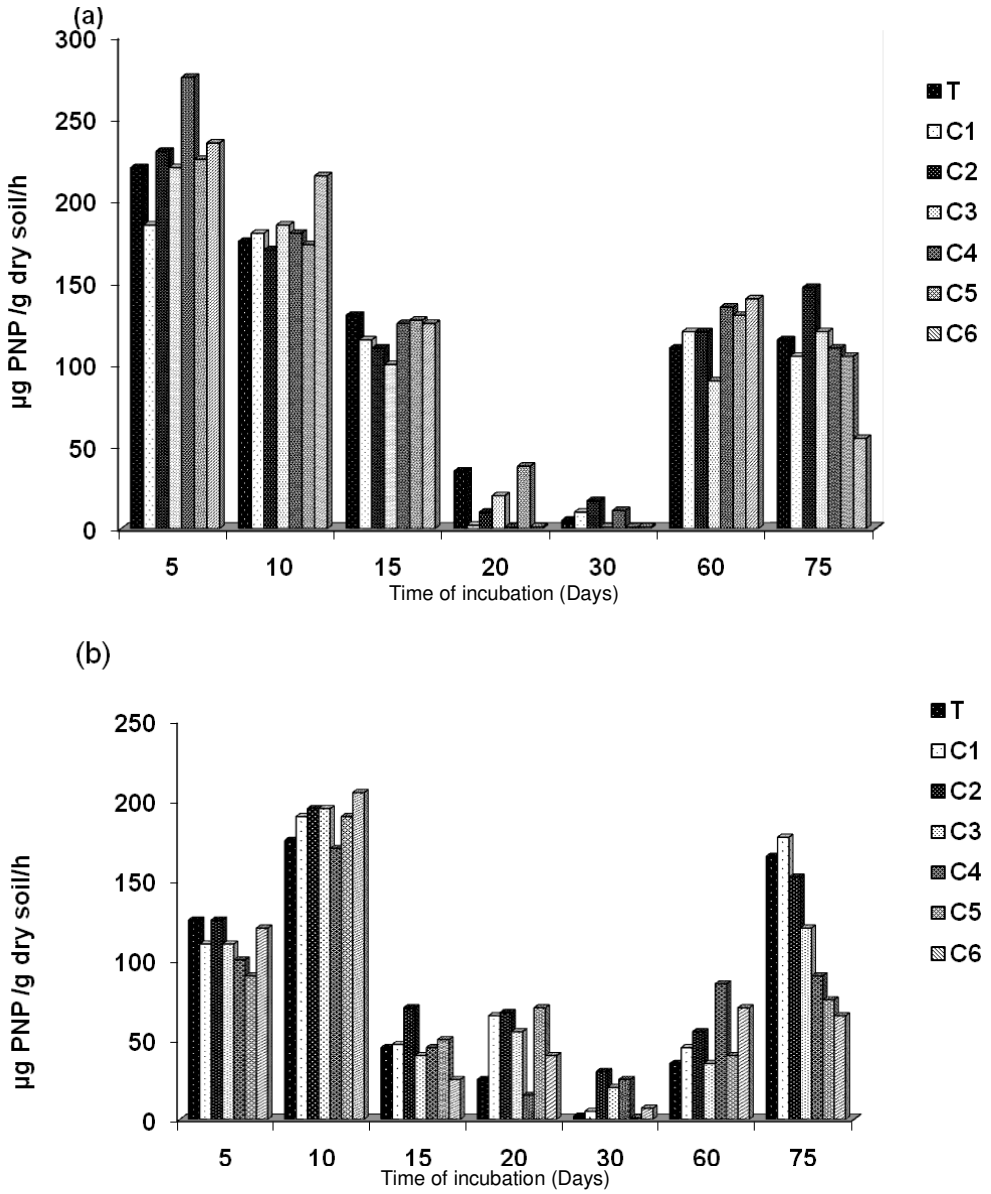

Figure 2. Effect of endosulfan on glucosidase activity of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

to the 30th day of incubation, characterized by the stimulation of this activity in the Mendong soil while in the Minkoa-Meyos soil, there was no measurable effect of endosulfan on the dehydrogenase activities and the other phase was after 60 days of incubation, where the addition of endosulfan in both soils inhibited the dehydrogenase activities. This inhibition showed dose-response results and was more sensitive at 75th day particularly in the Minkoa-Meyos soil. The inhibitory action was expressed as 50% inhibitory concentration (IC_{50}). At the 60th day, the IC_{50} was 28.53 and 5.96 µg/g of dry soil for the Minkoa-Meyos and Mendong soil, respectively. At day 75, the IC_{50} was 5.14 and 5.83 µg/g of dry soil for the Minkoa-Meyos and Mendong soil, respectively.

Effect of endosulfan on β-D-glucosidase activities

Before 60 days of incubation, addition of endosulfan seemed to increase the activities of β-D-glucosidase as compared with the control in Minkoa-Meyos soil (Figure 2a). However, no significant effect of endosulfan addition on the activity of β–D–glucosidase from Mendong soil was observed (Figure 2b). In both soils, at the 75th day of

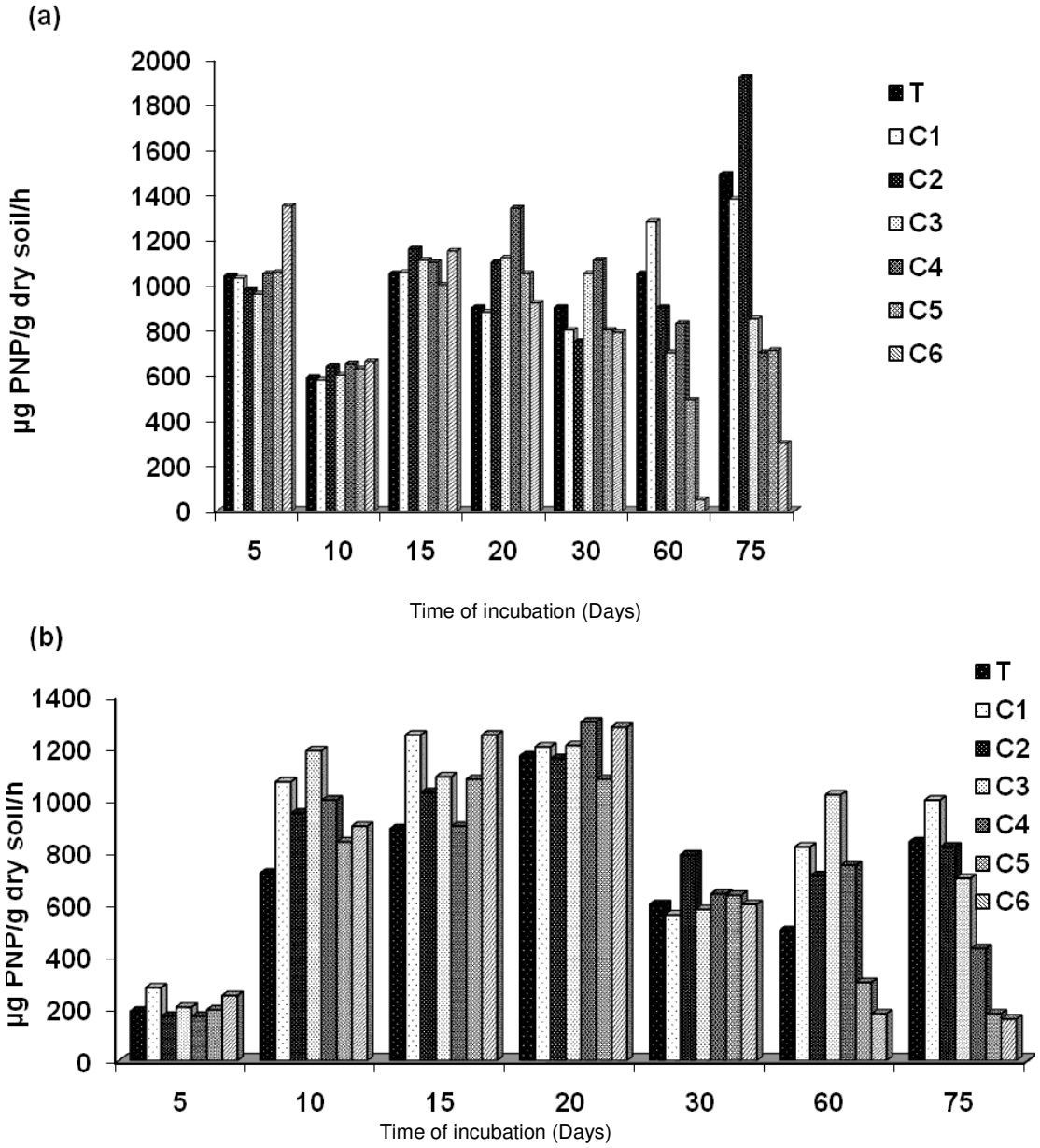

Figure 3. Effect of endosulfan on acid phosphatase activity of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

incubation, the increase of toxins in the soil decreased the activity of β-D-glucosidase. This inhibition showed dose-response results and the IC_{50} was 63.37 µg/g of dry soil (Figure 2).

Effect of endosulfan on phosphatase activities

Generally for short term (time of incubation ≤ 30 days), no significant change in the phosphatase activity in the Minkoa-Meyos soil was observed. However, the addition of endosulfan on Mendong soil increased the phosphatase activity. After 60 days of incubation, the addition of endosulfan on soil inhibited the activity of these enzymes, except in the Mendong soil, where the stimulation of this activity for concentrations ≤ 10 µg/g of dry soil was observed (Figure 3b). The inhibitory action was expressed as 50% IC_{50}. In the Minkoa-Meyos soil, at the 60th day of incubation, the IC_{50} was 18.71 and 10.1 µg/g of dry soil for acid and alkaline phosphatase activity, respectively. At day 75, the IC_{50} was 13.87 and 18.12 µg/g of dry soil, respectively. In the soil from Mendong at

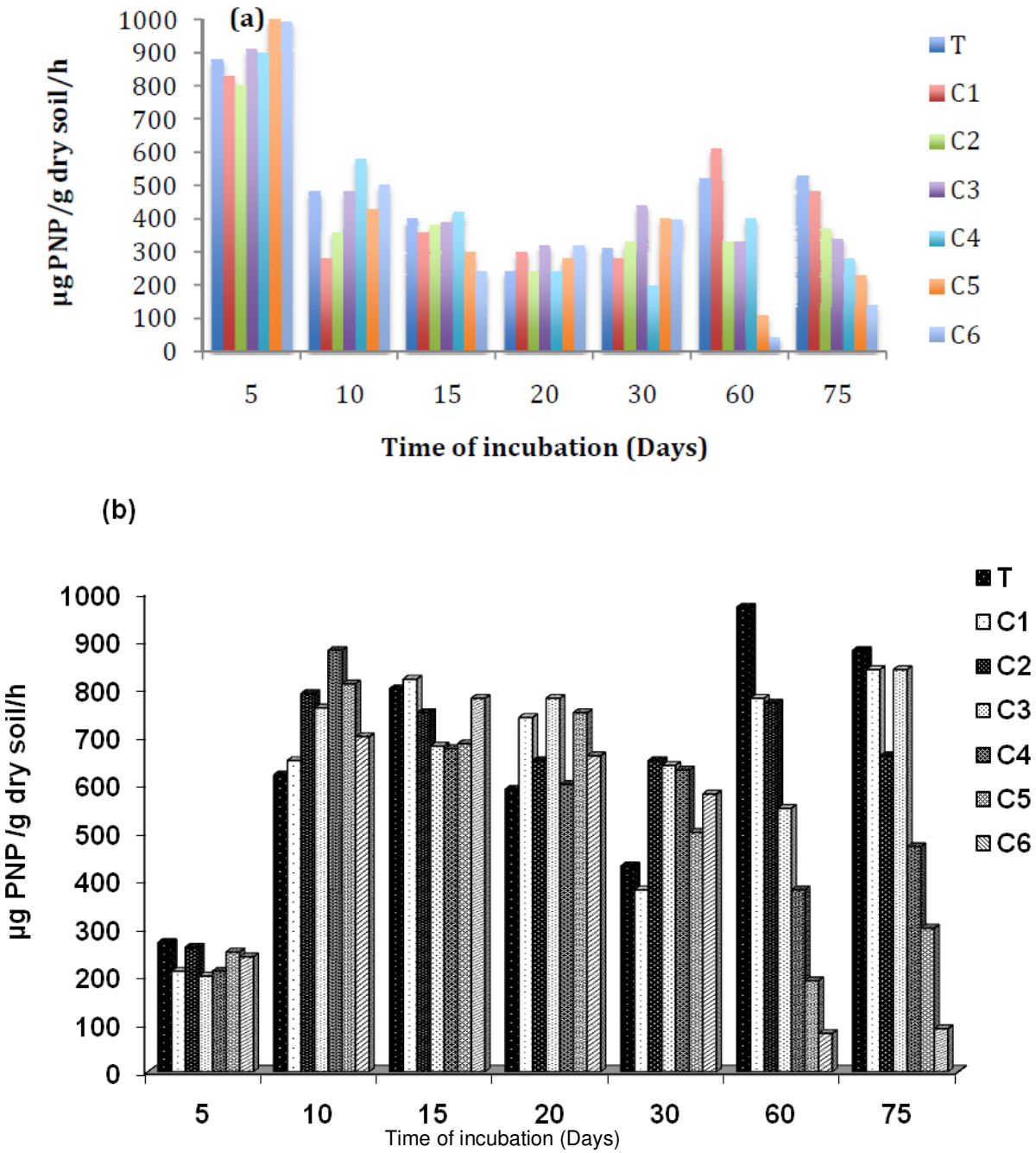

Figure 4. Effect of endosulfan on alkaline phosphatase activity of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

the 60th day of incubation, the IC_{50} was 13.87 and 18.12 µg/g of dry soil for acid and alkaline phosphatase activity respectively, while at day 75, the IC_{50} was 15.5 and 15.38 µg/g of dry soil for acid and alkaline phosphatase activity, respectively (Figure 4).

Effect of endosulfan on organic carbon content

The insecticide treatment schedule used in this investigation did not cause any adverse effect on the organic carbon content of the soil from Minkoa-Meyos

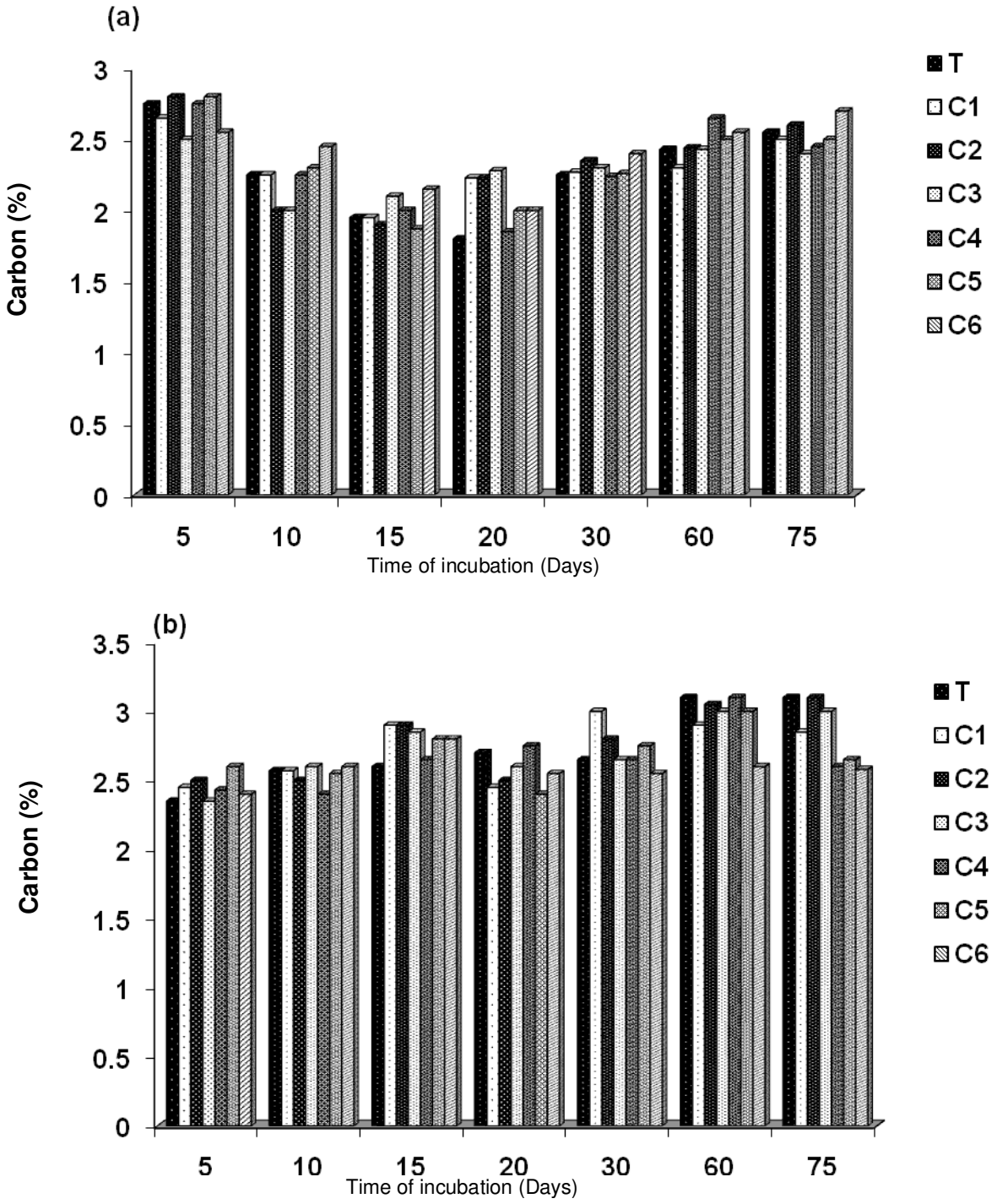

Figure 5. Effect of endosulfan on organic carbon content of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

after 75 days of incubation (Figure 5a). However, after 60 days, endosulfan significantly decreased the organic carbon content at the highest concentration used on the soil from Mendong (Figure 5b).

Effect of endosulfan on phosphorus availability

Generally, in both soils, after 30 days of incubation, the addition of endosulfan increased the availability of

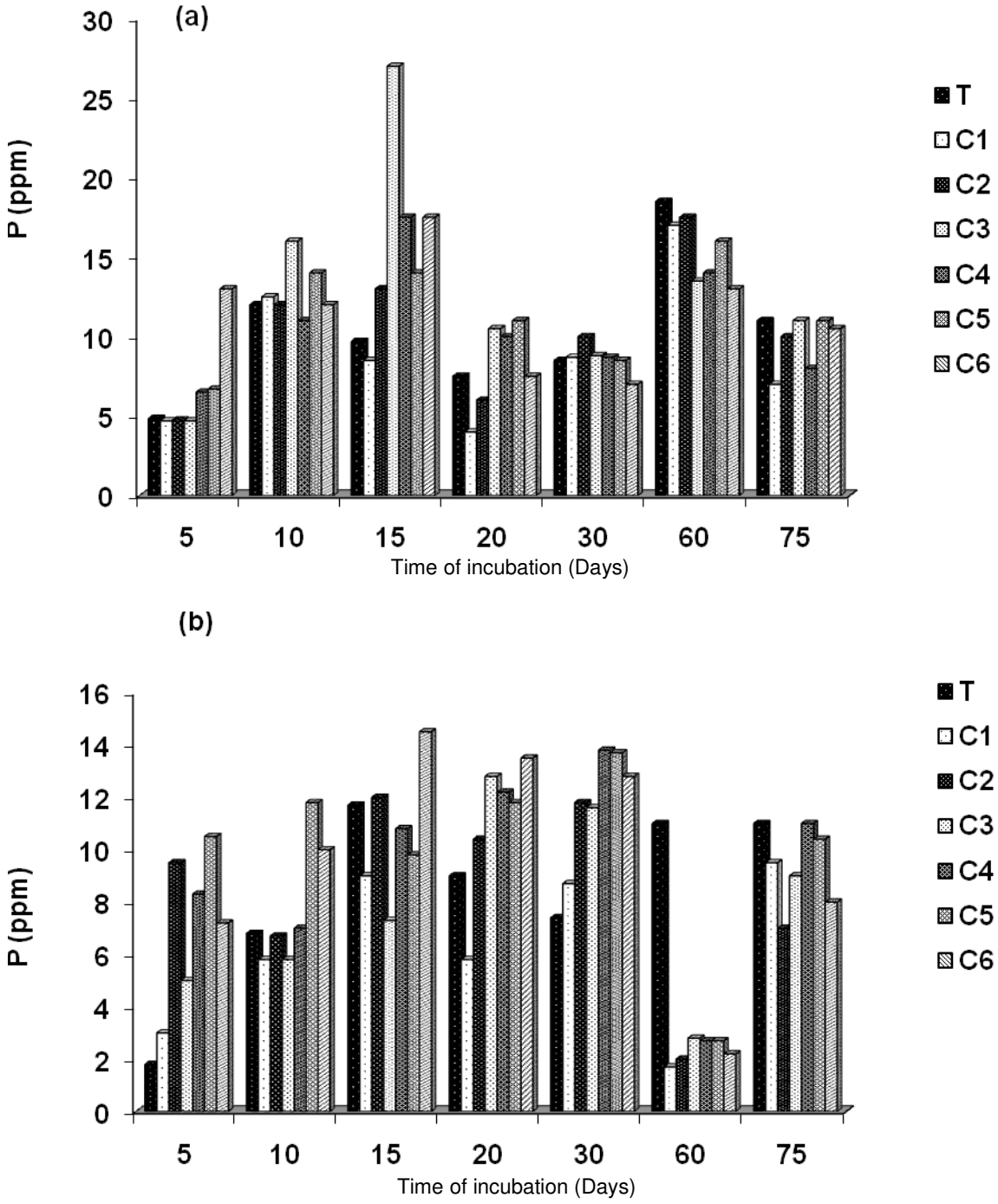

Figure 6. Effect of endosulfan on phosphorus availability of the soils from (a) Minkoa-Meyos, (b) Mendong after the addition of different rates of endosulfan.

phosphorus (Figure 6), and this increase was more pronounced in the soil from Mendong (Figure 6b). After 60 days of incubation, a decrease in phosphorus availability in both soils was observed, but this inhibition was moderated after 75 days of incubation (Figure 6).

Research of correlations between enzyme activity, organic carbon content and phosphorus availability

In both soils studied after 75 days of incubation, there was no significant correlation between the β-D-glucosidase

activity and the organic carbon content irrespective of the concentration. Furthermore, no significant correlation was observed between the phosphatase activity and the phosphorus available. Nevertheless, in the soil from Mendong, at the highest concentration (100 µg/g of dry soil), a significant correlation ($p < 0.05$) between acid phosphatase activity, alkaline phosphatase activity and phosphorus availability was apparent.

DISCUSSION

In this study, certain microbial functions were emphasized in order to estimate the non target effects of endosulfan on agricultural soils.

Effect of endosulfan on enzyme activities

The effect of endosulfan on enzyme activities studied generally revealed two phases: Firstly, after 30 days of incubation, endosulfan seemed to increase the activities of these enzymes in both soils. Usually, after an initial toxic effect, the mineralization of the insecticides leads to an increase in the substrates for microbial growth, which might be the reason for the increase in enzyme activities as observed in this investigation. In this case, endosulfan becomes a source of carbon for microorganisms (Iqbal et al*.,* 2001; Ikeda et al., 2005; Savadogo et al., 2007). In support to these findings, Hussan et al. (2005) reported that profenophos or profenophos and cypermethrin or alphamethrin stimulates the dehydrogenase activity. It was also found that, at this time of incubation, there were no significant effects of endosulfan on the dehydrogenase activity in the soil from Minkoa-Meyos and on β-D-glucosidase activity in the soil from Mendong. The effect of pesticides on microbial activities 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 enzyme activities indicated that, the time of incubation was insufficient to induce an effect. The effect of a pesticide on soil microorganisms is governed not only by the chemical and the physical properties of the pesticide itself, but also by the soil type, soil properties, and prevailing environmental conditions (Malkomes and Wohler, 1983). The dehydrogenase is an intracellular enzyme. β-D-Glucosidase is an extracellular enzyme and could be protected from degradation by adsorption on clays and humic substances (Skujins, 1976; Boyd and Mortland, 1990). Moreover, Cervelli et al. (1978) proved that an insecticide could stimulate enzymes activities in one soil and does not affect or inhibit these activities in another soil. It depends on the relationship between the pesticide and the microbial population, relationship between the pesticide and the enzymes and relationship between the pesticide and the soil colloids.

Secondly, after 60 days of incubation, endosulfan appears to be toxic to the enzymes activities studied. Soil microorganisms mostly produce soil enzymes. The inhibition observed could be explained by the fact that endosulfan inhibited microorganisms involved in this activities. In support to these findings, Nisha et al. (2006) reported that dehydrogenase have also recorded a gradual decline in its activity with increasing number of sprays of monocrotophos and endosulfan. Soil dehydrogenase activity was inhibited by methamidophos, fenpropathrin, endosulfan + dimethoate and bifenthrin + ethion (http://www-naweb.iaea.org/nafa/fep/public/pakistan-impact.pdf).

Pozo et al. (1995) also reported that chlorpyrifos, an organophosphorus insecticide, significantly inhibited the acid and alkaline phosphatase activities in the soil. This inhibition also showed dose-response results and was more sensitive at 75 days of incubation. Increasing inhibitory effect on enzyme activity which increases the concentration of endosulfan was also recorded by Lal and Yadav (2000).

Effect of endosulfan on microbial functions studied

The increase in phosphorus available content at the first phase of the incubation in both soils could be explained by the fact that the enzymes involved in the degradation of organic phosphorus were stimulated. On the contrary, the decrease in the phosphorus available content was due to the inhibition of microorganisms or enzymes involved in this transformation. Inactivation of nitrogen-fixing and phosphorus-solubilizing microorganisms was observed in pesticide-contaminated soils (Hussain et al., 2009).

The effects of endosulfan on phosphatase activity in Mendong soil at 100 µg/g of dry soil were in the same direction as for phosphorus available. In fact, the two parameters studied were positively correlated. Phosphatases are exoenzymes involved in the mineralization of organic phosphorus (Ajwa et al., 1999). The decrease in phosphorus availability at this concentration could be explained by the inhibition of acid and alkaline phosphatase activities. It may be that endosulfan treatment decreased the number of soil bacteria involved in the transformation of organic phosphorus. There was no measurable effect of endosulfan on organic carbon content in Minkoa-Meyos soil at the end of this experiment. The soils contained more clays and silt (31 and 24% respectively). Thus, the enzymes involved in the transformation of organic carbon like β- D- glucosidase could be protected from being degraded by adsorption of clays and humic substances (Skujins, 1976; Boyd and Mortland, 1990). Kumar and Philip (2006) showed that the content of organic matter

was correlated with the coefficients of adsorption. Clays can cause chemical conversion of adsorbed organic molecules. However, after 60 days of incubation, endosulfan significantly decreased the organic carbon content at highest concentration in the soil from Mendong. Endosulfan could stimulate the activity of organisms involved in the organic carbon mineralization. Several studies indicated that endosulfan degradation in soils was carried out by mushrooms, bacteria and Actinomycetes (Martens, 1976; Awasthi et al*.,* 2000). The importance of the respiratory activity was related to the soil carbon content. The organic carbon mineralization could be partially explained by the increase in the respiratory rate. Savadogo et al. (2009) reported that, endosulfan with amount of 6 µg/g induced a stimulation of the respiratory activity of the soils.

In this investigation, endosulfan was applied at different concentrations on the soil and was incubated for 75 days. Short-term changes (stimulation) or no changes were observed in the activities of the enzymes studied. However, at the end of the experimental period, these activities were significantly inhibited. Endosulfan appeared to have no measurable effect on the organic carbon content and the availability of phosphorus in Minkoa-Meyos soil. On the contrary, these two functions were inhibited in the soil from Mendong. Moreover, significant correlations in this soil at the highest concentration were obtained between phosphatase activities and the available phosphorus.

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